

DANMAP 2018

DANMAP 2018 - Use of antimicrobial agents and occurrence
of antimicrobial resistance in bacteria from food animals,
food and humans in Denmark



DANMAP 2018

Editors:

Birgitte Borck Høg (bibo@food.dtu.dk)
Johanne Ellis-Iversen (joell@food.dtu.dk)
Ute Wolff Sönksen (uws@ssi.dk)

Assisting editors:

Helle Korsgaard
Anna Emilie Henius
Karoline Skjold Selle Pedersen

DANMAP Steering Committee:

National Food Institute: Flemming Bager, Johanne Ellis-Iversen
Statens Serum Institut: Anders Rhod Larsen, Ute Wolff Sönksen

Layout:

Charlotte Brunholt Jensen, STEP PRINT POWER
Anja Bjarnum, Statens Serum Institut

Photos: Colourbox

Printing: STEP PRINT POWER

Contact:

National Food Institute,
Technical University of Denmark
Kemitorvet, Building 202, DK-2800 Kgs. Lyngby

Infectious Disease Preparedness - Bacteria, Parasites and Fungi,
Statens Serum Institut
Artillerivej 5, DK-2300 Copenhagen

DANMAP 2018 - September 2019 - ISSN 1600-2032

Text and tables may be cited and reprinted only with reference to this report:
DANMAP 2018 - Use of antimicrobial agents and occurrence of antimicrobial
resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032

The report is available from www.danmap.org

Authors:

National Food Institute

Valeria Bortolaia
Rene S. Hendriksen
Birgitte Borck Høg
Johanne Ellis-Iversen
Helle Korsgaard
Channie Kahl Petersen

Statens Serum Institut

Jeppe Boel
Tine Dalby
Anette M. Hammerum
Frank Hansen
Henrik Hasman
Anna Emilie Henius
Steen Hoffmann
Mette Bar Ilan
Hülya Kaya
Anne Kjerulf
Brian Kristensen

Anders Rhod Larsen
Eva Møller Nielsen
Stefan Schytte Olsen
Karoline Skjold Selle Pedersen
Andreas Petersen
Lone Jannok Porsbo
Louise Roer
Sissel Skovgaard
Hans-Christian Slotved
Ute Wolff Sönksen
Mia Torpdahl

**Danish Health Data
Authority**, Register of
Medicinal Statistics:
Maja Laursen

Authors of invited textboxes:

Nissrine Abou-Chakra
Øystein Angen
Maïken Cavling Arendrup
Raluca Datcu
Hans Henrik Dietz
Mette Fertner
Karen Geismar
Jan Berg Gertsen

Sophie Gubbels
Christian Fink Hansen
Rasmus Krøger Hare
Jette Holt
Barbara Juliane Holzknecht
Anne Birgitte Jensen
Vibeke Frøkjær Jensen
Sven Erik Jorsal

Elsebeth Tvenstrup Jensen
Lise Kristensen
Jonas Kähler
Jesper Larsen
Tove Larsen
Pimplapas Leekitcharoenphon
Inge Mortensen
Patrick Munk

Peter Poulsen
Malene Risum
Charlotte Mark Salomonsen
Tina Struve
Marianne Voldstedlund

DANMAP 2018

DANMAP 2018 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

Table of contents

1. Editorial	7
2. Summary	9
3. Introduction to DANMAP	15
3.1 Background	16
3.2 Information on demographics, food production and dataflow	18
4. Antimicrobial consumption in animals	23
Highlights	24
4.1 Introduction	25
Textbox 4.1 Regulation resulted in a shift between antimicrobial classes in pigs 2014-2018	27
4.2 Total antimicrobial consumption in animals	28
4.3 Antimicrobial consumption by animal species	28
Textbox 4.2 Establishment of an Advisory Committee on Veterinary Medicines	37
Textbox 4.3 OUA - an up and coming special production	38
Textbox 4.4 Antimicrobial use in mink - action plan/research projects	39
5. Antimicrobial consumption in humans	41
Highlights	42
5.1 Introduction	44
5.2 Total consumption	47
5.3 Primary health care	58
5.4 Hospital care	67
Textbox 5.1 Incidence of multi resistant bacteria and consumption of antimicrobial agents in Greenland	68
Textbox 5.2 Antibiotics in dentistry	70
Textbox 5.3 Infection prevention and control can combat antimicrobial resistance	72
Textbox 5.4 Candidaemia in Denmark: Epidemiology, antifungal consumption and resistance Azole resistance in <i>Aspergillus</i> spp. - results from a new surveillance programme	74 77
6. Resistance in zoonotic bacteria	79
Highlights	80
6.1 Introduction	81
6.2 <i>Campylobacter</i>	81
6.3 <i>Salmonella</i>	84
Textbox 6.1 Antimicrobial resistance in <i>Salmonella</i> from sow, multiplier and breeder pig herds	89
Textbox 6.2 Resistance in bacteria from diagnostic submissions from pigs	92
7. Resistance in indicator bacteria	97
Highlights	98
7.1 Introduction	98
7.2 Indicator <i>Escherichia coli</i>	98
7.3 ESBL/AmpC- and carbapenemase-producing <i>E. coli</i>	103
Textbox 7.1 Antimicrobial resistance in prawns and pangasius fillets imported from Asia	106
Textbox 7.2 ESBL- and pAmpC-producing <i>E. coli</i> - comparison of isolates of animal origin and isolates obtained from human bloodstream infections	109
Textbox 7.3 Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries	110

8.	Resistance in human clinical bacteria	113
	Highlights	114
8.1	Introduction	115
Textbox 8.1	MiBa, the Danish Microbiology Database - Now prepared for use in national AMR-surveillance	118
8.2	Surveillance based on MiBa data	122
8.2.1	<i>Escherichia coli</i>	122
8.2.2	<i>Klebsiella pneumoniae</i>	126
8.2.3	<i>Pseudomonas aeruginosa</i>	131
8.2.4	<i>Acinetobacter</i> species	132
8.2.5	Enterococci	133
8.3	Surveillance based on data from the reference laboratories	134
8.3.1	ESBL- and pAmpC-producing <i>E. coli</i> from bloodstream infections	134
8.3.2	Carbapenemase-producing bacteria	137
8.3.3	<i>VanA Enterococcus faecium</i>	138
8.3.4	Linezolid resistance and the <i>optrA</i> in enterococci	140
8.3.5	<i>Streptococcus pneumoniae</i>	141
8.3.6	Beta-haemolytic streptococci	143
8.3.7	<i>Haemophilus influenzae</i>	145
8.3.8	<i>Staphylococcus aureus</i> - MRSA	146
Textbox 8.2	LA-MRSA CC398 in animals and humans	151
8.3.9	<i>Neisseria gonorrhoeae</i>	153
9.	Materials and Methods	155
9.1	General information	156
	Animals and meat	
9.2	Data on antimicrobial consumption	156
9.3	Collection of bacterial isolates	158
9.4	Microbiological methods	159
9.5	Susceptibility testing	160
9.6	Whole genome sequencing	160
9.7	Data handling	160
	Humans	
9.8	Data on antimicrobial consumption	162
9.9	<i>Salmonella</i> and <i>Campylobacter</i>	165
9.10	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>Acinetobacter</i> spp. and enterococci	165
9.11	ESBL-producing bacteria	165
9.12	CPO in human patients	166
9.13	VRE in human patients	166
9.14	<i>Streptococcus pneumoniae</i>	166
9.15	Beta-haemolytic streptococci (Group A, B, C and G streptococci)	167
9.16	<i>Haemophilus influenzae</i>	167
9.17	<i>Staphylococcus aureus</i> - MRSA	167
9.18	Gonococci in humans	168
10.	Terminology	169
	List of abbreviations	170
	Glossary	171

Additional information and supporting data on antimicrobial consumption and antimicrobial resistance is presented in the web annex at www.DANMAP.org.

1. Editorial

In 1998, Copenhagen hosted an AMR conference, The Microbial Threat, which called for a joint European strategy to deal with increasing levels of antimicrobial resistance. Since then, AMR has gained importance on the global agenda with the United Nations calling for action. The financial costs of AMR are well documented and without effective and prompt interventions, the attainment of several of the UN Sustainable Development Goals by 2030 will be in grave jeopardy.

A window of opportunity has opened: In addition to national governments, several major philanthropic organisations have acknowledged that investing in AMR control worldwide is paramount. Currently, on a global scale several projects implement surveillance and control programmes of bacterial resistance and antimicrobial use with focus on low- and middle income countries.

Overall and regardless of the cultural setting, the strategy for reduction of AMR is targeting major drivers. For example, access to antimicrobials needs restriction to situations, where treatment of disease is necessary, not where use of antimicrobials is simply convenient.

Interventions to reduce AMR require population-wide behavioural changes driven by policies, awareness and good governance. The awareness of resistance development as a side effect to use of antibiotics is often low among the general population in large parts of the world. Communication efforts and courage of politicians are required to change the perception of antimicrobials among users globally.

In general, Danish veterinarians and medical doctors have good knowledge of correct antibiotic treatment. However, despite good management of the major drivers, levels of AMR are also increasing in Denmark. AMR is a global problem and the risks from imported resistant bacteria from foods, feeds and travels remain. Therefore, Denmark has a direct interest in endorsing and supporting the international recommendations and initiatives around the world. The experiences and know-how gained over the last three decades in Denmark are important to disseminate worldwide and Denmark is actively mentoring and sharing our experiences and knowledge with many countries.

Another key message is the improvement of hygiene such as biosecurity, sanitation and food safety to prevent diseases. Further, once improved hygiene principles are in place, they need to be maintained. These areas are often neglected, not necessarily due to lack of knowledge, but because antimicrobial use is a feasible and sometimes less expensive option than the more time demanding principles of infection prevention. In addition, infection prevention often lacks the innate interest of innovation and therefore struggles to find funding.

In Denmark, these key interventions are implemented at often very high levels and continue to improve. Food safety in Denmark is good and farmers and abattoirs take pride in high standards. The new scheme of 'raising pigs without antimicrobials' also seems sufficiently appealing for consumers to allow a premium price and to be economically viable. This creates incentives for farmers to abandon group treatments and to focus on the treatment of individual animals. Such schemes and other success stories are important to communicate to the world.

Presently, however, resistance monitoring systems on both veterinary and human side are challenged. Regardless of laboratory method applied, detailed surveillance of resistance mechanisms and clones demands bacterial isolates or at least biological samples. The introduction of easily used and quickly applied point of care tests in animal production units or in hospitals helps improving fast diagnostics of infectious conditions, but can be an antagonist to referral of samples to diagnostic laboratories, where data or isolates for DANMAP are submitted.

Already, very few veterinary diagnostic samples are available for DANMAP and surveys are often small, albeit frequent. The small sample sizes reduce the ability to maintain a surveillance that can readily detect changes in antimicrobial resistance levels as well as new emerging resistance mechanisms, which are pivotal for updating prudent use guidelines. Most significantly, the sample sizes do not permit the reliable detection of new resistance phenotypes, when these are at a low prevalence in the population. As some of the rare and potentially critical resistance phenotypes become more frequent in people and imported foods, the risk of introduction in food-producing animals also increases. The benefits of finding a new resistance early, of course, need balancing with the cost of analysing more samples.

The introduction of whole genome sequencing has been of great help in typing and characterisation of bacterial clones or resistance mechanisms such as plasmids. This has enabled very precise comparisons of microorganisms involved in hospital outbreaks as well as in foodborne transmissions. In DANMAP context, it is now used for many of the organisms under surveillance. The recent promising results obtained using metagenomics data, indicate that metagenomics in the future may lead to another new era in antimicrobial resistance surveillance.

DANMAP Steering Committee

Acknowledgements

DANMAP is based on a strong collaboration between several institutions and on the contributions from highly skilled staff from many specialties and professions. Without their knowledge and engagement, there would be no DANMAP surveillance.

The DTU National Food Institute, would like to thank the following:

- the meat inspection staff and the company personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful
- the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup, and the DTU National Veterinary Institute for making isolates of animal pathogens available to the programme
- the staff of the Regional Veterinary and Food Control Authorities for collecting food samples and isolating bacteria
- the Department of Medication Statistics and Research Support at the Danish Health Data Authority (formerly the Danish Medicines Agency and SSI) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies
- the Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, including statistics on consumption measured in tonnage
- the Danish Agriculture and Food Council for cooperation regarding the estimation of live biomass of production animals

Statens Serum Institut would like to thank the following:

- the staff of the Neisseria and Streptococcus Typing Unit at SSI for providing data on samples and resistance in beta-hemolytic streptococci, *H.influenzae* and *Neisseria gonorrhoeae*
- the staff of the Foodborne Pathogens Unit at SSI for providing data on resistance in *Campylobacter* and *Salmonella* from human clinical isolates
- the staff of the Staphylococcus Laboratory at SSI for providing data on invasive staphylococcal infections as well as all MRSA
- the staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI for providing data on resistance in the referred *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and vancomycin and linezolid resistant enterococci
- the staff at Unit of Mycology at SSI for providing resistance data for human *Candida* and *Aspergillus*
- Anne Kjerulf and co-authors for the textbox on the antimicrobial resistance and consumption in Greenland
- Erik Villadsen from the Danish Health Data Authority for providing data on bed-days and admissions

Especially SSI would like to thank

- Maja Laursen from the Danish Health Data Authority, the Register of Medicinal Products Statistics for providing antimicrobial consumption data: DDD, DID, number of users and prescriptions
- all Departments of Clinical Microbiology and the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance.

Participating members from DANRES

Anette Hammerum
Barbara Holzkecht
Bent Røder
Claus Østergaard
Dennis Schrøder Hansen
Jenny Dahl Knudsen
John Eugenio Coia
Jurgita Samulionienė
Lillian M. Søes
Mikala Wang
Niels Frimodt-Møller
Turid Snekløth Søndergaard
Ulrik Stenz Justesen

Laboratory technicians and medical doctors from the Danish DCM

Berit Have Kallesøe
Birgitte Tønning
Dorte Paulmann
Elly Kristensen
Hanne Wiese Hallberg
Henrik Duch Laursen
Ingrid Astrup
Jeanne Elin Storm
Kirsten Paulsen
Lena Mortensen
María Kristín Björnsdóttir
Marianne Bøggild Pedersen
Marlene Ballegaard

Tina Profft Larsen
Tine Besser
Anette Holm
Dennis Back Holmgaard
Hans Linde Nielsen
Henrik C. Schønheyder
Henrik Westh
Jens Kjølseth Møller
Karen Leth Nielsen
Kristian Schønning
Lars Erik Lemming
Leif Percival Andersen
Ram Dessau
Svend Ellermann-Eriksen
Thøger Gorm Jensen



2

SUMMARY



2. Summary

The Danish integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) was founded in 1995, providing a unique one-health platform for the continuous surveillance and research of antimicrobial consumption and resistance. A key objective of the DANMAP programme is to provide an evidence base for decision-making and to further understand the associations between antimicrobial usage (AMU) and the occurrence of antimicrobial resistance (AMR).

In Denmark, antibiotic treatment - of both humans and animals - is available by prescription only, and all prescriptions are recorded by the Register of Medicinal Statistics and available through national databases (MEDSTAT and VetStat). The registration is very detailed, covers the whole nation and dates back to the mid-90s (Medstat) and 2000 (VetStat), respectively.

The report DANMAP 2018 summarises the results of susceptibility testing of isolates obtained from hospitals, general practice, veterinary practice, food-industry laboratories and the Danish Veterinary and Food Administration; as well as records of the types and amounts of antimicrobials prescribed for animal and human treatment in Denmark during 2018. Human isolates cover all bacteraemias caused by the most important pathogenic bacteria, based on all available microbiological data on the first isolate per species per patient per year, representing complete data from all of Denmark. Also included, are microbiological data on *Escherichia coli* and *Klebsiella pneumoniae* isolates from urinary tract infections from hospitals and from general practitioners. In addition, the report includes results from typing and characterisation based on whole genome sequencing of isolates from reportable diseases or isolates carrying specific resistance mechanisms that were received at the reference laboratories. Since 2014, *Campylobacter*, *Salmonella* and indicator and ESBL/AmpC-producing *E. coli* from production animals and meat are collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. Furthermore, isolates collected from additional surveys and isolates from *Salmonella* control programmes are also included

Statens Serum Institut (SSI) collates and interprets data from the human sectors and the Technical University of Denmark (DTU) collates and interprets data from the food and animals sectors.

Antimicrobial consumption in animals

Information on consumption in animals is based on total amount of veterinary prescription medicine as registered by pharmacies, private companies, veterinarians and feed mills. Detailed data for each prescription item are sent to the VetStat database, and the antimicrobial use in animals is estimated

first as kg active compound and further transformed into defined animal daily doses (DADD), a national veterinary equivalent to the international defined daily doses (DDD) system applied in the human field.

Since 2013, there has been an overall decreasing trend in the use of antimicrobials in animals and in 2018 the use had been reduced by 14%, a reduction of almost 17 tonnes, compared with the antimicrobial use in 2013. The total use of veterinary prescribed antimicrobials amounted to just around 100 tonnes in 2018.

Patterns in antimicrobial use in animals are driven by the pig sector, because 75% of all antimicrobials used in animals are used for pigs, equivalent to 74.7 tonnes in 2018. Measured in DADD per 1000 animals per day (DAPD), the antimicrobial use in weaner pigs was reduced from 97 to 91 DAPD, meaning that on a given day in 2018, on average, approximately 9% of weaner pigs received antimicrobial treatment. In finishers, the use was reduced from 18 to 17 DAPD, while it remained at roughly the same level in sows and piglets, around 19 DAPD. The overall use for pigs, measured in DAPD and adjusted for changes in exports was reduced by approximately 4% from 24 to 23 DAPD in 2018, and has decreased by 32% since 2009.

The types of antimicrobials used in pigs also shifted notably. Since 2009, the use of tetracyclines in pigs reduced significantly and especially from 2016 to 2018, due to the implementation of the differentiated "Yellow card".

The differentiated "Yellow Card" initiative has also resulted in a close to zero use of colistin, since the first quarter of 2017. However, in weaners the decrease in the use of tetracyclines and colistin was somewhat mirrored by increases in the use of macrolides, pleuromutilines and aminoglycosides.

The overall use for cattle has fluctuated between 12 and 13 tonnes over the past five years. In 2018, more than two thirds were used to treat older cattle (>1 year) and approximately 500 kg were used as intramammary treatment. Measured in DAPD, the antimicrobial use for older cattle (>1 year) has decreased from 4 to 3 DAPD (-13%) over the past decade, while the use for younger cattle (<1 year) has increased from around 5 to 7 DAPD (+43%).

Antimicrobial use in poultry is relatively low (1,326 kg). Notable fluctuations may occur as a result of disease outbreaks in a few large flocks, which was the case in 2014 and 2015. However, since 2015, the use has been reduced each year and was 16% lower than in 2015. In contrast, the use of antimicrobials in the aquaculture industry more than doubled (1,860 kg more) in 2018 due to the very warm summer in 2018.

From 2014 to 2017, the use of antimicrobials in fur animals (mink) increased. However, in 2018 the industry increased focus on prudent use and developed an action plan to reduce the use within the production. The increased focus combined with low occurrence of disease in 2018 resulted in a 40% reduction in antimicrobial use for fur animals (2,467 kg less) compared with 2017.

Companion animals still use more critically important antimicrobials compared to other species. Almost all fluoroquinolones and more than half of the cephalosporins used in animals are used in dogs and cats. Despite a small increase in use from 2015 to 2016, there has been an overall decreasing trend in the use of antimicrobials in dogs and cats since 2011. In addition, the antimicrobial use in pets appears to have shifted away from the use of cephalosporins towards the broad spectrum penicillins.

Antimicrobial consumption in humans

Information on consumption in humans is based on total sales from primary pharmacies and hospital pharmacies, reported in volume per package size and transformed into defined daily doses (DDD), the assumed average maintenance dosage per given antimicrobial class as defined by the WHO ATC Center. Data from primary health care has been reported since 1994 and from hospitals since 1997. As per first of January 2019, the WHO ATC Center changed DDD values for several antibiotics, based on recommendations and results from an expert working group. In DANMAP 2018, the new DDD values were applied and all tables and figures updated ten years back.

In 2018, consumption of antimicrobials in humans in total, primary sector and hospital sector combined, was 15.95 DDD per 1000 inhabitants per day (DID), lower than the consumption in 2017 (16.67 DID) and lower than a decade ago in 2009 (17.47 DID). The highest consumption ever reported in Denmark was in 2011 (18.91 DID).

In 2009, the consumption of antimicrobial agents in the primary sector was 15.69 DID, which since decreased to 13.98 DID in 2018 (-11%). The hospital sector simultaneously saw an increase from 1.67 DID in 2009 to 1.92 DID in 2018 (15%).

For comparison with actual treatment dosages used in Denmark, the report also includes two figures presenting consumption in Danish adjusted daily dosage (DaDDD) for the primary sector and the hospital sector, respectively. When applying DaDDD, figures changed notably and the share of each antibiotic class was more correctly resembled in the total, overall showing a smaller use than with the standard DDD. For most figures in the report the standard DDD as defined by the WHO were used for comparison with other countries.

Decreases in the past ten years described for the **primary sector** were observed for all age groups (but less pronounced for the eldest > 80 years) and for both genders, regardless of

the indicators used. The biggest decrease was observed in the youngest (0 to 4 year olds), where the number of treated patients per 1000 inhabitants of the same age group decreased with 41%. In 2018, there were 272 per 1000 treated 0 to 4 year olds corresponding to 406 prescriptions redeemed per 1000 inhabitants.

In women, the number of treated patients per 1000 inhabitants decreased with 16% and in men with 20%. In 2018, the average number of patients treated (regardless of age and gender) was 243 per 1000 inhabitants corresponding to 459 prescriptions redeemed per 1000 inhabitants. When the number of prescriptions issued through hospital doctors is subtracted from the total number, the average number of treated patients (all age groups and genders) was 236 per 1000 inhabitants corresponding to 396 prescriptions redeemed per 1000 inhabitants.

The total antimicrobial **consumption at hospitals** was measured at 99 DBD and 292 defined daily doses per 100 admissions (DAD), respectively, a rise from 96 DBD and 282 DAD the previous year. From 2009 to 2018, the total consumption at hospitals increased with 41% and 10%, when measured in DBD and DAD, respectively.

Penicillins remained the most frequently used antimicrobial agents in both primary health care (66%) and in hospital care (54%), but the changes in consumption observed within this drug group in the last decade continued. Thus in 2009, beta-lactamase sensitive penicillins constituted 54% of all penicillins consumed in primary health care (5.31 DID of 9.45 DID), while in 2018 this had decreased to 39% (3.61 DID of 9.22 DID). Simultaneously, consumption of combination penicillins increased markedly in both sectors for that decade. Thus in 2018, combination penicillins constituted 4.7% of the antimicrobial consumption in primary health care and 16% of the consumption in hospital care. From 2017 to 2018, decreases in the consumption of most penicillins were observed.

Together with the penicillins with extended spectrum, combination penicillins were in 2018 the largest antimicrobial drug group consumed at hospitals.

In Denmark, **fluoroquinolones, cephalosporins and carbapenems** are defined as antimicrobials of critical interest and cephalosporins and carbapenems are only used at hospitals. In 2018, the consumption of the three drug classes constituted altogether 20% of the consumption at hospitals, a decrease from 23% observed the year before, and a larger decrease from 32% in 2009. Carbapenems decreased from 2.83 DBD in 2017 to 2.77 DBD in 2018, a decrease of 2.3 %. Cephalosporins decreased from 2.83 DBD in 2017 to 2.77 DBD in 2018, a decrease of 16%. This decrease was expected since the consumption increased during 2017 due to a shortage of piperacillin with tazobactam.

Fluoroquinolones continued the decreasing trend observed since 2013, but with more pronounced reductions for the last three years. In 2017, fluoroquinolones accounted for a consumption of 6.98 DBD, corresponding to 7.9% of the total consumption at hospital. In 2018, this had decreased to 6.81 DBD, corresponding to 7.2% of the consumption at hospitals. In primary care, fluoroquinolones accounted for 0.41 DID equivalent to 3.0% of the total consumption in 2018.

Resistance in zoonotic- and indicator bacteria

In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea including salmonellosis and campylobacteriosis, due to the self-limiting nature of the diseases. If needed, it is recommended that patients are treated with macrolides (azithromycin and erythromycin).

Isolates from animals and meat for susceptibility testing are mainly collected by repeated, representative, national surveys conducted at Danish slaughter houses.

Resistance to quinolones remained the most common resistance type found in *Campylobacter jejuni* from all populations: broilers, cattle and humans. Around one third of all isolates of animal origin and domestically acquired human cases were resistant to ciprofloxacin, whereas 83% of the travel associated human isolates were ciprofloxacin resistant. The majority of ciprofloxacin resistant isolates from poultry and humans were also resistant to tetracycline and over the last ten years resistance to both tetracycline and ciprofloxacin increased in broilers. No erythromycin resistance was found in *C. jejuni* isolates from animals or human patients in 2018.

Salmonella isolates from pigs and Danish pork were included in DANMAP 2018, and *S. Derby* and *S. Typhimurium* remained the most prevalent serotypes. The resistance profiles were dominated by a large proportion of monophasic *S. Typhimurium* isolates, exhibiting resistance to tetracycline, ampicillin and sulfonamides. The trend in resistance to these three antimicrobials has been increasing steadily with approximately 30% increase since 2009. Resistance to ciprofloxacin and 3rd generation cephalosporins was present in low levels in human isolates and not found in pigs or Danish pork. In 2018, the level of azithromycin resistance in *S. Typhimurium* isolates from humans was less than 1%, and only 5% in Danish pork.

In 2018, the most prominent changes in resistance in indicator *Escherichia coli* from food-producing animals were a reduced occurrence of multidrug-resistance in isolates from broilers and pigs and an increase in fully susceptible isolates from pigs compared to previous years. Resistance patterns and levels in indicator *E. coli* from poultry, pigs and cattle were overall similar to previous years. No resistance to cefotaxime and ceftazidime was detected when using the non-selective isolation method and no resistance to colistin, meropenem and tigecycline was found.

Imported chicken meat was more likely to contain **ESBL/AmpC-producing *E. coli*** than Danish meat and broilers. The levels were comparable to those of 2016. The most common ESBL/AmpC enzymes identified across the broiler sources were again the AmpC enzyme, CMY-2 and the ESBL enzyme, CTX-M-1. As previously, all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

Resistance in human clinical bacteria

The national surveillance of resistance in human clinical bacteria, is based on either data from routine diagnostics performed at the 10 departments of clinical microbiology (DCMs) in Denmark, or on resistance and typing results from isolates received at the reference laboratories at SSI for further characterisation. Isolates are received either based on a mutual agreement on voluntary submission of specific species and/or types of resistances or as part of a mandatory surveillance program of diseases made notifiable by the Danish Health Authority. In DANMAP 2018, data from the 10 DCMs were extracted directly from the Danish Microbiology Database (MiBa).

Resistance in human clinical bacteria -surveillance based on MiBa data

Since the beginning of DANMAP and particularly during the past decade the number of human invasive infections has increased remarkably. The number of blood cultures taken as registered in the Danish Microbiology Database increased equivalently.

For *Escherichia coli*, the number of invasive cases increased from approximately 61.8 cases per 100,000 inhabitants in 2010 to 93.4 cases in 2018. Until 2017, the resistance trends were rather stable despite the increasing number of invasive cases. However, the resistance trends for cephalosporins in invasive *E. coli* reversed in 2017 and continued these slight increases also in 2018. In addition, an increasing trend in cephalosporin resistance was observed for urinary cases coinciding with an increasing total number of urinary cases from primary Health, from approximately 30,000 cases in 2010 to 80,000 cases in 2018. As for blood cultures taken, the increase in the number of positive urine samples equalled an increase in the total number of urine samples from primary health care submitted for culturing at the DCMs.

For *Klebsiella pneumoniae*, the number of invasive cases increased from approximately 14.4 cases per 100,000 inhabitants in 2010 to 22.1 cases in 2018. Figures and time trend analyses revealed that resistance rates have decreased markedly over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with lesser or insignificant decreases for the past five years. For urinary cases, the notable increase in resistance rates for mecillinam and sulfonamide observed in 2017 was repeated in 2018 with approximately the same resistance rates (16-17% for mecillinam and 22-25% for sulfonamide) as in 2017. In 2018, rather

steep increases in resistance to piperacillin/tazobactam and ciprofloxacin were observed in urinary cases as well.

Regarding resistance in invasive *Pseudomonas aeruginosa*, the situation in Denmark remained stable with resistance rates below 5% to ciprofloxacin, gentamicin, ceftazidime, meropenem and piperacillin/tazobactam. The number of invasive cases has also remained relatively stable but with a small increase during the last four years from approximately seven cases per 100,000 inhabitants in 2014 to 8.5 cases in 2018.

For *Acinetobacter species* the total number was approximately one invasive case per 100,000 inhabitants in 2018 corresponding to a low total number of 55 invasive cases. Of these, two cases had combined resistance to ciprofloxacin, gentamicin and meropenem and further two cases were resistant to ciprofloxacin only and one case to gentamicin only.

The number of invasive cases of *Enterococcus faecium* increased from 9.4 cases per 100,000 inhabitants in 2010 to 13.6 cases in 2018. For invasive *Enterococcus faecalis* the number has remained stable with 10.7 cases per 100,000 inhabitants in 2010 compared with 10.5 cases in 2018. An alarming increase in invasive cases of vancomycin-resistant and -variable *E. faecium* was observed. While 0.5% of invasive *E. faecium* were reported vancomycin resistant in 2008, this rate had increased to 12% in 2018. Part of the increase was due to the detection and spread of vancomycin-variable *E. faecium* since 2017.

Resistance in human clinical bacteria -surveillance based on data from the reference laboratories

Since 2014, the Danish departments of clinical microbiology have voluntarily submitted 3rd generation cephalosporin resistant *E. coli* isolates from bloodstream infections, for characterisation to SSI. In 2018, 24 different ESBL-, pAmpC- and carbapenemase-enzymes were detected among the 352 **ESBL- and pAmpC-producing *E. coli* from bloodstream infections**. As in previous years, CTX-M-15 was the most prevalent enzyme, with a significant increase from 164 cases in 2017 to 200 cases in 2018 ($p = 0.032$), whereas the presence of CTX-M-14 and CTX-M-55 decreased significantly from 48 and 13 cases in 2017 to 31 and 4 cases in 2018, respectively. In five cases, a carbapenemase-enzyme was detected along with an ESBL-enzyme. The most common sequence type (ST) was ST131 (54%), followed by ST69 (8%) and ST38 (6%).

In recent years, Danish departments of clinical microbiology have voluntarily submitted carbapenem resistant isolates (both clinical and screening) for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at SSI. The Danish Health Authority made **carbapenemase-producing organisms (CPO)** notifiable as of September 5th 2018. During 2018, 177 CPOs were detected from 160 patients compared with 123 CPOs from 115 patients in 2017, equivalent to a 44% overall increase of submitted CPO isolates

compared to 2017. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. The 177 CPOs consisted of 153 CPEs (mainly *E. coli*, *K. pneumoniae* and *Citrobacter freundii*), 18 *Acinetobacter* spp. and three *Pseudomonas* spp. Several outbreaks with CPE were seen during 2018.

In recent years, *E. faecium* harbouring the *vanA* gene complex, but phenotypically vancomycin susceptible, has been described in different countries. These enterococci are referred to as **vancomycin-variable enterococci (VVE)**. In 2017, VVE isolates were included in the **vancomycin-resistant enterococcus (VRE)** surveillance. However, VVE diagnostics differ substantially in the different regions. For the 2017 and 2018 reports, the number of submitted isolates was supplemented with the number of VRE/VVE registered in the Danish Microbiological Database (MiBa), which resulted in a total of 600 VRE/VVE isolates from 599 patients in 2018 compared to 510 VRE isolates from 508 patients in 2017. From 2013, a steep increase in clinical VRE isolates has been observed. The increase has mostly been seen for *vanA E. faecium*. In 2017, the VVE clone ST1421-CT1134 *vanA E. faecium* accounted for 3% of the *E. faecium* isolates. In 2018, 34% ($n = 173$) of the *vanA E. faecium* isolates belonged to ST1421-CT1134.

During 2015-2018, eight **linezolid resistant *E. faecium* (LRE)** isolates and eight **linezolid resistant *E. faecalis* (LRE)** isolates were sent to SSI (only one isolate per patient were included). No **linezolid-vancomycin resistant *E. faecalis* (LVRE)** were detected, whereas, six linezolid-vancomycin resistant *E. faecium* (LVRE) were detected. The findings of LRE and LVRE are of concern. Linezolid is used for treatment of VRE. Only a limited number of antimicrobial agents are available for treatment of infections with LVRE.

Streptococcus pneumoniae causes close to eight hundred cases of invasive pneumococcal disease (IPD) annually in Denmark, of which bacteraemia are the most frequent and meningitis counts for 50-60 of the cases. DANMAP has included data on susceptibility for IPD on penicillin and erythromycin from 1990 and onwards. Non-susceptibility to both penicillin and erythromycin increased gradually from less than 1% in the early nineties to 5-6% in recent years. Non-susceptibility to penicillin has varied without a clear pattern throughout the past six years and was 3.8% in 2018. Only one isolate (0.1%) was resistant to penicillin in 2018. The level of erythromycin non-susceptibility has decreased steadily since 2014 and reached 2.5% in 2018. Antimicrobial susceptibility in *S. pneumoniae* is closely related to serotypes. The prevalence of IPD-associated serotypes are also influenced by the PCV vaccines, which were introduced in the childhood immunization programme in Denmark in 2007. The large variation in antimicrobial susceptibility in IPDs seen in recent years is most likely driven by changes in serotype prevalence due to the effect of vaccines and by the natural cycles of different serotypes.

The surveillance of invasive infections caused by **beta-haemolytic streptococci (BHS)** in Denmark is based on voluntary submission of invasive isolates from the departments of clinical microbiology. During the last five years, the number of isolates of BHS has increased from 556 in 2014 to 873 in 2018. The corresponding increases for individual serogroups were: group A; 36%, group B; 32%, group C; 51%, and group G; 30%. The erythromycin resistance rate showed a small increase from 2014 to 2018 for groups A and G, while a small decrease was observed for groups B and C. The clindamycin resistance rate increased for groups A and G but was unchanged for groups B and C. All BHS isolates, irrespective of serogroup, were fully susceptible to penicillin.

Surveillance of invasive ***Haemophilus influenzae*** is mandatory for type b, but the majority of isolates of all types are voluntarily submitted to the reference laboratory at SSI. Serotyping is performed at SSI while the data for antimicrobial susceptibility is collected through MiBa. 121 cases were identified in 2018 of which 17% were type b and the majority (73%) were non-capsular. Susceptibility data are described in this report for the five most frequently tested antimicrobials. Of the isolates with available data for susceptibility in MiBa, 26% were registered as resistant to penicillin, 1% to ciprofloxacin, 20% to ampicillin, 16% to cefuroxime and 10% to amoxicillin/clavulanic acid. One hundred isolates were received at SSI, and 17 of these possessed the TEM-1 gene for beta-lactamase.

The number of bloodstream infections with ***Staphylococcus aureus*** increased from 2,104 cases in 2017 to 2,276 cases in 2018, of which 1.6% were methicillin-resistant (MRSA). Resistance to penicillin has decreased slowly during the last decade and was 72% in 2018.

The number of new **methicillin-resistant *Staphylococcus aureus* (MRSA)** cases was 3,669 in 2018, a small increase from 2017 (3,579 new cases). The number of livestock-associated MRSA CC398 was 1,215 (1,212 in 2017) and most of them were found in patients with contact to livestock production.

The national surveillance of antimicrobial resistance in ***Neisseria gonorrhoeae*** in Denmark is based on the voluntary submission of gonococcal isolates from the departments of clinical microbiology. From 2011 to 2016 the annual number of received isolates increased followed by a decrease in 2017 and 2018. Concomitantly with these changes, the rate of ciprofloxacin resistance decreased to 18% in 2016 and increased to 40% in 2018. Ceftriaxone resistance has never been diagnosed in Denmark, except from one case in 2017 with a marginally increased MIC (0.25 mg/L).

Future improvements and developments

DANMAP demonstrates that a well-established surveillance programme is important to understand the development of AMR and to point out where prudent use of antimicrobials is necessary.

Antimicrobial use in humans and food animals is relatively low and well regulated in Denmark compared to EU and the rest of the world. This contributes to relatively stable resistance patterns in production animals and in Danish meat compared to the big reductions observed when growth promoters were banned in the 90s.

Over the last decade, we have observed increasing numbers of multi resistant bacteria in humans and introduction of new critical resistance such as ESBL in food animals and Danish meat. International travel and trade plays an important part in introducing new bacteria and resistance in the Danish populations, where they may be maintained and spread. Monitoring critically resistant bacteria such as MRSA, ESBL, CPE and VRE in all relevant reservoirs provides essential information on when and where control measures are needed.

This DANMAP report provides a robust overview of the status on antimicrobial use and antimicrobial resistance in Denmark in 2018. The long history of the report adds certainty to its conclusions, whilst the DANMAP programme continues to evolve and develop as new opportunities and challenges appear.



3

INTRODUCTION TO DANMAP

3. Introduction to DANMAP

3.1 Background

DANMAP - the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme was established in 1995 to predict and counteract the emergence of antimicrobial resistance in both animals and humans in Denmark. In the 90s, many European countries were focusing on probable and possible relationships between antimicrobial use (AMU) and the development of antimicrobial resistance (AMR). The Danish initiative thus coincided with an overall strive to reduce or prohibit the use of animal growth promoters in the European Union and was paralleled by the introduction of similar programmes of surveillance in other northern and middle European countries within the decade.

The focus was on producing evidence for the linkage between AMU and AMR, point out possible knowledge gaps and establish monitoring systems that would create data for action. Connected research today focuses on evolution of bacteria and their survival mechanisms and resistance traits. Simultaneously, research in mechanisms of spread has gained increasing interest as has improvement of diagnostic methods.

The key objectives of DANMAP are:

- To establish the "state of the nation" on to the use of antimicrobial agents in food animals and humans
- To collect and collate available national data on the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin (e.g. meat) and humans.
- To identify areas for further research e.g. in transmission of resistance or possible associations between antimicrobial consumption and antimicrobial resistance
- To ensure data availability for veterinarians, medical doctors and other health professionals for the development of antibiotic guidelines for treatment
- To act as a knowledge base for authorities and politicians when performing risk assessment and management, thus supporting decision making in the prevention and control of resistant bacterial infections

The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of Environment and Food.

The risk of AMR

Bacteria can be inherent resistant or become resistant either by spontaneous mutation or by transfer of resistance genes from other bacteria. Resistant strains are favoured, when use of antimicrobial agents provide a selective pressure. This occurs in humans as well as in animals undergoing antimicrobial treatment. Resistant bacteria can spread between humans in the community, at healthcare centres and at hospitals. Furthermore, resistant bacteria from animals can be transmitted to humans either through direct contact with animals and their environment or through ingestion of contaminated food or other contaminated vehicles.

Antimicrobial treatment failure may occur if the ingested resistant bacteria are a direct cause of disease, or if resistance determinants are transferred to pathogenic bacteria causing the disease. Bacteria may be resistant to several - sometimes all - antimicrobial agents available for treatment, increasing the risk of treatment failure.

Currently there is only a limited number of antimicrobial agents, with novel modes of actions, under development by the pharmaceutical industry. Therefore, it is vital for public health organisations to ensure the continued effectiveness of compounds considered critically important to human treatment by ensuring prudent use for both humans and animals.

Prudent use should include considerations on possible restrictions of critical antimicrobial agents, so these can be reserved for use in humans primarily, to consider the introduction of new compounds for use in one sector only, as well as to eliminate all overuse. Only humans and animals suffering from an infection responsive to antimicrobial treatment should be exposed to antimicrobial agents.

The DANMAP surveillance system

DANMAP surveillance relies on four equally important components: well-established and well-functioning diagnostic systems, well-designed and representative surveys, reliable registers as well as mutual trust and openness between all collaborators.

The monitoring programme was initially developed using a bottom-up approach by researchers based on frequent discussions and exchange of knowledge and results from research. Since then, DANMAP has evolved into a governmentally supported organisation. However, much of the design of the programme, including participation of the human laboratories and referral of strains is still based on a voluntary principle.

A positive side effect of the regular meetings and exchange between stakeholders is that these prove helpful in other aspects, for example, by contributing to a common knowledge pool regarding laboratory methods. This ensures and contributes to continuous improvements and harmonisation of much of the laboratory work. Meetings across sectors and between different stakeholders also contribute to a better mutual understanding, facilitating development of the current system and increasing the willingness to listen to each other and work towards mutual goals.

DANMAP today is a governmentally supported programme with objectives, roles and tasks. While participation from several stakeholders continues to be voluntary, DANMAP is primarily fi-

nanced by the two ministries. Support from the ministries has also helped building the databases and ensuring the registers, which the current surveillance system relies upon. The system builds on transparency, mutual agreements and a standardised approach, which ensures consistency and continuity. For further information, please read chapter 2, "DANMAP - A 20 year perspective" in DANMAP 2015.

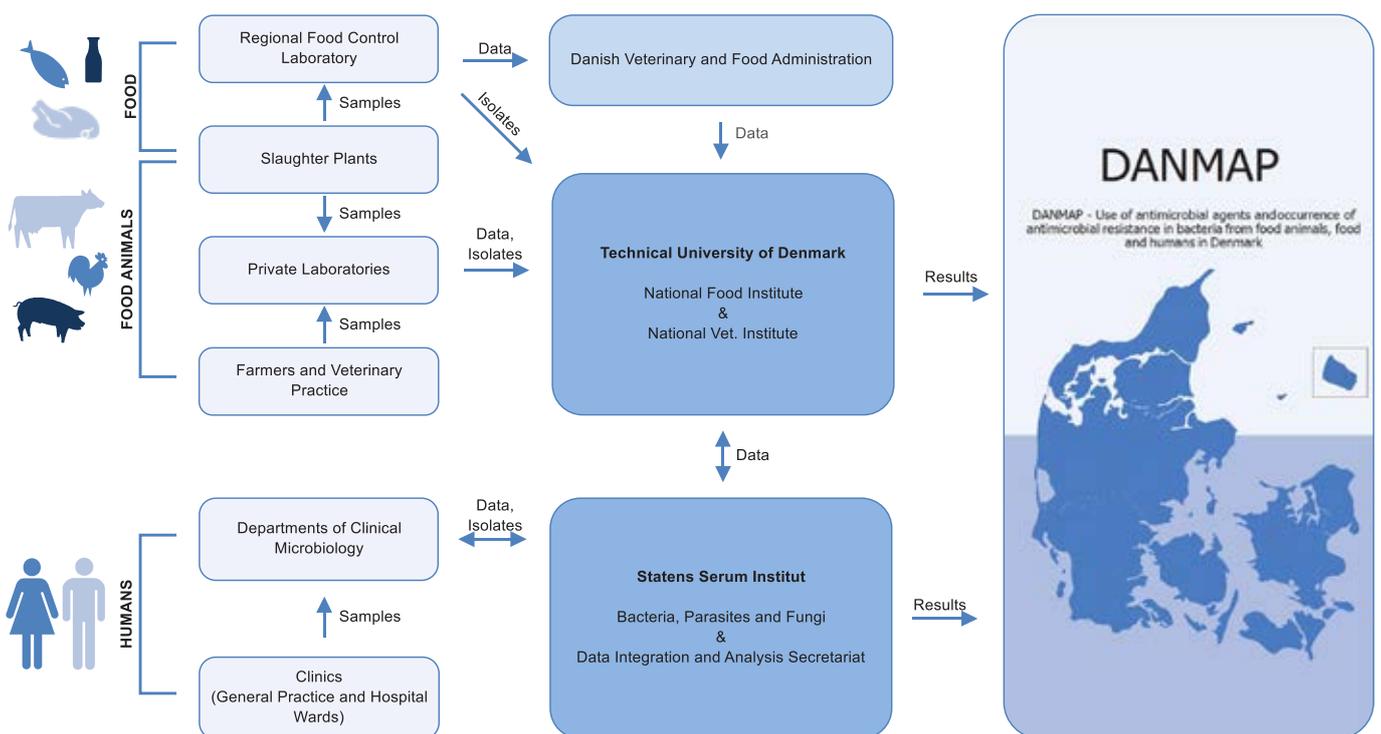
Surveillance is a complex undertaking and DANMAP encompasses many different surveillance components and covers resistance in different populations and contexts. Three categories of bacteria are always included in DANMAP:

- Human clinical isolates to reflect the antimicrobial resistance levels in the human population that seek medical care.
- Food borne zoonotic bacteria along the whole farm-to-patient chain to monitor the levels of antimicrobial resistance in shared pathogens
- Indicator bacteria, enterococci and *E. coli*, from healthy food-producing animals to monitor status of antimicrobial resistance in the animal reservoirs.

Since 1995, a main purpose of DANMAP has been to surveil the entire chain from farm to fork to sickbed. The organisation and collection of DANMAP resistance data is presented in Figure 3.1. The diagram shows the interdisciplinary collaboration between sectors and organisations.

Figure 3.1 Organisation of the DANMAP collaboration regarding resistance data and data flow

DANMAP 2018



For surveillance purposes and in outbreak situations the recent introduction of whole genome sequencing (WGS) has been a big step forward. In the clinical situation, the phenotypical testing may still be highly relevant, more feasible, cheaper and sometimes faster. Phenotypical testing is also extensively used in combination with WGS to describe and determine which resistance genes are relevant to look for when using molecular analyses.

Bacterial isolates from food, food animals and humans are submitted to Statens Serum Institut, the Regional Food Control Laboratory or the Technical University of Denmark for further phenotypic and genotypic characterisation (Figure 3.1). In 2018, WGS is performed on selected, single isolates. These isolates are analysed for clonal relationship, as well as antimicrobial resistance genotypes (including ESBL and AmpC genes), and the presence of mobile elements such as plasmids. The outcomes are used for surveillance as well as detection of outbreaks. Furthermore, when specific clones carrying the same antimicrobial resistance genes and plasmids are found among both food and human isolates, genomic data analysis such as core genome multilocus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) calling, are used to examine possible transmission between the reservoirs. The choice of the varying methods in surveilling different bacteria and infections is described in more detail in the different chapters and sections of the report.

3.2 Information on demographics, food production and data flow

The following sections present some general information about the human population in Denmark in 2018, the production of food animals and meat. It also provides an overview of the antimicrobial agents for systemic and intramammary therapeutic use in humans and animals in 2018.

3.2.1 Populations and productions Human population and healthcare system

During the past two decades, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to approximately 5.8 million in 2018 (www.dst.dk). Simultaneously, the average age increased (Figure 3.2). In 2018, the national average age was 41.5 years. The population, which could potentially have received antimicrobial treatment in 2018, is shown as regional distribution in Figure 3.3. In Denmark, microbiological analyses are carried out by altogether ten departments of clinical microbiology (DCMs), situated at the main regional hospitals in Denmark, also presented in Figure 3.3. Analyses cover all samples from public hospitals and most samples from general practitioners (GPs). In addition, some GPs perform culturing of urinary samples from their patients. In the Capitol region one private laboratory also performs analyses for the GPs.

Figure 3.2 Changes in regional distribution of average age, Denmark

DANMAP 2018

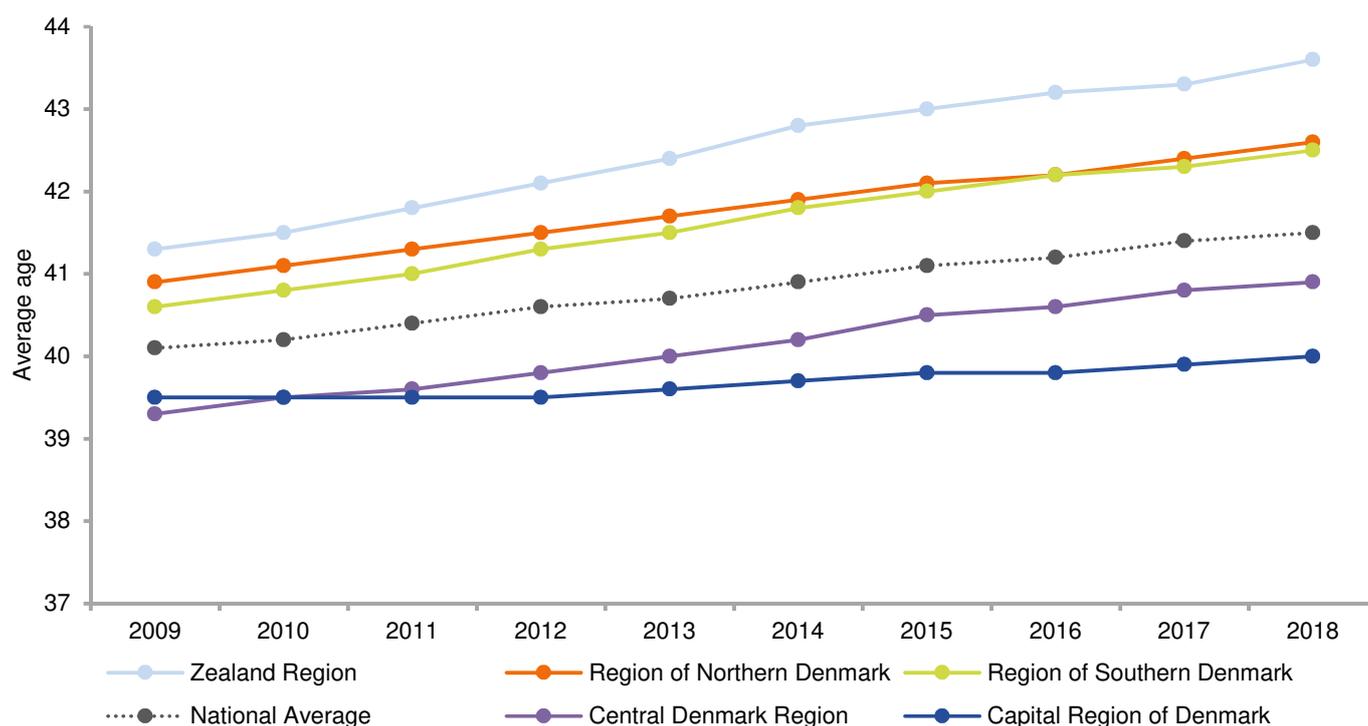
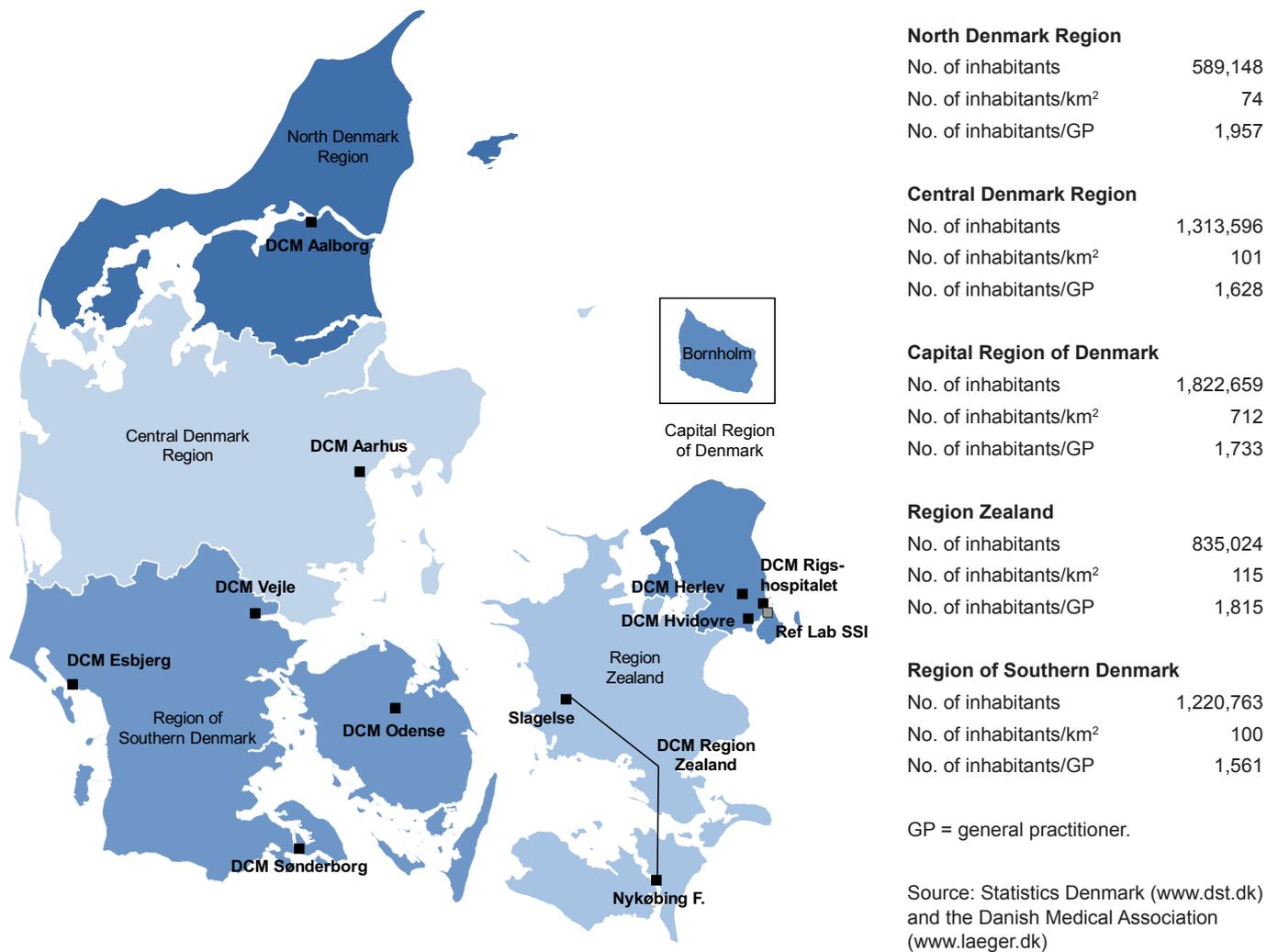


Figure 3.3 The five Danish healthcare regions and their respective population distributions. In addition, the ten DCM are marked by black squares. The grey square indicates the reference laboratory situated at SSI

DANMAP 2018



Animal population and food production system

Denmark is an agricultural country, with more than half of its area managed by the agricultural sector. The agricultural sector contributes to employment with around 146,000 jobs in the primary production and processing, and contributes around 25% of the Danish export earnings. Livestock is of great importance and approximately 25% of the agricultural enterprises are specialised in the production of livestock, mainly pigs, cattle, chicken and mink [Danish Agriculture and Food Council, 2017].

The production of food animals and the production of meat and milk are presented in Table 3.1 and 3.2. In 2018, the number of pigs produced increased by approximately 3% compared to 2017, and the number of exported fattening pigs (15-50 kg) continued to increase by approximately 6%. Since 2004, the total exports of fattening pigs have increased more than seven-fold [Statistics Denmark, Danish Agriculture and Food Council, 2017].

From 2017 to 2018, the cattle production experienced a slight increase in general, the number of cattle slaughtered increased by approximately 5%, the number of dairy remained approximately the same level, while the amount of milk produced increased by approximately 2,5% [Statistics Denmark].

The number of broilers produced increased and approximately 15% of the broilers produced in Denmark in 2018 were exported for slaughter. The production of turkeys has fluctuated considerably over the past decade. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus the majority of turkey meat available for sale in Denmark is listed as imported.

3.2.2 Registered antimicrobial agents

Table 3.3 shows the antimicrobial agents registered to treat bacterial infections in humans and animals respectively. Some of these are listed on the highest priority list of critically important antimicrobial agents for the treatment of bacterial in-

fections in humans, according to definitions made by a working group under the World Health Organization [AGISAR, 6.revision, WHO 2019]. In order to be considered critically important, an antimicrobial must be the only- or one of a limited number of compounds available to treat serious human disease. Critically important antimicrobial agents are also used to treat diseases in food animals and pets, so the reservoir of resistance-potential bacteria is not restricted to humans only. Since bacteria may be transmitted from animals to humans, and bacteria that cause human disease are capable of acquiring resistance genes from bacteria of animal origin, resistance against the critically important antimicrobials can be spread widely.

In the newest revision from 2019 five drug classes were considered to be critically important: and of highest priority:

fluoroquinolones, 3rd, 4th and 5th generation cephalosporins, macrolides, glycopeptides and polymyxins. In Denmark, in food animals the use of these drug classes has in general been low or been reduced through either voluntary or legislative restrictions, apart from macrolides, see chapter 4 for more information. For trends and traditions in the antimicrobial treatment of humans and information on the national action plan from 2017 see chapter 5.

Growth promoters are no longer used for animals in Denmark and are shown in parentheses in Table 3.3. Most of these influenced Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat have been used as a measure of resistance towards growth promoters.

Table 3.1 Production (1000' heads) of food animals and mink, Denmark

DANMAP 2018

Year	Pigs		Cattle		Poultry		Fur animals - mink	
	Total	Exported ^(a)	Slaughter cattle	Dairy cows	Broilers	Turkeys	Females	Kits
2009	27603	6642	507	569	108851	1175	2675	14768
2010	28505	7074	519	574	117653	1184	2657	14638
2011	29399	7632	551	575	115454	960	2776	15325
2012	29047	8794	539	580	111080	1103	2936	16147
2013	28996	9318	551	574	117315	692	3143	17634
2014	29926	10517	556	563	115497	595	3296	17963
2015	30874	11563	513	561	114238	598	3387	18798
2016	31660	12771	540	571	120685	834	3161	17260
2017	31662	13679	509	570	117602	601	3410	18378
2018	32558	14028	533	575	122268	642	3385	17634

Source: Statistics Denmark (www.dst.dk) and Copenhagen Fur. Export data for 15-50 kg live pigs from the Danish Agriculture and Food Council
a) Export of 15-50 kg live pigs. These are included in total number of heads

Table 3.2 Production (mill kg) of meat, milk and fish, Denmark

DANMAP 2018

Year	Pork	Beef	Broiler meat ^(a)	Turkey meat	Milk	Farmed fish ^(b)	
						Fresh water	Marine
2009	1898	137	165	11	4734	29	11
2010	1974	142	178	14	4830	28	10
2011	2008	145	175	9	4801	28	11
2012	1902	138	168	12	4928	28	13
2013	1896	140	177	8	5025	28	12
2014	1924	143	174	9	5113	30	11
2015	1954	135	172	9	5278	32	12
2016	1943	142	182	10	5376	33	12
2017	1896	135	178	7	5478	33	14
2018	1966	141	185	10	5615	33	14

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Export data for poultry from Statistics Denmark (personal communication)

a) Export data for poultry from Statistics Denmark (personal communication). Assumes a final slaughtered weight of 1.51 kg per broiler produced (Danish Agriculture and Food, 2013)

b) The numbers for 2018 are not final. The production of farmed fish includes fish transferred from one production facility to another

Table 3.3 Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark

DANMAP 2018

ATC / ATCvet codes ^(a)	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA, QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, tetracycline, tigecycline
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB, QJ51DB	First-generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin, cefazolin
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD, QJ51DD	Third-generation cephalosporins incl. comb. with beta-lactamase inhibitors	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, ceftazidime/avibactam
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem
J01DI	Fifth-generation cephalosporins incl. comb. with beta-lactamase inhibitors		Ceftaroline fasamil, ceftolozan/tazobactam, ceftobiprol
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb. of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxazol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine, telithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX ^(b)	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA, QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ ^(b)	Quinoxalines	(Carbadox, olaquinox)	
J01XA, A07AA / Not in ATCvet ^(b,c)	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin, dalbavancin, oritavancin
J01XB / QA07AA ^(b)	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD, P01AB ^(c)	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, tedizolide, fosfomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet ^(b)	Oligosaccharides	(Avilamycin)	
Not in ATCvet ^(b)	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes start with a Q

b) Animal growth promoters used before 1999 are listed in parentheses

c) Intestinal anti-infectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) were, for the first time, included in DANMAP 2014, since their widespread use in the treatment of *Clostridium difficile* infections makes them belong to the most used antibiotics in human infections in Denmark





4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals



Highlights: There has been an overall decreasing trend in the use of antimicrobials in animals since 2013 and in 2018 the use had been reduced by 14%, equivalent to almost 17 tonnes, compared with 2013. The total use of antimicrobials in animals amounted to approximately 100 tonnes in 2018.

Approximately 75% of all veterinary prescribed antimicrobials are used in the pig sector, which makes pigs the main driver of trends in antimicrobial use in animals. The export of weaner pigs continued to increase in 2018, while the number of pigs slaughtered in Denmark remained approximately at the same level. Measured in treatment intensity (DAPD), the overall use, when adjusted for export, was reduced by 4% from 24 to 23 DAPD in 2018. This means that on a given day in 2018 an estimated 2,3% of all pigs received antimicrobial treatment. In the different agegroups the use was reduced from 97 to 91 DAPD in weaner pigs, from 18 to 17 DAPD in finishers, while it remained at the same level for sows and piglets around 19 DAPD.

The types of antimicrobials used in pigs has also shifted notably. The use of tetracyclines in pigs has been reduced significantly since 2009, and in particular from 2016 to 2018, following the implementation of the differentiated "Yellow card". This initiative also resulted in a close to zero use of colistin, since the first quarter of 2017. However, the reduction in the use of tetracycline and colistin, was mirrored by clear but less marked increases in the use of macrolides and aminoglycosides, especially in weaners.

The overall use for cattle has fluctuated between 12 and 13 tonnes over the past five years. In 2018, more than two thirds were used to treat older cattle (>1 year) and approximately 500 kg were used as intramammary treatment. Measured in DAPD, the antimicrobial use for older cattle (>1 year) has decreased from 4 to 3 DAPD (-13%) over the past decade, while the use for younger cattle (<1 year) has increased from around 5 to 7 DAPD (+43%).

Antimicrobial use in poultry is relatively low (1,326 kg). Notable fluctuations may occur as a result of disease outbreaks in a few large flocks, which was the case in 2014 and 2015. However, since 2015, the use has decreased each year. In contrast, the use of antimicrobials in the aquaculture industry more than doubled (1,860 kg more) in 2018 due to the very warm summer in 2018.

From 2014 to 2017 the use of antimicrobials in fur animals (mainly mink) has increased. In 2018 the industry increased focus on prudent use and developed an action plan to reduce the use within the sector. It is likely the increased focus combined with low occurrence of disease in 2018 resulted in the observed 40% reduction in antimicrobial use for fur animals (-2,467 kg) in 2018.

Companion animals still use more critically important antimicrobials compared with other species. Almost all fluoroquinolones and more than half of the cephalosporins used in animals are used in dogs and cats. Despite a small increase in use from 2015 to 2016, there has been an overall decreasing trend in the use of antimicrobials in dogs and cats since 2011, with a marked reduction in the use of cephalosporins.

4.1 Introduction

The DANMAP programme began monitoring the national use of antimicrobial agents in humans and animals in 1995. Since the early 1990s, there has been both political and public focus on the consumption of antimicrobial agents in the Danish animal production, which resulted in the discontinued use of antimicrobial agents for growth promotion in the years 1995-1999. The focus on antimicrobial use has continued to increase; more recent initiatives include a voluntary ban on the use of cephalosporins in the pig and cattle production, as well as regulatory legislation regarding therapeutic use.

Figure 4.1 shows the total use of antimicrobials in animals and humans since 1994 and 1997, respectively. Changes in the patterns of antimicrobial use in animals can be explained in part by an increase in pig production over the years, but risk management measures to reduce consumption have also contributed. In addition, the increasing export of pigs at 30-40 kg live weight has also affected the overall use of antimicrobials in animals.

The prescription patterns for animals have clearly been influenced by risk management decisions during the period. For example, the decrease in antimicrobial consumption after 1994 was likely the result of 1) limitation of veterinary practitioners' profit from sales of medicine; 2) implementation of Veterinary Advisory Service contracts (VASCs) with regular visits from the veterinarian in order to promote preventive veterinary strategies and optimise antimicrobial use, and 3) enforcement of the so called "cascade rule" [Order (DK) 142/1993], which limits the use of (cheaper) extemporaneously produced medicines.

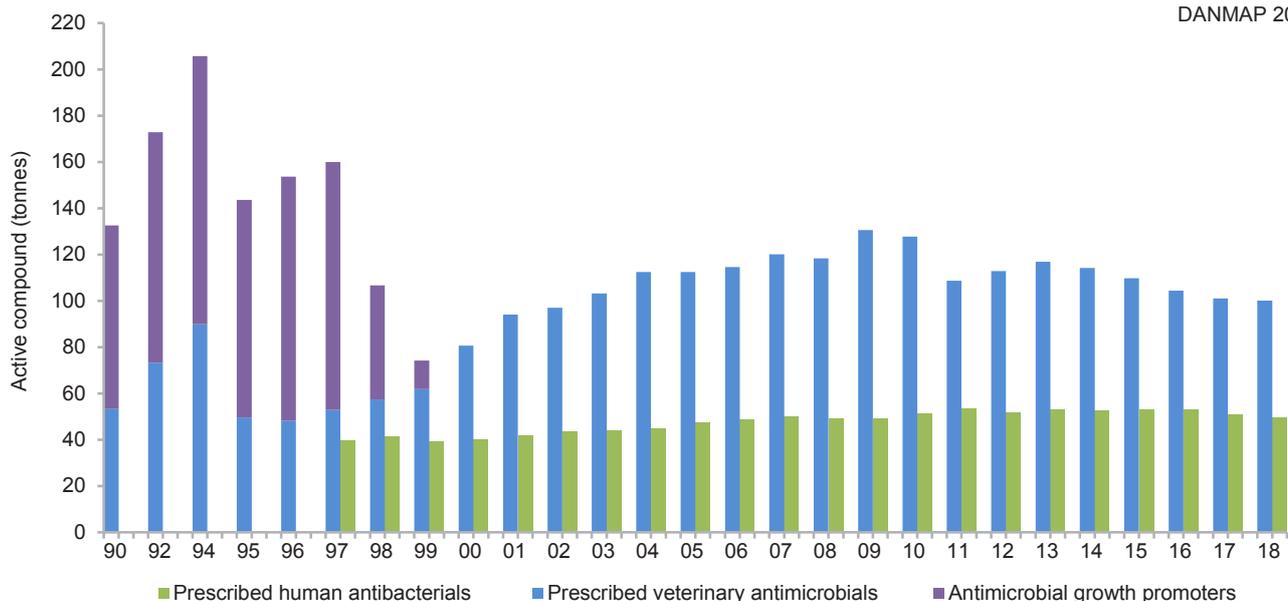
Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003, and the voluntary ban on the use of cephalosporins in the pig industry in 2010 followed by a similar initiative in the dairy cattle industry in 2014.

As a part of the national action plan against antimicrobial resistance, a 10% reduction of antimicrobial use in farm animals by 2014 compared to the 2009 level was set as a national target. To achieve this, the introduction of threshold values for antimicrobial use was adopted with the "Yellow Card" Initiative in 2010. This enforces legal action on pig farmers with high antimicrobial use per pig [DANMAP 2010], and as a result, a decrease in consumption was seen from 2010 to 2011. Effects from other parts of the legislation may be less obvious, but are also likely to have affected prescription patterns. As an example, the rules for group medication in pig herds were tightened in 2014, calling for thorough laboratory diagnoses and frequent veterinary visits when antimicrobials are prescribed for groups of pigs. Furthermore, in 2015 the national action plan to reduce livestock associated MRSA called for a 15% reduction in antimicrobial use in pigs from 2015 to 2018.

In 2016, the "Yellow Card" Initiative was revised, adding on multiplication factors to adjust the consumption of certain antimicrobials. Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10. Tetracyclines were given a multiplication factor of 1.2, which was further adjusted in 2017, to a factor of 1.5 [DANMAP 2017]. The effects of this are described in Textbox 4.1.

Figure 4.1 Prescribed antimicrobial agents for humans and all animal species, tonnes active compound, Denmark

DANMAP 2018



Sources: Human therapeutics: The Danish Medicines Agency. Antimicrobials for animals: Data are based on reports from the pharmaceutical industry of total annual sales (until 2001), from the Federation of Danish pig producers and slaughterhouses (1994-1995), from the Danish Medicines Agency and Danish Plant Directorate (1996-2000) and since 2001 from VetStat. For DANMAP 2018, consumption data for 2004-2018 were extracted from VetStat on 3rd March 2019 and includes all antimicrobial agents registered for use in animals

In 2017, The Danish Ministry of Environment and Food and The Danish Ministry of Health presented a new One Health strategy against antimicrobial resistance, setting the framework for reducing the development and occurrence of AMR. At the same time, two national action plans to reduce AMR were introduced, setting specific targets to further reduce the antimicrobial use for both humans and animals in the coming years. As part of the political agreement on the veterinary strategy 2018-2021 (Veterinærforlig III), an Advisory Committee on Veterinary Medicines was established in 2018, Textbox 4.2.

Official treatment guidelines for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the appropriate antimicrobial treatment of all common indications in the major production animal species. Since 2005, DVFA have updated the guidelines in collaboration with stakeholders and university experts. The guidelines were updated in 2010, when new dynamic evidence based treatment guidelines for pigs were launched [DANMAP 2010, www.fvst.dk], and were further revised in 2017 and the new version published in April 2018. In 2012, the Danish Veterinary Association published treatment guidelines to promote prudent use of antimicrobials in dogs and cats. The guidelines were prepared by clinical specialists and expert scientists from the Faculty of Health and Medical Sciences at the University of Copenhagen and DTU National Food Institute. The treatment guidelines for dogs and cats were revised in 2017 and new guidelines were published in 2018. Similarly, the Danish Veterinary Association published treatment guidelines for use of antimicrobials in horses in 2017.

Finally, in 2017 the EU Commission decided that use of medical zinc has to be phased out completely by 2022 at the latest. Medical zinc is used extensively in the pig production to prevent diarrhoea in weaner pigs.

4.1.1 Data sources

In Denmark, antimicrobials are available by prescription only, and data on antimicrobial use at product level have been collected in Denmark since 1996, including historical data back to 1990.

Since 2001, data on all medicines prescribed for use in animals, including vaccines, antimicrobial growth promoters (no longer permitted) and coccidiostatic agents (non-prescription) have been recorded in the national database VetStat. Since 2010, the VetStat database is hosted and maintained by the Danish Veterinary and Food Administration (DVFA). The data presented in this report were extracted from VetStat on 3rd of March 2019 and have been summarised for DANMAP by the National Food Institute at DTU.

4.1.2 Methods

Metrics of antimicrobial use are numerous, each with its own advantages and limitations. Therefore, the selection of metrics used for monitoring must depend on the monitoring objective and the information available.

The overall amount of antimicrobial agents is measured in kg active compound and is used in section 4.2 for the purpose of an overall crude comparison of antimicrobial use in the veterinary and human sectors (Figure 4.1).

Since 2012, we have further presented 'defined animal daily dose' (DADD) and 'proportion of population in treatment per day' (DAPD) to monitor trends in antimicrobial consumption. These metrics are defined below, and for additional information on methodology, please refer to chapter 9 and the web annex [www.Danmap.org].

DADD - Defined Animal Daily Dose.

DADD is the average maintenance dose per day for the main indication of a drug in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species; and when appropriate age group. The DADDs have been defined specifically for use in DANMAP based on current knowledge (section 9.2) and may vary from the prescribed daily dose or the recommended dosage in the Summaries of Product Characteristics (SPC) or in the VetStat database.

DAPD - Proportion of population in treatment per day

Trends in antimicrobial usage in pigs, cattle and fur animals are presented in DAPD.

DAPD=DADD per 1,000 animals per day, where 'animals' are represented by their live biomass and adjusted for life-span. The estimated live biomass is expressed as the number of standard animals with an estimated average weight on a given day. This may also be referred to as the 'standard-animals-at-risk'. This metric allows for comparison of antimicrobial use between species with large differences in body-mass and life-span.

The estimated treatment proportion, DAPD, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs means that an estimated 1% of the pig population, on average, receives a certain treatment on a given day (see section 9.2). In principle, DAPD as a metric is analogous to DID (defined daily dose per 1,000 inhabitants per day), the metric used to measure antimicrobial consumption in the human sector. Please refer to section 9.8 for a description of DID. In DANMAP 2018 we calculated treatment proportions in pigs, cattle, and fur animals.

Textbox 4.1

Regulation on antimicrobial classes resulted in a shift between antimicrobial classes in pigs 2014-2018.

The 'Yellow card' initiative, with threshold values for antimicrobial use for pigs, has been in place in Denmark since 2010. In 2015, a political decision to promote a more prudent use of antimicrobials was imposed by further regulation. This included assigning weights to the different antimicrobial classes. Three levels of weights were applied in 2016 and modified in 2017: Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10. Tetracyclines were given a multiplication factor of 1.2, which was adjusted to a factor of 1.5 in 2017.

The use of fluoroquinolones and 3rd/4th generation cephalosporins in food animals has been negligible in Denmark for several years; therefore, in reality only the use of colistin was affected when critically important antimicrobials were weighted with a factor 10.

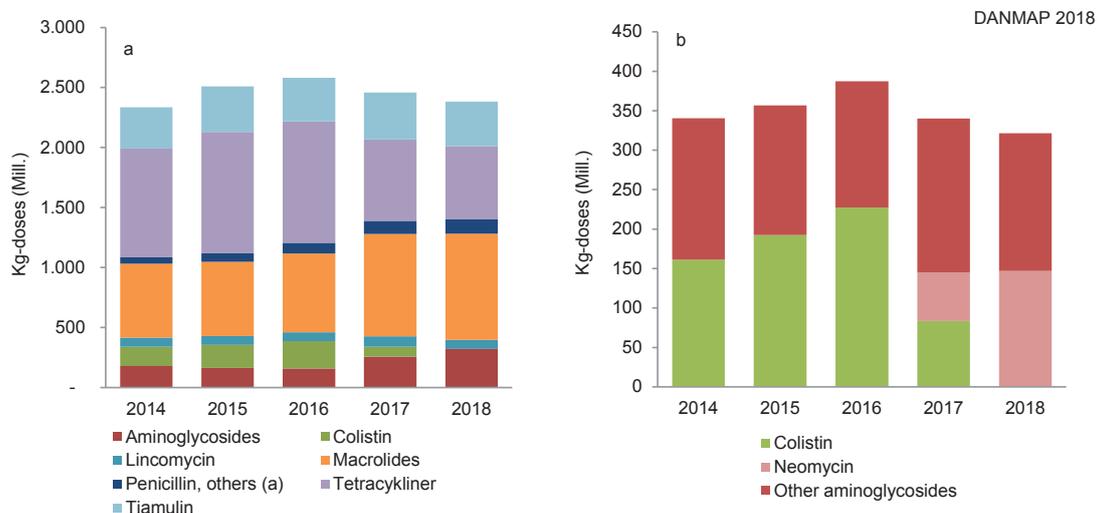
Tetracyclines and colistin are mostly used for gastrointestinal disorders in weaner pigs. The assignment of the differentiated weights resulted in an immediate change in usage (Figure 1a). The use of tetracycline was reduced from approximately 12 to 7 tonnes active compound from 2016 to 2018 and colistin dropped from approximately 1 ton to less than 1 kg active compound over the same period. In both cases, the use reduced in antimicrobial classes assigned with a higher factor and increased other classes assigned a lower factor. The reduction in the use of tetracyclines resulted in a marked increase in use of macrolides and the reduction in the use of colistin shifted to an increase in the use of aminoglycosides (Figure 1b).

Figure 1 shows the effect of the differentiation between antimicrobial classes. A marked reduction in the use of tetracyclines (40%) was replaced by an increased use of macrolides (35%, Figure 1a). Figure 1b illustrates the reduction and shift between colistin and aminoglycosides, mostly neomycin. Neomycin was reintroduced to the Danish market in 2017 and seems to be the preferred alternative to colistin. The almost complete phasing out of colistin caused the use of aminoglycosides to double between 2016 and 2018 (measured in kg-doses).

The regulatory intervention clearly triggered a shift between antimicrobial classes. If an important antimicrobial is restricted, it appears more likely to be replaced by another class or active substance than to result in an overall reduction in use. This is an important lesson that needs to be considered for future restrictions. The effect of the current shifts in consumption on antimicrobial resistance is not possible to predict, but will be monitored carefully for possible adverse effects in the future.

*Elisabeth Okholm Nielsen, Danish Veterinary and Food Administration
For further information: Elisabeth Okholm Nielsen, eloni@fvst.dk*

Figure 1. The most frequently used antimicrobials to treat gastrointestinal disorders in weaner pigs (a) and shift in use of colistin, neomycin and other aminoglycosides (b), million kg-doses per year



4.2 Total antimicrobial consumption in animals

The total use of antimicrobial agents in all animals, amounted to 100.1 tonnes active compound, representing a 1% (808 kg) decrease compared with 2017, Figure 4.1.

In 2018, the antimicrobial use in pigs, cattle, fur animals and poultry comprised approximately 75%, 13%, 4% and 1% of the total antimicrobial consumption for animals, respectively (Figure 4.2). The pig industry is the main driver of antimicrobial usage in animals in Denmark, due to the size of the production. Cattle and pigs comprise almost equal proportions of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

Historically, the overall use of kg active compound was 51% lower in 2018 compared with 1994. A major part of this reduction can be explained by the discontinued use of growth promoters from 1994 to 1999.

Between 2000 (start of VetStat) and 2009 the amount of kg active compound increased by 62% (Figure 4.1). During this period the number of pigs produced also increased as did the proportion of pigs exported live at approx. 30 kg. Since then, the proportion of exported live pigs has continued to increase, while there has been an overall gradual decreasing trend in the use of antimicrobials in animals and in 2018 the antimicrobial use was approximately 23% lower than in 2009.

4.3. Antimicrobial consumption by animal species

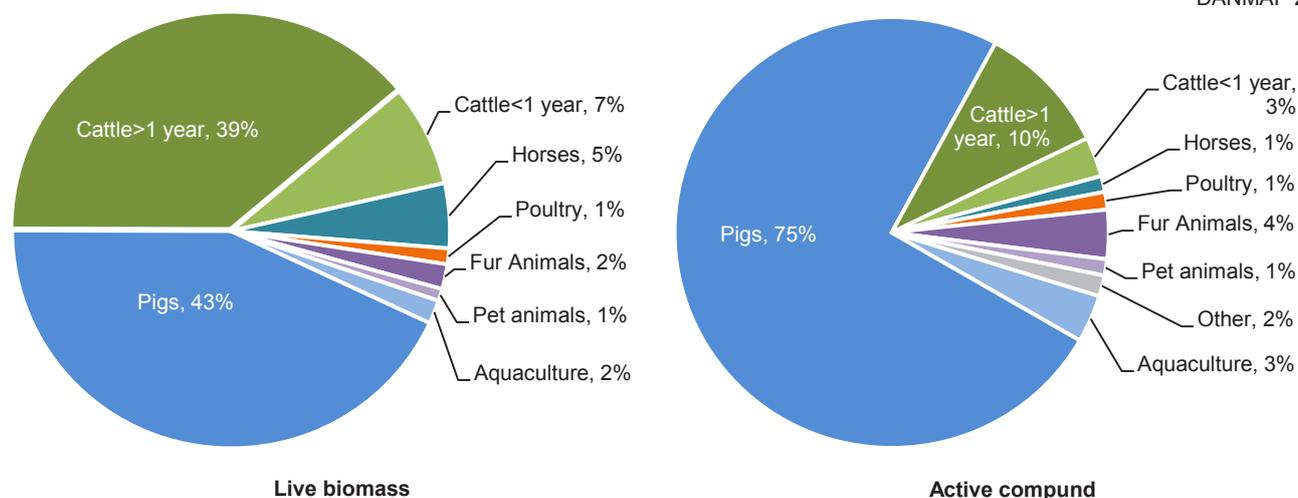
4.3.1 Antimicrobial consumption in pigs

The majority of antimicrobial use in animals is used in the production of pigs. The total antimicrobial consumption in pigs (sows and piglets, weaners, finishers) was 74.7 tonnes active compound (Table 4.1), which was 277 kg less than in 2017.

The treatment proportion (DAPD) of the total population reflects the trends in selection pressure within the population. The treatment intensity is much higher in weaners than in finishers and sows. The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figures 4.3 and 4.4 and the DADD's are shown in the web annex (Table A4.1 and in the DADD description).

The large differences in DAPDs between age groups affects the DAPD of the total population and trends are influenced by changes in population structure. As an example, increased export of live pigs just after weaning could lead to an increase in DAPD in the total pig population, since the exported pigs were only in the country, when the treatment proportion was highest. Approximately 42% of the pigs produced in 2018 were exported as live pigs at approximately 30 kg (Table 3.1), in 2009 this percentage was approximately 24%. When estimating DAPD for all age groups in the pig production, we account for changes in export of weaners by calculating an adjusted treatment proportion, referred to as DAPD_{adjr} see section 9.2.2.

Figure 4.2 Distribution of live biomass and antimicrobial consumption in main animal species, tonnes, Denmark



Note: The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals, aquaculture). For poultry, the figures comprise only the biomass for the main production types (turkeys and broilers). The live biomass estimates for poultry, aquaculture, horses and pet animals are based on 2012 data and may well be underestimated. The estimation procedures are described in section 9.2

Historically, the treatment proportion (DAPD) increased from 2004 to 2009, followed by a decrease in 2010 and 2011, which is considered a result of the “Yellow Card” initiative (DANMAP 2010). Since 2013, there has been a gradual decrease in treatment intensity for all age groups (Figure 4.3).

In 2018, the antimicrobial consumption in pigs, measured in DAPD_{adjr} decreased from approximately 24 to 23 (Figure 4.3) when adjusted for export. Also, measured in DAPD_{adjr} the antimicrobial use in pigs was 32% lower in 2018 than in 2009 (Figure 4.3).

Within the different age groups, the most remarkable change was seen for weaners, where the treatment proportion decreased from 97 to 91 DAPD, a decrease not apparent when

inspecting crude consumption data in Table 4.1. The treatment proportion was also reduced in the finishers, from 18 to 17 DAPD, but remained at the same level in the sows and piglets around 19 DAPD (Figure 4.3). Thus, on a given day in 2018, approximately 2% of sows and piglets, 1-2% of finisher pigs and just around 9% of weaner pigs were treated with antimicrobials.

Changes to the “Yellow Card” initiative were implemented in 2016 and 2017, i.e. multiplication factors of 1.5 and 10 were applied to the use of tetracyclines and colistin, respectively, to promote further reduction (see Textbox 4.1).

The National MRSA Action plan aimed to reduce the antimicrobial use in pigs by 15% in 2018, compared to 2014. In 2018,

Table 4.1. Antimicrobial use (kg active compound) by animal species and age group, Denmark

DANMAP 2018

Therapeutic group	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others(a)	Pleuromutlins	Sulfonamides and trimethoprim	Tetracyclines	Total 2017	Total 2018
Pigs	8296	388	<1	0	1978	12056	<1	0	16551	8715	7627	6080	12965	74935	74658
Sows and piglets	1854	279	<1	0	441	624	<1	0	8764	3605	937	4654	1174	21752	22331
Finishers	173	9	<1	0	701	3457	0	0	5867	734	3827	236	3475	19354	18479
Weaners	6270	100	<1	0	837	7976	<1	0	1920	4376	2863	1190	8316	33828	33848
Cattle	901	837	79	<1	11	245	3	0	7627	730	0	879	1553	12370	12865
Intramammarys	38	0	68	0	9	0	<1	0	287	131	0	<1	0	502	533
Cows and bulls	215	12	10	<1	<1	97	<1	0	6627	470	0	767	894	9056	9093
Calves <12 months	614	803	<1	<1	<1	145	3	0	574	118	0	105	615	2557	2978
Heifers and steers	34	22	<1	0	<1	3	0	0	139	11	0	7	44	255	261
Poultry	51	0	0	<1	26	162	0	0	323	212	<1	37	516	1491	1326
All poultry excl. turkeys	45	0	0	<1	23	95	0	0	216	121	<1	37	163	851	699
Turkeys	6	0	0	0	3	67	0	0	107	91	0	0	353	640	627
Other production animals	164	330	<1	<1	62	468	<1	896	11	2144	<1	2701	497	7879	7274
Aquaculture	0	323	0	<1	<1	0	0	896	0	45	0	2293	<1	1697	3557
Fur animals	161	6	0	<1	62	466	<1	0	5	2095	0	402	491	6156	3689
Other	2	1	<1	<1	<1	2	<1	0	6	4	<1	6	5	25	28
Companion animals^(b)	7	<1	97	15	62	2	41	1	29	681	<1	1441	41	2472	2418
Pets ^(c)	6	<1	97	15	62	2	41	1	20	681	<1	261	37	1287	1224
Horses	<1	<1	<1	0	0	<1	<1	0	10	<1	0	1179	4	1184	1194
Unspecified^(d)	278	7	4	1	9	17	2	7	801	140	4	175	98	1744	1541
Total	9697	1563	180	16	2147	12951	46	904	25341	12622	7632	11313	15669	100890	100082

Note: Data for 2018 were extracted from VetStat 3rd March 2019. Only the ATCvet group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds

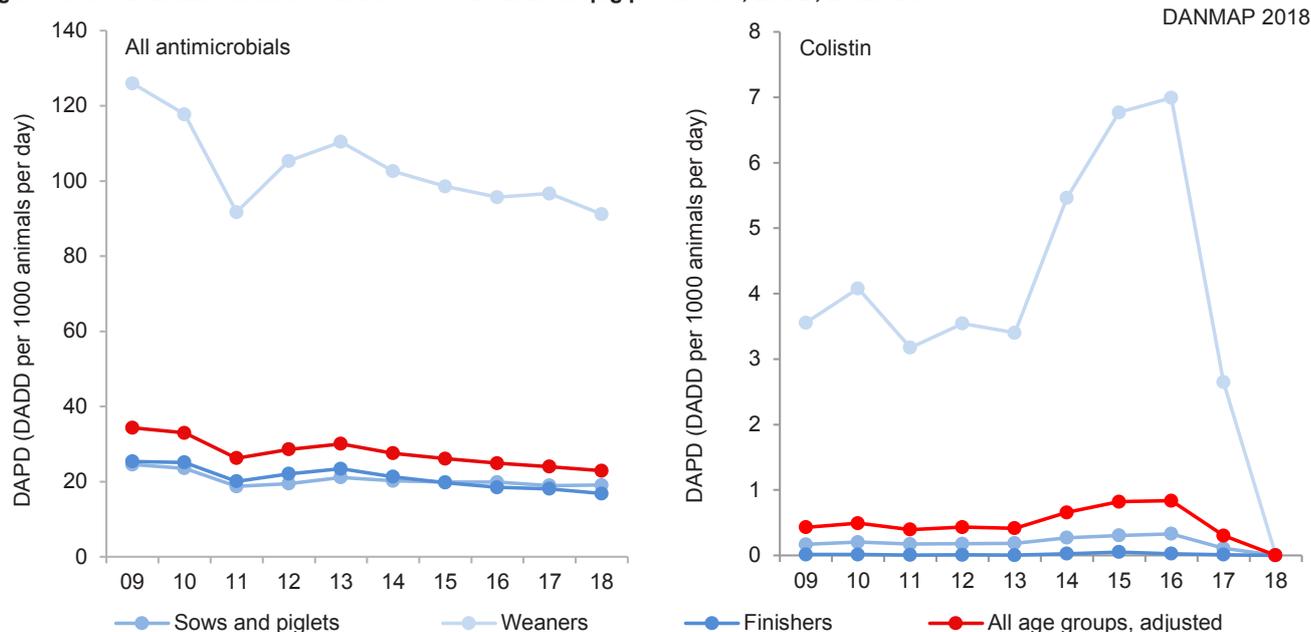
a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

b) In DANMAP 2016, new principles were applied to estimate the antimicrobial use for companion animals, see section 4.2.2

c) Approximately 242 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

d) This includes data on sheep and goats (13 kg), data where the animal species has not been defined or where the age group does not apply to the designated animal species

Figure 4.3 Total antimicrobial use and use of colistin in the pig production, DAPD, Denmark



Note: Sows and piglets includes treatment in boars. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group of the total population (in tonnes). DAPDs for "all age groups" are adjusted for export of pigs at 30 kg (see text)

the overall use in the pig production was reduced by approximately 13% when measured in kg active compound.

Tetracyclines has been one of the most commonly used antimicrobials in the Danish pig production for more than a decade. It is almost exclusively administered orally, and is especially used for treatment of gastrointestinal disease in weaners and finishers. The overall use of tetracyclines has decreased since 2013 and in 2018 the treatment proportion was at the lowest levels registered in the last 14 years, with the most marked changes following the recent adjustments changes to the "Yellow Card" initiative (Figure 4.4). Measured in $DAPD_{adj}$, the use of tetracyclines, in all age groups was reduced by 49% from 2015 to 2018 and by 63% since 2009. The proportion of weaners treated with tetracyclines on any given day has decreased from approximately 5% in 2009, to less than 2% in 2018. Also, the use of colistin, formerly one of the "first choice" antimicrobials for treating gastroenteritis, was almost completely stopped, as a consequence of the most recent adjustments to the "Yellow Card" initiative. In contrast, the use of other antimicrobial agents has increased, particularly the use of aminoglycosides (mainly neomycin) and macrolides, see Figure 4.3 and 4.4 and Textbox 4.2.

Use of the critically important antimicrobial agents, fluoroquinolones and 3rd and 4th generation cephalosporins was close to zero in 2018 (Figure 4.5).

Use of medical zinc in pigs

In the latest issues of DANMAP, we have presented the use of medical zinc in pigs (Figure 4.6). This is relevant, because its use may select for antimicrobial resistance in some bacteria, including MRSA. Medical zinc, in the form of zinc oxide, is prescribed to piglets after weaning to prevent or treat diarrhoea. Following a steady increase, the use of zinc for pigs peaked at 548 tonnes in 2015. In 2017, the European Commission announced an EU wide withdrawal of medical zinc for pigs effective from June 2022. Already in 2016, the Danish pig industry launched an action plan to help the pig producers reduce the use of zinc. This was followed up by an updated action plan in 2018. The use of medical zinc was reduced by 4% from 533 to 509 tonnes in 2018.

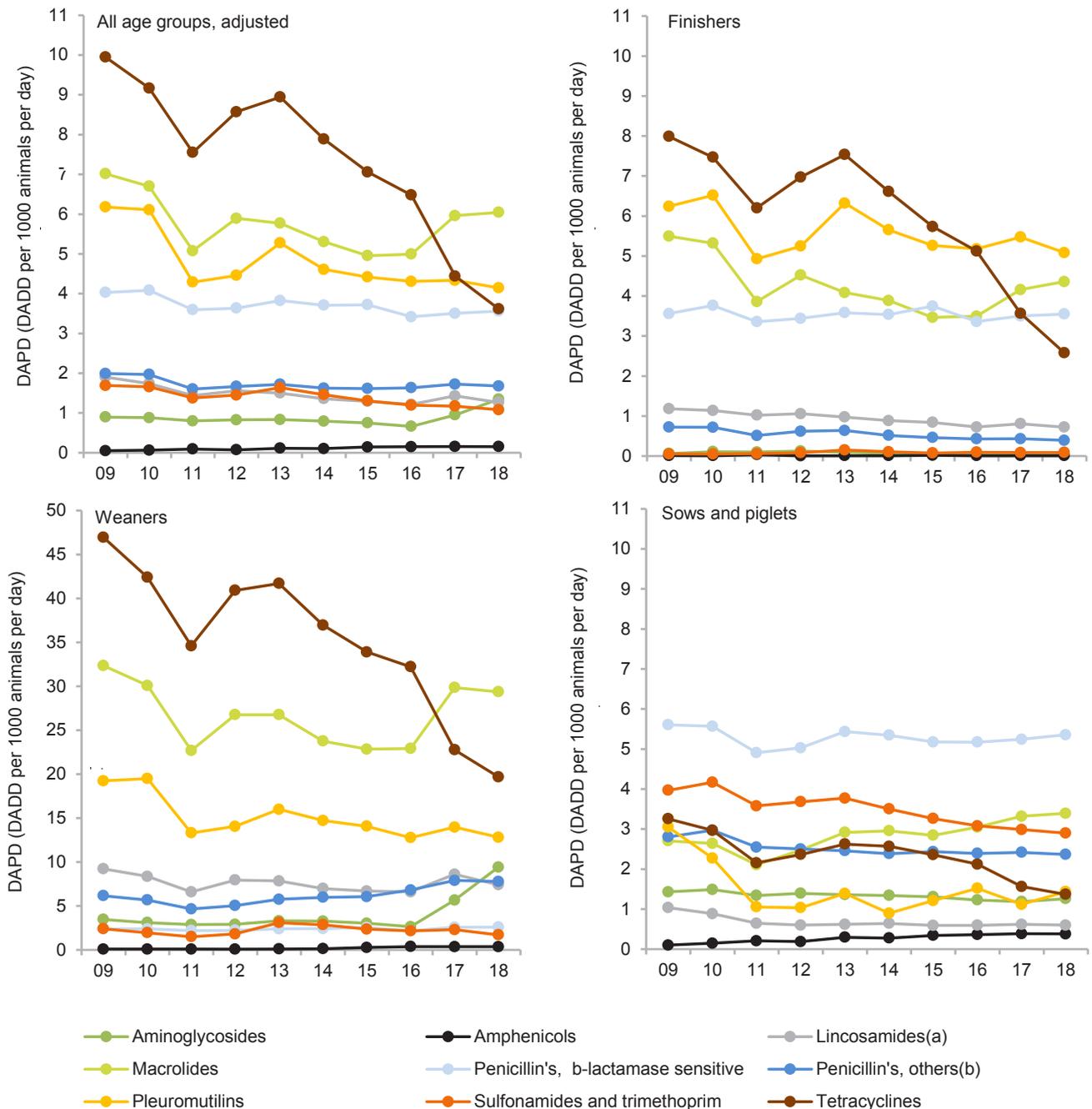
4.3.2 Antimicrobial consumption in cattle

Legislation supported thresholds for antimicrobial use in cattle have been in place since 2011. The overall consumption of antimicrobials in cattle has fluctuated between 12 and 13 tonnes for the past 5 years. In 2018, approximately 13 tonnes were registered for use in cattle, of which approximately 500 kg were used for intramammary treatment, either for therapeutic use or for dry cow treatment. More than two-thirds of the kg active compound were used to treat cows and bulls older than 24 months old (Table 4.1).

The production of veal and beef has remained relatively stable over the past 5-10 years, while the production of milk has increased steadily, Table 3.1.

Figure 4.4. Antimicrobial use in the total pig production and in each age group, DAPD, Denmark

DANMAP 2018

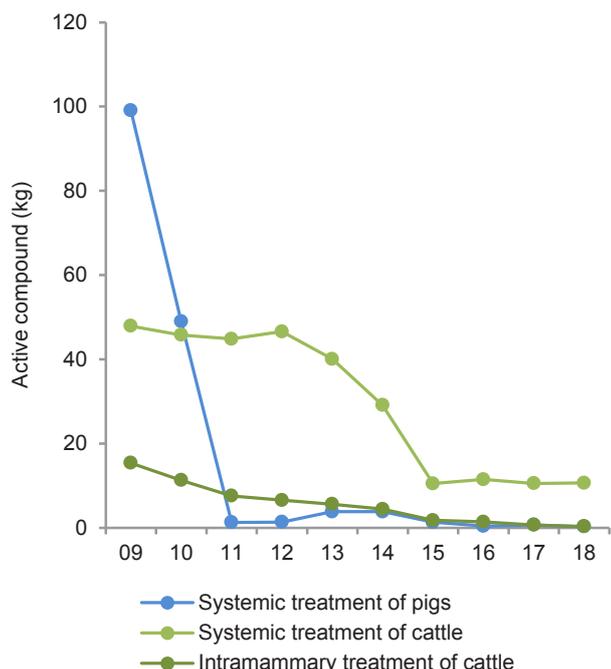


Note: Intramammaries, gynecologicals and topical drugs not included. The age group "sows and piglets" includes treatment in boars. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes). DAPDs for "all age groups" are adjusted for export of pigs at 30 kg (see text).
 a) Lincosamide/spectinomycin combinations comprise 76% of this group
 b) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

Since 2010, there has been an overall decrease in systemic treatment for adult cattle (>12 months) of 16% measured in DAPD. The main indication for systemic treatment in adult cattle was mastitis and beta-lactam sensitive penicillin accounted for approximately two thirds of the antimicrobials used in this age group followed by tetracyclines (17%). The use of macrolides constituted 2% in 2018, Figure 4.7 and Figure 4.8.

For young cattle (<12 months) antimicrobial use has increased by 43% from 2009 to 2018 when measured in DAPD. The main indication for systemic treatment in calves is respiratory disease followed by joint/limb infections and gastrointestinal diseases. The DAPD of amphenicols (florfenicol) has increased steadily over the past decade and have become the most frequently prescribed antimicrobial class

Figure 4.5 Use of 3rd and 4th generation cephalosporins in pigs and cattle, kg active compound, Denmark
DANMAP 2018



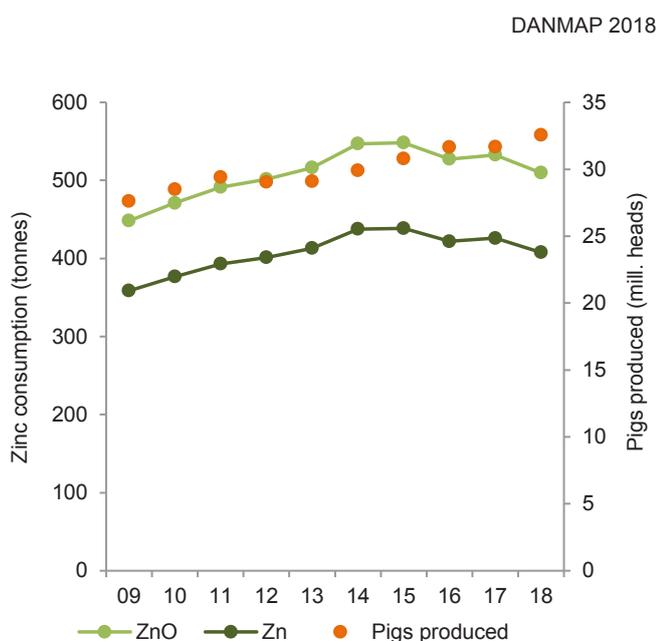
(28%), followed by tetracyclines and macrolides (25% and 20%, respectively).

The use of fluoroquinolones in cattle has been close to zero for the last decade. Fluoroquinolones may only be prescribed in food producing animals, as a last-line drug, based on microbiological analysis and susceptibility testing in an accredited laboratory. Use of fluoroquinolones in food producing animals is also notifiable to the DVFA.

In 2014, the cattle industry decided to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment (orally and parenterally), which caused the use to drop significantly in 2015, but has since then stabilised at approximately 10 kg per year. In 2018, the use of cephalosporins for systemic treatment in cattle and calves had been reduced by 64% and 51%, respectively, compared with 2015. The use of 3rd and 4th generation cephalosporins is shown in Figure 4.5.

The board of Danish dairy and beef producers has recently renewed its strategy for good udder health. The goals are a 20% reduction in use of antimicrobials for treatment of mastitis and other cattle diseases, as well as a lowering of geometric mean bulk tank cell counts to 150.000 by the year 2020. In addition, the dairy industry will promote use of dry-cow therapy and mastitis treatment with simple penicillins.

Figure 4.6 Usage (in tonnes) of medical zinc - zinc oxide (ZnO) and zinc (Zn) - in the pig production, Denmark
DANMAP 2018



Note: The most commonly used product is zinc oxide (ZnO) which contains 80% zinc and which is largely insoluble in water

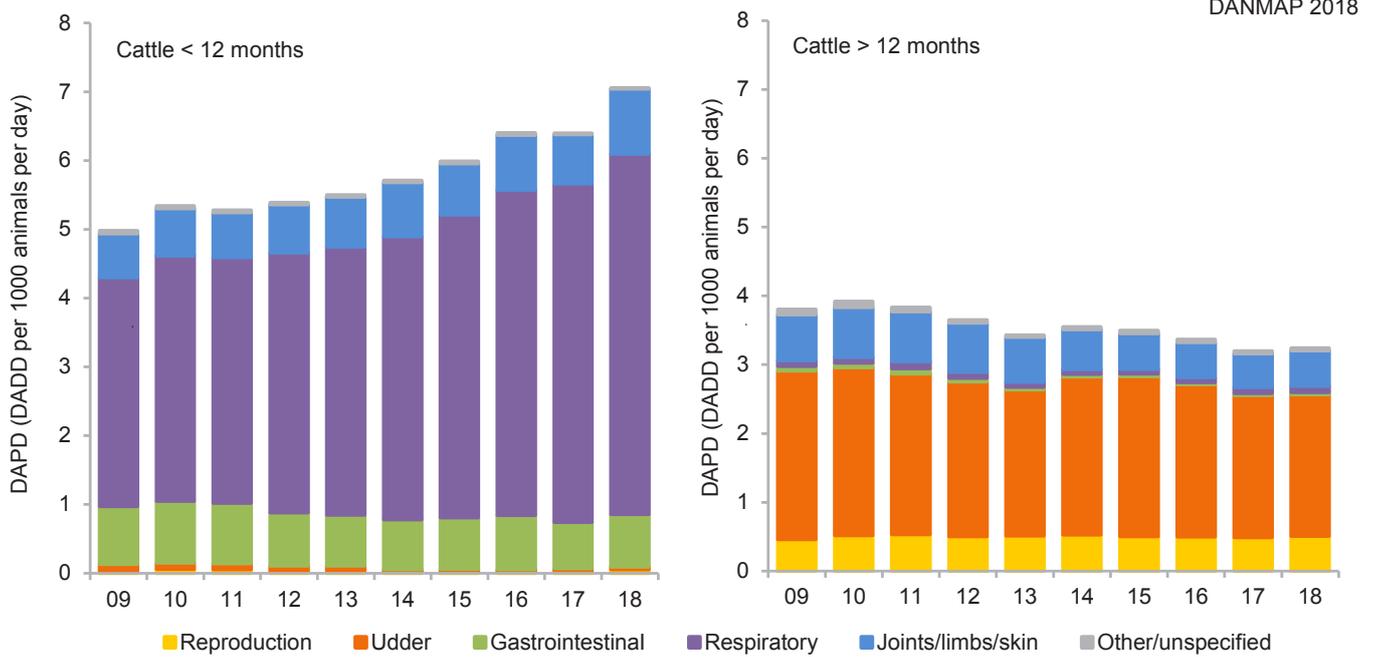
The majority of antimicrobials administered parenterally in cattle are used in dairy cows (Table 4.1) primarily to treat mastitis. The use of intramammary treatment is shown in Tables 4.2 and 4.3. The overall use in total DADD per cow per year has remained at the same level for the past decade, but the usage pattern has shifted away from the 3rd and 4th generation cephalosporins.

The number of dry-cow antibiotic treatments has increased since 2009. The relative proportion of dry-cow treatment versus therapeutic treatment has shifted markedly from 22% versus 78% in 2010 to 48% versus 52% in 2018 (Table 4.3). Dry-cow treatment is only allowed following diagnostic testing, where the presence of bacteria causing mastitis has been confirmed (Order nr 1647, 18/12/2018).

4.3.3 Antimicrobial consumption in poultry

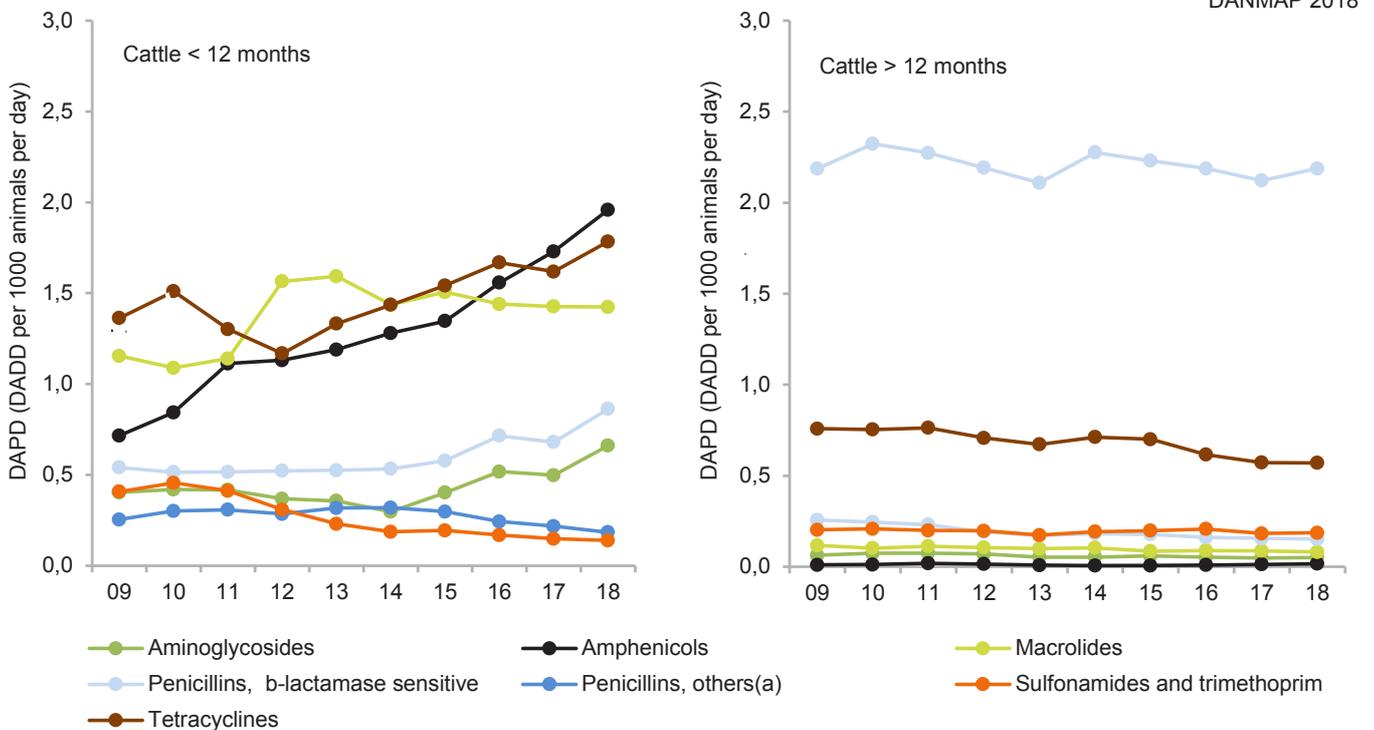
The poultry production comprises broiler production, egg layers and turkey production in sequence of magnitude. In addition, there is a small production of ducks, geese, and game birds. Danish broiler farms have a very high level of biosecurity and the antimicrobial consumption in broiler production is generally low compared with other species. Accordingly, disease outbreaks in just a few farms can markedly affect and cause considerable fluctuations in the national statistics on antimicrobial usage in the broiler sector (Table 4.4).

Figure 4.7 Indications for use of antimicrobials in cattle, DAPD, Denmark



Note: Intramammaries, gynecologicals and topical drugs not included. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group (in tonnes)

Figure 4.8 Use of antimicrobial agents in cattle, DAPD, Denmark



Note: Intramammaries, gynecologicals and topical drugs not included. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group (in tonnes)

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

Table 4.2. Use of antimicrobial agents for intramammary application in cattle, 1000s DADD, Denmark

DANMAP 2018

Doses per antimicrobial class	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Aminoglycoside-benzylpenicillin(a)	169	135	94	70	69	103	143	154	206	180
Cephalosporins, 1st gen.	97	97	103	111	117	113	96	89	86	113
Cephalosporins, 3rd and 4th gen.	73	53	36	31	26	21	8	6	3	1
Penicillins(b)	191	238	271	288	287	292	275	262	216	251
Others(c)	17	15	12	9	9	11	11	10	11	19
Total DADD	570	559	516	510	508	540	0	521	522	563
Total DADD per cow per year	1.0	1.0	0.9	0.9	0.9	1.0	0.0	0.9	0.9	1.0

Note: For intramammary treatment, 1 DADD is defined as the dose to treat two teats for 24 hours

a) Mainly dihydrostreptomycin-benzyl penicillin combinations; includes also combinations of penicillin/aminoglycoside with bacitracin or nafcillin (QJ51RC)

b) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations (QJ51CE, QJ51CF, QJ51RC)

c) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

Table 4.3. Number of treatments with antimicrobial agents for intramammary application in cattle, 1000s DADD, Denmark

DANMAP 2018

Total doses per indication	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Dry-cow treatment (4 teats)	75	84	94	110	125	140	152	160	168	193
Therapeutic treatment (2 teats)	397	370	329	290	258	259	229	202	186	176
Dry-cow treatment, porportion of total	16%	19%	22%	27%	33%	35%	40%	44%	48%	52%

In late 2014 and throughout 2015 several outbreaks increased the total use. In 2016, use of antimicrobials in poultry (excl. turkeys) decreased sharply again and in 2018 the usage was further reduced to 699 kg (Table 4.1). For the past decade, cephalosporins have not been used in the poultry industry, the use of fluoroquinolones has been close to zero and the use of colistin less than 10 kg per year.

VetStat does not allow differentiation of the use of antimicrobials between different sectors of the poultry production. The consumption in turkeys was identified by combining information from the Central Husbandry Register and collating this with information from VetStat.

The annual usage in turkeys can also be notably affected by disease outbreaks in few flocks. In 2018, the antimicrobial use was approximately at the same level as in 2017, of which more than half (56%) was tetracyclines (Table. 4.1).

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

Antimicrobial consumption in aquaculture is mostly influenced by the summer temperatures, because bacterial diseases are more likely to occur when temperatures are high. In recent years, the aquaculture industry has developed new and better vaccines and improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment. The

Table 4.4 Use of antimicrobial agents in poultry, kg active compound, Denmark

DANMAP 2018

	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutlins	Sulfonamides and trimethoprim	Tetracyclines	Total
2014	21	9	<1	10	359	2	0	120	326	<1	83	617	1548
2015	258	4	1	129	114	7	0	184	500	<1	446	796	2441
2016	60	5	<1	24	153	6	0	239	225	<1	111	749	1571
2017	65	5	<1	32	206	0	1	321	293	<1	85	483	1491
2018	51	0	<1	26	162	0	0	323	212	<1	37	516	1326

Note: Data were extracted from VetStat 3rd March 2019. VetStat does not differentiate between use in the different sectors of poultry production.

summer of 2018 was exceptionally warm and this was clearly reflected in a antimicrobial use that more than doubled from 1,697 kg in 2017 to 3,557 kg in 2018. However, the use was still lower than in 2014, when the use in aquaculture peaked at 5,116 kg (Table 4.5).

Mainly three compounds are used in aquaculture; sulphonamide/trimethoprim (64%), 1st generation quinolones (25%) and amphenicols (9%). Compared with 2017, it seems that the usage pattern has shifted towards use of sulphonamide/trimethoprim (40% in 2017) and away from quinolones and amphenicols (38% and 21% in 2017, respectively) (Table 4.4).

The use of antimicrobials in mink has a distinct seasonal variation, with high use from the spring, when the mink kits are born and again when they are weaned. Furthermore, there is usually an increase in antimicrobial use again in the autumn. The production of mink has increased over the last decade, peaking at 18.8 million in 2015. In 2018, 17.6 million mink were produced in Denmark, Table 3.1 (Source: Copenhagen Fur).

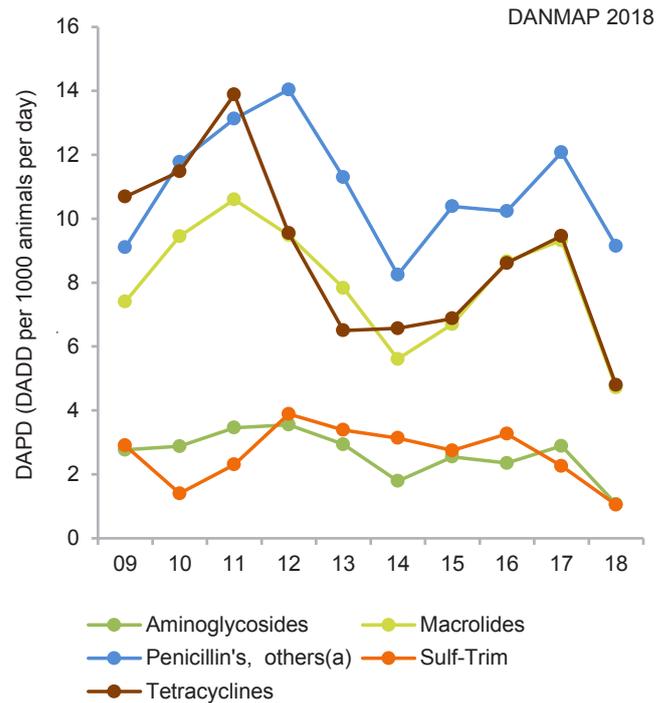
With the exception of 2013 and 2014, the use of antimicrobial agents in mink production increased every year for more than a decade until 2017, from less than two tonnes in 2004 to more than 6 tonnes in 2017. As a response, the industry increased focus on reducing the antimicrobial use and developed an antimicrobial action plan in cooperation with the Danish Food and Veterinary Administration, The Danish Veterinary Association and the veterinary practitioners. Remarkably, already in 2018 the use was reduced by 40%, from 6,156 kg in 2017 to 3,689 kg in 2018, or 42% when measured in DAPD, (Figure 4.10). The action plan and ongoing research projects are described in Textbox 4.4.

Table 4.5 Use of antimicrobial agents for aquaculture measured in kg active compound, Denmark DANMAP 2018

	Amphenicols	Other quinolones	Sulfonamides and trimethoprim	Other AB	Total
2014	297	1678	3132	9	5116
2015	311	1005	1650	5	2970
2016	315	893	1086	12	2307
2017	350	637	679	31	1697
2018	323	896	2293	46	3557

Note: Data were extracted from VetStat 3rd March 2019. Other AB include mainly penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors (98%) and tetracyclines (2%)

Figure 4.10 Use of antimicrobial agents in fur animals, DADP, Denmark



Note: Intramammaries, gynecologicals and topical drugs not included. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the total population (in tonnes)

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

It is particularly the use of tetracyclines, penicillins with extended spectrum, combinations penicillins and macrolides that have fluctuated over the past three years (Figure 4.10). Use of fluoroquinolones and cephalosporins in the fur animal production has been close to zero for more than a decade.

The information available on antimicrobial consumption in companion animals was not as complete as for production animals, because antimicrobials used for companion animals may be registered in VetStat without defining target animal species. It is assumed that a substantial part of the prescriptions, where no animal species is given have been used for companion animals. This proportion has been estimated using similar principles as described in DANMAP 2016. The estimated antimicrobial use for horses and pets are shown in Tables 4.6 and 4.7.

The overall antimicrobial use for horses appears to have a slightly increasing trend over the past five years from 1,047 kg active compound in 2014 to 1,194 kg in 2018. Sulphonamide/trimethoprim, administered mainly as oral paste, constitutes the majority of antimicrobial use in horses (98%). In addition 242 kg active compound sulphonamide/trimethoprim (oral paste for horses) was registered for use in cats and dogs (Table 4.7).

Table 4.6. Estimated use of antimicrobial agents for horses, kg active compound, Denmark DANMAP 2018

	Penicillins, b-lactamase sensitive	Sulfonamides and trimethoprim	Tetracyclines	Other AB	Total
2014	15	1024	6	2	1047
2015	10	1049	4	4	1067
2016	8	1117	5	1	1131
2017	9	1172	3	1	1184
2018(a)	10	1179	4	1	1194

Note: Data were extracted from VetStat 3rd March 2019.

The estimates include all antimicrobial agents registered, by either the pharmacy or veterinarians for use in horses. Antimicrobial agents, where no animal species was given, were allocated to horses based on relevant type of preparation (e.g. oral paste) or registration.

Antimicrobials administered parenterally - with no information on animal species - are not included.

Other AB include mainly aminoglycosides (67%), cephalosporins (13%) and macrolides (11%)

a) In 2018, additionally 242 kg of the sulfonamides and trimethoprim registered for pets were products (oral paste) typically used for horses (included in Table 4.6)

A large proportion of antimicrobials used for dogs and cats are prescribed for the treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, the repeated use of critically important antimicrobials may pose a risk to the owners.

The use of fluoroquinolones for use in pets, mainly dogs and cats, was 15 kg, which represented the majority of fluoroquinolones used in all animals. Similarly, the pets accounted for more than half (97 kg or 54%) of the use of cephalosporins used in animals (Table 4.7). However, antimicrobial use in pets appears to be shifting away from the use of cephalosporins towards broad spectrum penicillins, in particular amoxicillin with beta-lactamase inhibitor.

Since the treatment guidelines by Danish Veterinary Association were first published (November 2012, revised version 2018), the use of cephalosporins has been reduced by 64%. The guidelines recommend that use of critically important antimicrobials should be reduced as much as possible, demonstrating the effect of science-based treatment guidelines to control antimicrobial use.

Birgitte Borck Høg and Helle Korsgaard

For further information: Birgitte Borck Høg, bibo@food.dtu.dk

Table 4.7 Estimated use of antimicrobial agents for dogs and cats measured in kg active compound, Denmark

DANMAP 2018

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others(a)	Pleuromutlins	Sulfonamides and trimethoprim	Tetracyclines	Total
2014	8	<1	213	14	69	6	35	1	31	653	<1	300	35	1366
2015	7	<1	157	14	68	5	33	0	25	655	1	235	39	1240
2016	6	<1	137	15	69	3	31	<1	20	718	<1	276	40	1318
2017	6	1	111	14	67	2	31	0	19	718	<1	280	38	1287
2018(b)	6	<1	97	15	62	2	41	1	20	681	<1	261	37	1224

Note: Data were extracted from VetStat on 3rd March 2019. Data include all antimicrobial agents registered, by either the pharmacy or veterinarians for use in pets. Furthermore, antimicrobial agents, where no animal species is given, were allocated to pets based on relevant type of preparation (eg. tablets, eye- or eardrops) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

b) In 2018, approximately 242 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

Textbox 4.2

Establishment of an Advisory Committee on Veterinary Medicines

In 2017, as part of a broad political agreement between all the parties in parliament, it was decided to set up an Advisory Committee on Veterinary Medicines. This impartial Committee initiated its work autumn 2018. The Advisory Committee on Veterinary Medicines comprises veterinary and human medicine experts from University of Copenhagen, Danish Technical University, the Danish Medicines Agency, the Danish Health Authority, Statens Serum Institut, the Danish Veterinary Association and the Danish Veterinary and Food Administration.

The Council will discuss and contribute to the solution of specific tasks in the field of antimicrobials and offer guidance to initiatives on antimicrobial use and resistance. Its objective is to provide evidence-based professional advice for the Minister of Environment and Food in relation to the use of veterinary medicine and to tackle related issues proactively.

One of the first issues faced by the Committee is new national targets for antimicrobial consumption. The current national target was a 15-percent reduction in antimicrobial use for pigs by the end of 2018; new targets are needed for the coming years and the Committee will advise the Minister on these targets.

In the coming years the Advisory Committee on Veterinary Medicines will also be addressing the following issues:

- The Committee will assess the situation and advise on possible actions, if the presence of livestock MRSA at herd level for a given animal species exceeds 10%.
- The Committee will prepare “good clinical practice” for the veterinary practitioners’ activities.
- The Committee will regularly contribute with recommendations as a basis for decisions in relation to prudent use of veterinary medicine, including treatment guidelines.
- The Committee will assist the Danish Veterinary and Food Administration in assessing and clarifying risk assessments in order to ensure evidence-based decisions in relation to appropriate use of antibiotics in livestock production.
- The Committee will propose criteria and weighting in the Yellow card initiative for pig producers.
- The Committee may contribute to evaluations in connection with the resistance monitoring of all animal species.

The Advisory Committee on Veterinary Medicines is working in line with a One Health approach, and supplements the Danish National Antimicrobial Council established in 2010, and can be contacted at vetmed@fvst.dk

*Hans Henrik Dietz, Dept. of Veterinary and Animal Sciences, University of Copenhagen
For further information: Hans Henrik Dietz, hhd@sund.ku.dk*

Textbox 4.3**OUA - an up and coming special production**

OUA is the Danish acronym for “Opdrættet uden antibiotika”, which means raised without the use of antibiotics (in English: RWA = Raised Without Antibiotics). This type of production, now well established as a special production in Danish pig production, was initiated to meet a growing customer demand particularly from countries outside Denmark.

Danish Crown, which identified a market for this type of production, first embarked on the OUA project in collaboration with SEGES Danish Pig Research Centre in 2014. Initial trials were conducted at two farms on the island of Bornholm where it was shown that a large number of pigs could be produced without the use of antibiotics, with the proviso that the production of such pigs from birth to slaughter involves additional costs, which ultimately means higher meat prices in order for OUA production to be viable.

Since the experiment was completed, several pig farms have been converted to OUA herds and now counts 51 herds with more to come. In 2018, approximately 6,000 OUA pigs were slaughtered per week. This figure has increased to 7,000-7,500 pigs per week since the start of 2019.



Photo: Danish Crown

Facts: What is OUA?

OUA is the Danish acronym for “Opdrættet uden antibiotika”, which means bred without the use of antibiotics. Such production requires the following:

- No antibiotics from birth to slaughter;
- Only vegetable-based feed although milk and dairy products are allowed;
- The pigs are tagged with special OUA tags, which are removed if they are treated;
- An annual, independent production audit is carried out.

Higher price for OUA pigs

As with other special productions, OUA pigs require a market and consumers who are willing to pay a higher price for the meat. OUA production demands extra close monitoring and thus additional working hours for the teams working in the housing units, which, in turn, means higher production costs. As well as the increased need for monitoring the pigs, OUA production needs better disease prevention, including more frequent use of vaccines and more expensive feed. That said, sick pigs that require treatment are treated accordingly - just like any other pig. If a pig is treated with antibiotics just once, it is no longer classified as an OUA pig and will not be sold as such.

The future of Danish OUA

There is support for OUA production from the pig industry as long as it remains a special production for which there is a market. In recent years, Danish Crown has seen a growing demand for OUA pigs, particularly from abroad. Here, there is strong awareness of Danish special productions because of the great confidence in what Denmark is already known for, i.e. high and uniform quality, low antibiotic consumption and traceability back to the producer.

OUA pigs have also attracted interest in Denmark. From the start of 2019, a large, nationwide supermarket started to sell pork from Danish OUA herds only. Other countries also raise pigs without the use of antibiotics, but the requirements differ from country to country. In some, the set of rules defining this type of production is somewhat loose, while in other countries they are much stricter.

OUA production - can we improve?

The art of raising OUA pigs is, of course, to produce as many pigs as possible without the use of antibiotics. Reports from many of these herds indicate that some of the challenges are the same - there are certain infectious diseases that are quite difficult to manage and prevent. Danish Crown, SEGES Danish Pig Research Centre in collaboration with university researchers have therefore embarked on a research partnership supported by GUDP (Green Development and Demonstration Programme). The aim is to solve some of these disease challenges - both for the benefit of OUA producers and for all conventional pig herds, with the ultimate aim of paving the way for even better OUA production in Denmark in the future.

*Christian Fink Hansen, Sector Director, SEGES Danish Pig Research Centre
For further information: Christian Fink Hansen, cfha@seges.dk*

Textbox 4.4

Antimicrobial use in mink - action plan/research projects

Antimicrobial use in the Danish mink industry has increased significantly over the last decade. From 2008 to 2017 the total amount of prescribed antimicrobials increased substantially (Figure 1). As almost no antimicrobial agents are registered for use in mink, the prescription is mostly off-label. This, combined with the lack of a dosage regime makes it critically important with a guideline for prudent use of antimicrobial agents for mink.

As a response to the increase in antimicrobial use, the Danish mink breeders organization (Kopenhagen Fur) initiated an antimicrobial action plan in cooperation with the Danish Veterinary and Food Administration, the Danish Veterinary Association and the veterinary practitioners. The action plan was launched in autumn 2018, with an overall aim to phase out the use of medical zinc oxide and to reduce the antimicrobial use for mink to 4,000 kg by 2022. The action plan includes 18 targets related to Benchmarking and Best Practice.

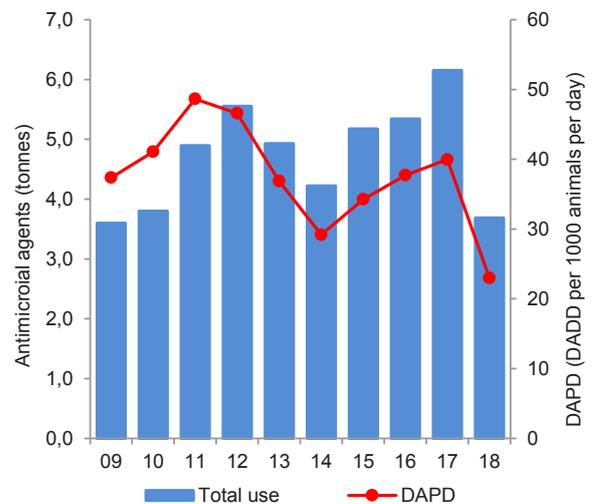
Benchmarking. To promote the highest degree of transparency, the antimicrobial use for Danish mink farms will be quantified in a comparable manner (DADD/biomass) and benchmarked across farms, veterinarians and feed producers. Recent studies have found that veterinarians and feed producers have a significant impact on the antimicrobial use. Therefore, the antimicrobial use relating to veterinarians and feed producers, will be quantified based on the antimicrobial use prescribed for mink on affiliated farms. The antimicrobial use in the individual mink farms, should be assessed annually by the farmer in cooperation with the farm veterinarian. Preferably, this annual assessment will be incorporated into the Veterinary Advisory Service contracts (VASCs).

Furthermore, the antimicrobial use by Danish mink farms will be included on an internal performance list used to rank farms in terms of productivity (reproduction and fur quality).



Oral administration of antimicrobial treatment to female mink. Photo: Julie Melsted Birch, Kopenhagen Fur

Figure 1 Use of antimicrobial agents in fur animals, Denmark
DANMAP 2018



continued ... Textbox 4.4

Best Practice. A substantial part of the scientific foundation of the action plan is based on recent and ongoing research projects carried out in collaboration with the Danish universities. In 2017 Kopenhagen Fur, DTU and Copenhagen University initiated two industrial Ph.d. projects supported by Innovationsfonden and Pelsdyravlernes Forskningsfond. The aim of the projects is to provide data on pharmacokinetics and antimicrobial resistance as well as data for determination of MIC break-points. Together, these two projects initiated in early 2017 are expected to provide the evidence based knowledge needed to support a best practice guideline with dosage and product recommendations for antimicrobial use in mink. However, the antimicrobial action plan already makes provision for the voluntary stop for use of zinc oxide, while the use of tetracyclines should be supported by an antimicrobial susceptibility test.

Antimicrobial group treatment must be supported by diagnostic tests carried out by a competent laboratory, while the Danish Veterinary and Food Administration has been requested to reduce the prescription period related to group treatments to a maximum of five days. In addition, practical possibilities to treat smaller groups of animals on-farm are being investigated.

Finally, the antimicrobial action plan includes points on preventive measures, which may result in a reduced antimicrobial use. This includes studies on breeding more robust mink, alternative treatment methods such as probiotics, as well as the impact of feed and feeding strategies on the occurrence of diarrhea. Furthermore, the plan includes points giving an increased focus on disease prevention, especially during the spring and summer, where a large part of the antimicrobial consumption occurs.

The effect of the antimicrobial action plan is dependent on a high level of commitment, from the breeders, the Danish Food and Veterinary Authorities, The Danish Veterinary Association and the veterinary practitioners. Therefore, a continuous focus on communication and sharing of knowledge is essential. To keep momentum, the Danish Veterinary Association has chosen to make a Mink theme at the upcoming annual meeting for Danish Veterinarians in 2019, where the antimicrobial use and latest research on the topic will be presented.

*Mette Fertner and Tina Struve, Kopenhagen Fur
For further information: Tina Struve, tst@kopenhagenfur.com*

5

ANTIMICROBIAL CONSUMPTION IN HUMANS



5. Antimicrobial consumption in humans



Highlights

Total: In 2018, consumption of antimicrobials in humans for both primary sector and hospital sector combined was in total 15.95 defined daily doses per 1000 inhabitants per day (DID), lower than the consumption in 2017 (16.67 DID) and lower than a decade ago in 2009 (17.47 DID). The highest consumption ever reported in Denmark was in 2011 (18.91 DID). In 2018, the primary sector accounted for 13.98 DID, a decrease of -18% compared to the consumption in 2011 (17.06 DID), while the hospital sector accounted for 1.92 DID, an increase of 9% (1.76 DID in 2011).

Trends in consumption: Trends in antimicrobial consumption are driven by treatment of patients in the primary health sector, accounting for approximately 90% of all antimicrobials consumed. When measured in number of prescriptions redeemed per 1000 patients, 20 years ago (in 1999) the consumption was at a national average of 535 prescriptions per 1000 inhabitants per year, increasing until the first peak of 630 prescriptions per 1000 inhabitants per year in 2007 and the second, highest peak of 638 prescriptions per 1000 inhabitants per year in 2011. Since 2011, antimicrobial consumption in the primary health sector has decreased for all five health regions and in all municipalities. Marked decreases were observed since 2016, possibly related to the development and introduction of national goals for the reduced consumption of antimicrobials in humans as defined in the National action plan from the Danish Ministry of Health published July 2017.

Decreases in consumption in primary healthcare since 2011 were mainly observed for beta-lactamase sensitive penicillins and macrolides, the beta-lactamase sensitive penicillins decreasing from 5.29 DID to 3.61 DID and the macrolides from 2.44 DID to 1.46 DID during the years 2011 to 2018, respectively. Decreases were also observed for fluoroquinolones and tetracyclines, declining from 0.57 DID to 0.41 DID and from 1.74 DID to 1.40 DID, respectively for the same period. From 2011 to 2018, beta-lactamase resistant penicillins were the only antimicrobials with continued increased consumption observed, from 1.22 DID to 1.60 DID. Decreases were noted for both genders and all age groups, most pronounced among the youngest and less pronounced among the eldest above 80 years of age.

Simultaneously, the **consumption at hospitals increased** during the decade, from 70.35 DDD per 100 bed days (DBD) or 264.29 DDD per 100 admissions (DAD) in 2009 to 99.31 DBD or 291.63 DAD in 2018, respectively, corresponding to increases of 41% in DBD and 10% in DAD.

Consumption of the **antimicrobials of special critical interest** (cephalosporins, fluoroquinolones and carbapenems) in total at hospitals decreased in the beginning of the decade, from a combined 22.19 DBD in 2009 to 19.59 DBD in 2018. In 2018, they constituted altogether 20% of the antimicrobials consumed at hospitals, corresponding to 10.2 DBD for cephalosporins, 6.81 DBD for fluoroquinolones and 2.77 DBD for carbapenems, respectively. Consumption showed no notable changes between 2016 and 2018, despite a defined national goal of a 10% decrease from 2016 to 2020.

For DANMAP 2018, the new WHO DDD values were applied and all calculations and figures updated 10 years retrospectively. Due to changes in DDD values for several important antimicrobials, the numbers and figures in this report are not comparable to former reports.

5.1 Introduction

In Denmark, all consumption of human medicine including antimicrobials is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority. This includes sales data from all public and private healthcare providers. The primary sector has submitted antimicrobial sales data since 1994, whereas the hospital sector has submitted data since 1997.

In Denmark only medical doctors, veterinarians and dentists can prescribe antibiotics and only publicly registered and approved pharmacies are allowed to sell. Recording of the consumption in the primary sector is based on the total sales from pharmacies to individuals and private clinics. For all sales, data contain information on the ATC code, formulation, package size and number of packages sold. For sales to individuals, additional information is available from the prescription registry; this includes information on the prescriber and on the age, gender and address of the patient. Since 2004, it also includes the indication for prescribing the medication. No over-the-counter sale takes place. This enables an almost complete surveillance of all systemic antimicrobials used in Denmark.

For the hospital sector, primarily data from public somatic hospitals with acute care function is included in the report – data from psychiatric hospitals, private hospitals and hospices has traditionally been excluded, since consumption at these facilities is only minor (0.5 DID in 2018) and no good denominator for measuring the consumption in these patient populations exists. But for Figure 5.1a (total consumption measured in defined daily doses (DDD) per 1000 inhabitants per day) all consumption data were included to give a complete picture on human antimicrobial consumption in Denmark.

For more detailed information on data reporting and registration, please see chapter 9.8, materials and methods.

In this chapter, the term ‘antimicrobial agents’ covers all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code J01. The only other antimicrobials included are metronidazole (ATC code P01AB01) and vancomycin (ATC code A07AA09), since these contribute with valuable information regarding antibacterial treatments as well. Their consumption has been included in DANMAP since 2014. Tuberculostatica, antiviral and antifungal drugs are not included, but textbox 5.4 (page 74 to 78) deals with the consumption of antifungal compounds and resistance patterns in human invasive isolates of *Candida* and *Aspergillus*.

A major change in DANMAP 2018 is the correction for the new DDD values for some of the commonly used antimicrobials, changed by the WHO Collaborating Centre for Drug Statistics Methodology in Oslo per January 2019. All numbers in the figures and tables were updated 10 years retrospectively with the new DDDs. Due to these changes, figures are no longer comparable between the present and former reports.

The most important changes in relation to the Danish patterns in consumption were the new DDD values for oral dosing of amoxicillin and amoxicillin with clavulanic acid and for parenteral dosing of meropenem, ciprofloxacin and colistin. Denmark has a strong tradition for using penicillins for the treatment of patients in both primary care and at hospitals. Over time, doses given per treatment have increased due to new knowledge in pharmacodynamics. Thus, for most penicillins the former WHO DDD values were no longer in correlation with actual recommendations on dosages, and the recent changes in the DDD were very welcome.

However, changes for the other groups of penicillins are still needed. In this report, for demonstration of differences and for use in National monitoring, Danish adjusted DDDs (DaDDD) were developed for both primary care data and hospital data and applied in two of the figures (Figure 5.4 and Figure 5.13, respectively). For more information regarding DaDDD, see Table 9.5 and 9.6 in chapter 9.8, materials and methods.

Further information and further numbers on the use of antimicrobials in Denmark can be found at www.medstat.dk and <http://esundhed.dk/sundhedsregistre/LSR/ANT/Sider/ANT.aspx>.

The Danish healthcare system has undergone significant changes since the DANMAP collaboration began in 1995. Most notable are the establishment of a more centralised hospital system, concentrating highly specialized functions in few tertiary care hospitals, paralleled by a reduction in the number of geographically more peripherally situated secondary care hospitals. Thus, during the last two decades the number of hospitals in Denmark offering 24 h acute care has diminished from 80 public somatic hospitals in 1995 to 41 in 2015. For 2020, it is expected that all acute care function can be merged further to 21 public hospitals.

Changes in organisation of hospital functions also happened to the overall organisation of surgical and medical treatment. Many surgical procedures are today performed in an ambulatory setting with only short time stay at the hospital. This also applies to internal medicine, where tasks have moved to ambulatory care or been outsourced to the general practitioner. A new political plan for the Danish Health system from 2018 focuses on enforcement of the primary sector by moving many functions from hospital ambulatory care back to the municipalities. This demands a restructuring and strengthening of collaboration between all sectors. It may affect monitoring systems, since bed days become more difficult to measure or less correct when it comes to describing the actual activity in the health care sector in total. Definitions of what is included in hospital activity become less clear and boundaries between primary and hospital sector become more fluent. It also challenges the comparison of consumption over time.

For DANMAP 2018, hospital consumption was measured in DDD per three different denominators: per 100 bed days, per 100 admissions and per 1000 inhabitants per region for more transparency and a clearer picture of the consumption in total.

Initiatives on the control and reduction of antimicrobials in the human health sector

The National Action Plan on the reduction of antibiotics in humans launched in July 2017 aims at fulfilling three measurable goals, two directed at the consumption in primary care and one focusing on hospital care. The first goal targets an overall reduction in antimicrobial consumption measured in the number of prescriptions redeemed at pharmacies in Denmark, from 462 prescriptions per 1000 inhabitants in 2016 to 350 prescriptions per 1000 inhabitants in 2020. In focus are general practitioners, medical specialists and dentists; prescriptions issued through hospital doctors are omitted from calculations, due to the assumption that these in most cases resemble continuation of a treatment begun at the hospital and thus should be counted there. The second goal aims at a more prudent choice of antimicrobials by focusing on an increase in the share of beta-lactamase sensitive penicillins used in primary care to 36% by 2020, thus emphasizing the importance of beta-lactamase sensitive penicillins as the continued drug of choice in many common infections. Also this goal is directed at general practitioners, medical specialists and dentists. The third goal aims at a 10% reduced consumption of the three antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) at hospitals from 2016 to 2020, measured in DBD. In 2017, this goal was challenged through shortages of piperacillin/tazobactam, which brought cephalosporins back as a first line treatment of septic patients. In 2018, no difficulties in deliverance of important antimicrobials were reported, but for beta-lactams of different formulations it is anticipated to happen again. During 2018, one and a half year into the plan, the following results had been achieved:

- For goal one the number of prescriptions from primary care (general practitioners, medical specialists and dentists) were reduced to 397 prescriptions per 1000 inhabitants per year
- For goal two, the proportion of beta-lactamase sensitive penicillin (based on number of prescriptions issued from general practitioners, medical specialists and dentists) had remained unchanged (31%) from 2016 to 2018
- At hospitals, the consumption of antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) had decreased by 2% (from 20.24 DBD in 2016 to 19.79 DBD in 2018)

The preliminary results from the initiatives based on the National Action Plan highlight the well-known fact that it is easier and faster to achieve overall reductions in consumption than to change habits towards a more prudent use, the latter taking more time and efforts.

The National Action Plan was issued by the Danish Ministry of Health and supported by the National antibiotic council representing all relevant health institutions, organisations and specialties working with AMR and treatment or control of infections in Denmark. Together with the National Action Plan, a One Health Strategy was published, building on the existing National Action Plan on the control of antimicrobial resistance from 2010. Both are available at the Danish Ministry of Health's homepage at www.SUM.dk.

Reducing the amount of antimicrobials consumed can only be achieved through parallel actions on the continued improvement of diagnostics and through infection control measures. The National Center for Infection Control (NCIC) at Statens Serum Institut supports many of the National antibiotic initiatives through recommendation guidelines aimed at hospitals and health care settings, (textbox 5.3, page 72)

5.2 Total consumption (Primary healthcare and Hospital care combined)

Historically, the consumption of antimicrobials in Denmark showed no significant trends during the first five years of systematic registration from 1996 to 2000, where consumption was estimated to be between 13.40 and 13.63 DDD per 1000 inhabitants per year (DID; based on former WHO DDD values and therefore not directly comparable to newer calculations). After these stable years, steady increases were observed until 2011. Since then the consumption has first levelled off and since decreased markedly.

For DANMAP 2018, calculations for figures were based on the new WHO DDD values, (Table 9.4 materials and methods) and data updated ten years back in time. Further back, data are not specified into the different drug classes and thus could not be updated.

The total consumption of systemic used antimicrobials in 2018 was 15.95 DID, which is 4.6% less than the consumption in 2017 (16.67 DID) and 9.5% less than the consumption a decade ago in 2009 (17.47 DID), (Figure 5.1a, total consumption of private and public health care, DID). The total consumption in 2018 corresponds to 49.786 kg active compound consumed (Table A5.1 in web annex).

This is the second consecutive year with a marked decrease in the total consumption in Denmark. The decrease is driven by reduced prescribing in primary health care, which accounts for approximately 90% of all antimicrobials used in humans in Denmark.

Fig 5.1b presents consumption data from primary care and acute care hospitals divided into the five regions. Although consumption per inhabitant differs between the regions, for all five regions marked decreases were observed in the primary sector since 2016. The two neighbouring regions, the Capital region and the region of Zealand showed highest total consumptions of 15.89 DID and 16.32 DID, respectively; for the

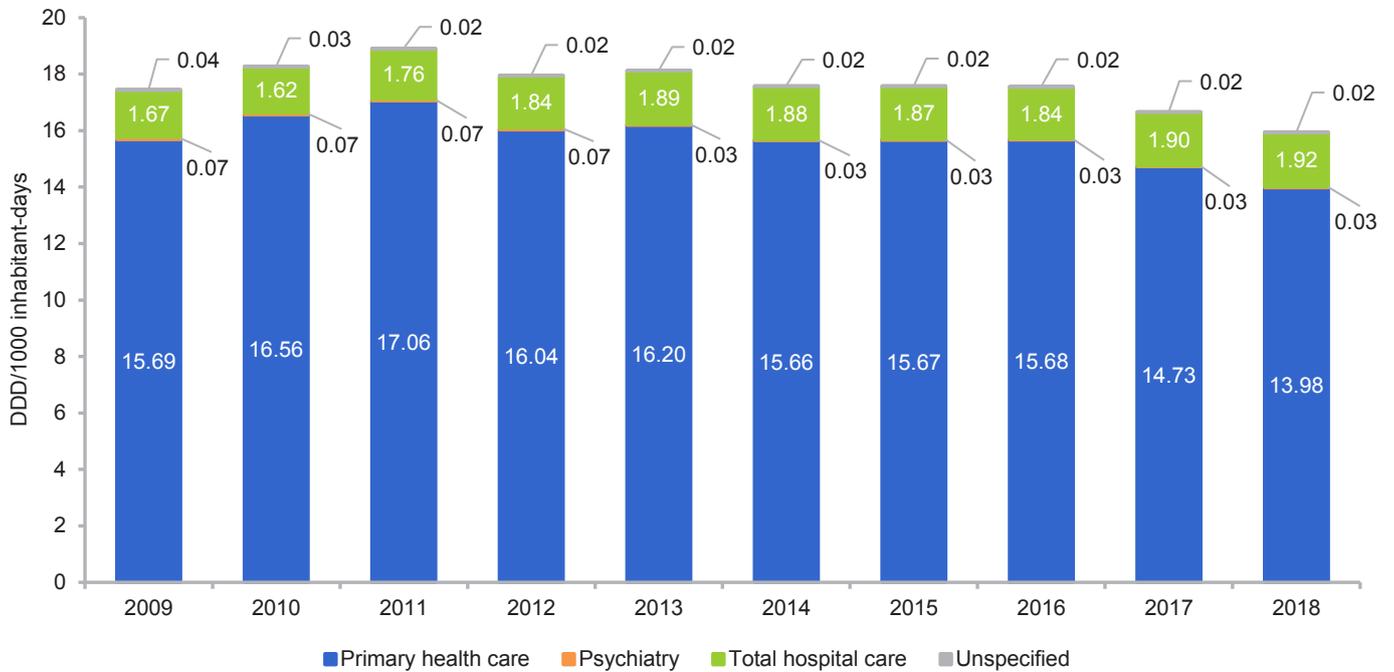
Capital region due to a relatively high consumption at hospitals and for region Zealand due to a comparably high consumption in primary care. The Central and Northern Region had the lowest total consumption with 14.23 DID and 14.70 DID, respectively. They were similar in an overall low consumption in both primary and hospital care. For more information on population

size and hospital activity in the five health regions, see Figure 3.2 and Table 5.7.

Figure 5.1c presents use of the main antimicrobial drug classes divided into primary care and hospital care, respectively.

Figure 5.1a Total consumption of all systemic antimicrobial agents used for humans, DID, Denmark

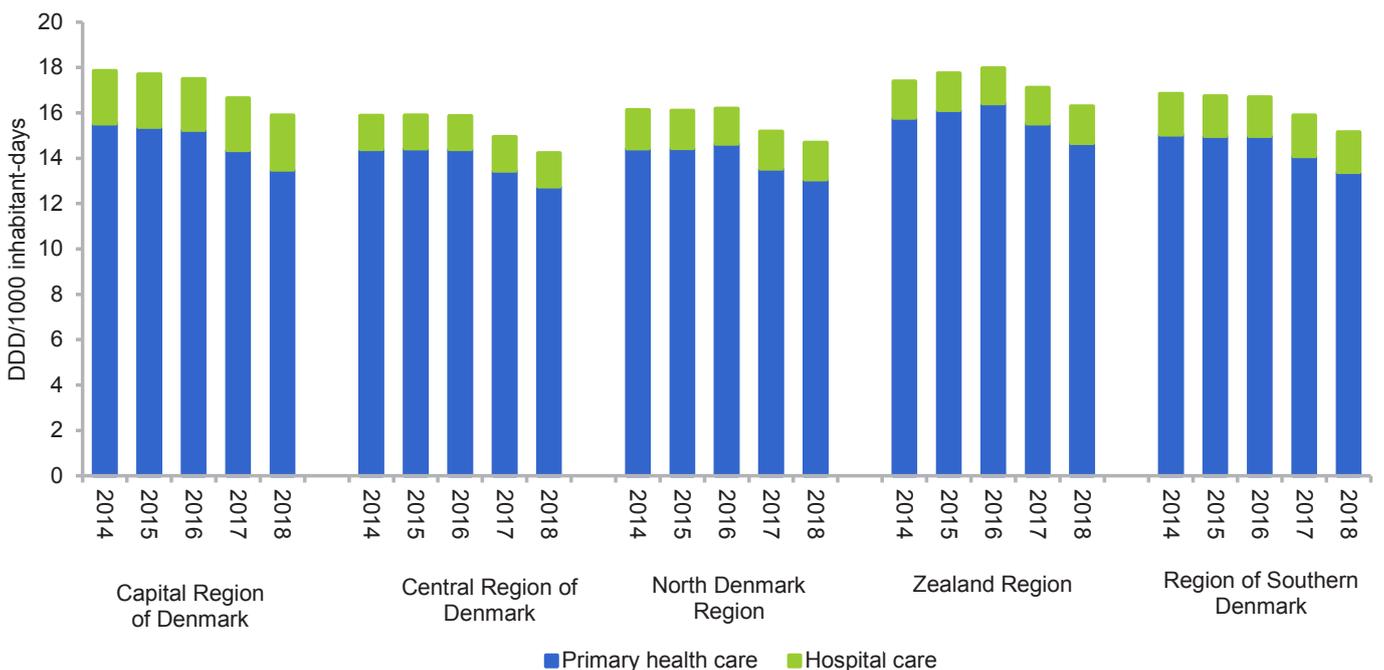
DANMAP 2018



Data for this figure is based on the total sales in Denmark
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.1b Total consumption of systemic antimicrobial agents used in primary care and at acute care hospitals, divided in regions, DID, Denmark

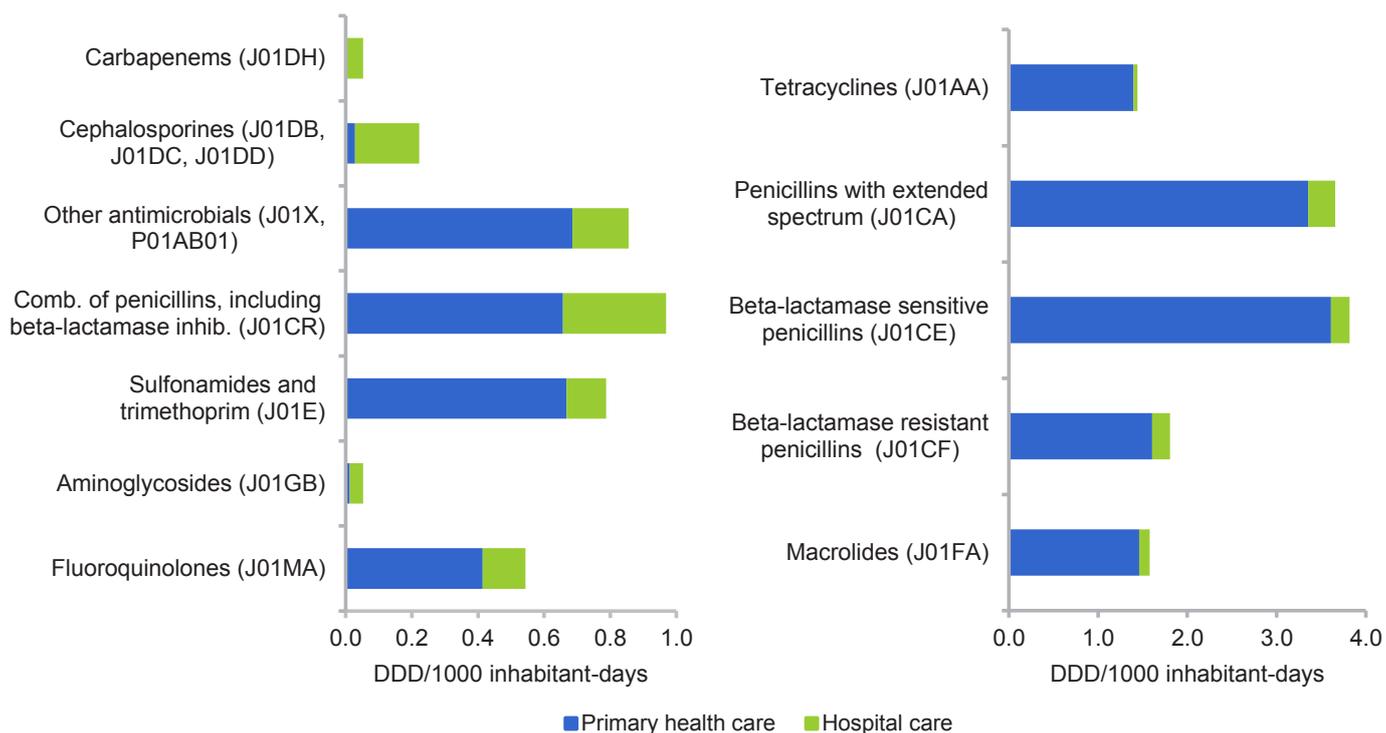
DANMAP 2018



Data used in this figure is based on registered sales to individuals and consumption at acute care public somatic hospitals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.1c Distribution of consumption of the main antimicrobial classes used for humans, DID, Denmark

DANMAP 2018



Data used in this figure is based on registered sales to individuals and consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care (DID), Denmark

DANMAP 2018

ATC group	Therapeutic group	Year									
		2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	1.62	1.70	1.74	1.76	1.96	1.66	1.60	1.62	1.42	1.40
J01CA	Penicillins with extended spectrum	2.88	3.02	3.11	3.03	3.12	3.20	3.28	3.33	3.36	3.35
J01CE	Beta-lactamase sensitive penicillins	5.13	5.26	5.29	4.68	4.65	4.38	4.33	4.16	3.88	3.61
J01CF	Beta-lactamase resistant penicillins	1.14	1.17	1.22	1.21	1.30	1.36	1.38	1.48	1.56	1.60
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.30	0.45	0.60	0.70	0.81	0.87	0.95	0.95	0.79	0.66
J01D	Cephalosporins and other betalactam antibiotics	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.48	0.51	0.50	0.52	0.53	0.55	0.56	0.56	0.56	0.53
J01EB	Short-acting sulfonamides	0.27	0.26	0.24	0.22	0.22	0.21	0.18	0.16	0.15	0.14
J01EE	Combination of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.21	2.44	2.60	2.20	1.94	1.79	1.77	1.82	1.62	1.46
J01FF	Lincosamides	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06	0.06	0.06
J01GB	Aminoglycosides	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.52	0.57	0.57	0.55	0.52	0.50	0.49	0.48	0.44	0.41
J01XC	Steroid antibacterials (combination fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
J01XE	Nitrofurans derivatives (nitrofurantoin)	0.49	0.51	0.50	0.50	0.49	0.48	0.45	0.43	0.26	0.15
J01XX	Other antibacterials (metheamine >99%)	0.26	0.27	0.26	0.25	0.24	0.24	0.25	0.27	0.28	0.29
J01XD and P01AB01	Nitroimidazole derivatives (metronidazole)	0.26	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.25	0.24
J01 and P01AB01	Antibacterial agents for systemic use (total)	15.69	16.56	17.05	16.03	16.19	15.64	15.66	15.67	14.71	13.97

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Data used for this table is based on total sales in Denmark (individuals and clinics)

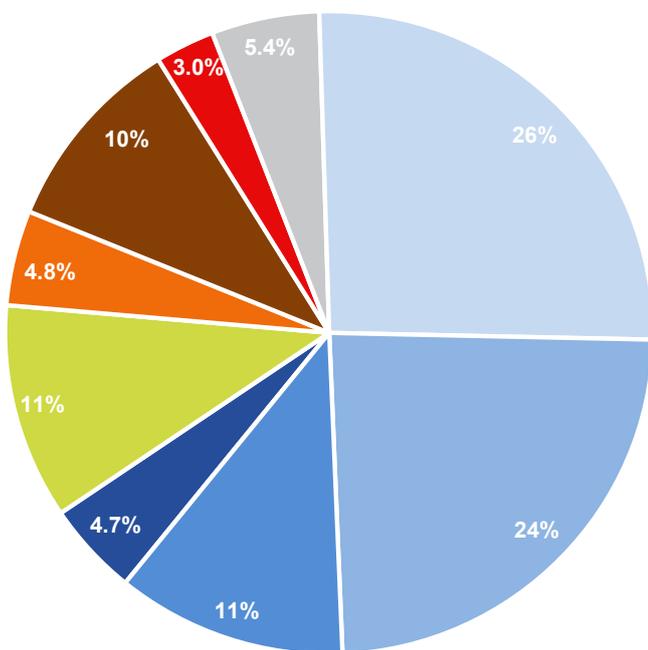
5.3 Primary healthcare

5.3.1 Total consumption in primary healthcare in DID

In 2018, the consumption of antimicrobials in primary healthcare based on total sales from pharmacies was 13.98 DID, a decline of 5.4% from 2017 (14.73 DID). This is the second year in a row with a significant decline since 2012 and a reduction of altogether 18% since the peak of 17.06 DID in 2011, (Figure 5.1a). Within the decade, the consumption has decreased overall 11% from 15.69 DID in 2009.

Beta-lactamase sensitive penicillins continued to be the biggest group consumed with 3.61 DID (accounting for 26% of

Figure 5.2 Distribution of the total consumption of antimicrobial agents in primary healthcare, based on DDD, Denmark
DANMAP 2018



- Beta-lactamase sensitive penicillins (J01CE)
- Penicillins with extended spectrum (J01CA)
- Beta-lactamase resistant penicillins (J01CF)
- Comb. of penicillins, incl. beta-lactamase inhib. (J01CR)
- Macrolides, lincosamides and streptogramins (J01F)
- Sulfonamides and trimethoprim (J01E)
- Tetracyclines (J01AA)
- Fluoroquinolones (J01MA)
- Other antimicrobials (J01D, G, X, P01AB)

Data used for this figure is based on total sales in Denmark (individuals and clinics)
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

the total consumption in primary care, Figure 5.2). They were followed closely by penicillins with extended spectrum with a consumption of 3.35 DID (corresponding to 24% of the total consumption). Beta-lactamase resistant penicillins surpassed macrolides in being the third biggest group consumed with 1.60 DID (accounting for 11% of the total consumption). Macrolides and tetracyclines were fourth and fifth biggest groups with 1.46 (11%) and 1.40 DID (10%), respectively, (Figure 5.2 and Table 5.1).

5.3.2 Trends in consumption of the leading antimicrobials in DID

The decreases in consumption in primary health care observed since 2016 apply to seven of the nine main antimicrobial classes, (Figure 5.3). For the beta-lactamase sensitive penicillins, macrolides and fluoroquinolones this is a continuation of a decreasing trend observed since the peak of consumption in 2011. Within these seven years the consumption of beta-lactamase sensitive penicillins decreased from 5.29 DID in 2011 to 3.61 DID in 2018 (- 32%), while consumption of macrolides decreased from 2.60 DID to 1.46 DID, (-44%). From 2017 to 2018 alone, the corresponding decreases were 7.1% and 10%, respectively. Fluoroquinolones also decreased steadily from 0.57 DID in 2011 to 0.41 DID in 2018, (-28%), Table 5.1.

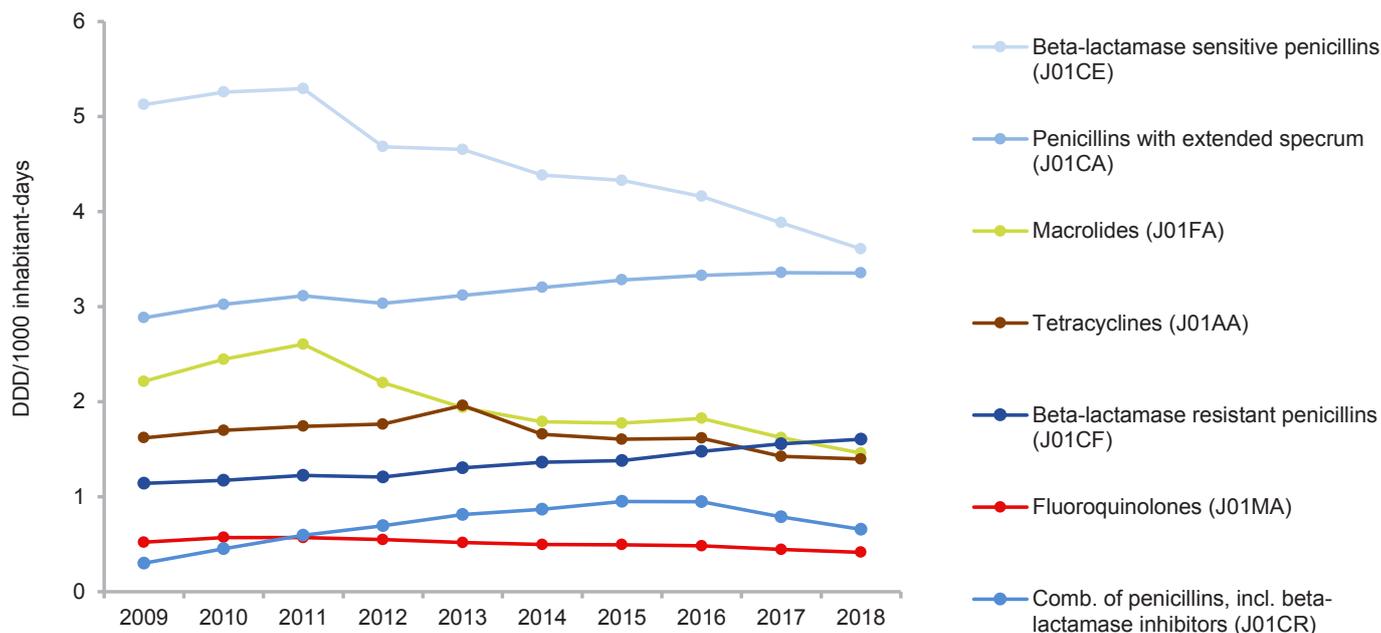
Fluoroquinolones represent the smallest drug class among the leading antimicrobials, for the last decade accounting for a stable 3% of the total consumption, when measured in DID, (Figure 5.2). In Denmark, fluoroquinolones are to be solely used for treatment of very few specific infections, where they are considered the drug of choice (e.g. exacerbation in a patient with chronic obstructive lung disease and known penicillin allergy). They are also recommended in the case of infection with multidrug-resistant bacteria, where microbiological results point towards a fluoroquinolone to be the best or only choice.

For all three drug classes (beta-lactamase sensitive penicillins, macrolides and fluoroquinolones) the decreases in consumption are paralleled by a corresponding decline in the number of treated patients and redeemed antimicrobial prescriptions, (Table 5.3 and 5.4). It also coincides with different initiatives on a more rational use of antibiotics in Denmark. Examples of these are the establishment of the National Antibiotic Council, 2012, recommendations regarding the use of antibiotics issued through the Danish Health Authority, also 2012, and 'happy audit' and other initiatives on better diagnostics undertaken by general practitioners in recent years. In addition, since 2012 antibiotic campaigns aimed at the public to create more knowledge and awareness regarding AMR were launched annually by the Ministry of Health.

The only antibiotics for which consumption showed continuing increasing trends during the decade (2009 to 2018), were the penicillins with extended spectrum and the beta-lactamase resistant penicillins. These increased from 2.88 DID to 3.35 DID (16%) and from 1.14 DID to 1.60 DID (33%), respectively.

Figure 5.3 Consumption of leading antimicrobial groups for systemic use in primary health care, DID, Denmark

DANMAP 2018



Data used for this figure is based on total sales in Denmark (individuals and clinics)
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Noteworthy for 2017 and 2018 were decreases in consumption for combination penicillins and tetracyclines. Combination penicillins had been steadily increasing since their introduction to the Danish market in 2003, but decreased from 0.95 DID in 2015 and 2016 to 0.66 DID in 2018 (-31%). Tetracyclines presented with increases until the peak of 1.96 DID in 2013 but then levelled off and decreased from 1.62 DID in 2016 to 1.40 DID in 2018 (-14%), (Table 5.1).

Penicillins

In Denmark, penicillins are the only beta-lactams used in primary health care; other beta-lactams such as cephalosporins, monobactams and carbapenems are solely used in hospital care and primarily at somatic hospitals with surgical or acute care functions.

In 2018, the four groups of penicillins accounted for 9.22 DID, 66% of all antimicrobials consumed; a decade ago in 2009, they accounted for altogether 9.45 DID, 60% of the total antimicrobials consumed that year. However, due to shifting trends in the usage of the different penicillin drug classes, the consumption of beta-lactamase sensitive penicillins in 2009 constituted 54% of all penicillins, while in 2018 they only constituted 39%, (not shown).

The increases described for the penicillins with extended spectrum are primarily due to increases in the consumption of pivmecillinam, in 2018 accounting for about 75% of this drug class, (Figure 5.4a). While pivmecillinam increased with 36% from 1.57 DID in 2009 to 2.47 DID in 2018, pivampicillin

decreased simultaneously with 74% from 0.44 DID to 0.16 DID and amoxicillin with 26% from 0.85 DID to 0.67 DID. From 2017 to 2018, pivampicillin continued to decrease (-15%), while trends for pivmecillinam and amoxicillin reverted for the first time, pivmecillinam decreasing with 0.7% and amoxicillin increasing with 5.9%, respectively.

The increased consumption of beta-lactamase resistant penicillins (dicloxacillin and flucloxacillin) was paralleled by an increased use at hospitals as well and followed the increased occurrence of staphylococcal infections observed in recent years (see section 8.1.3 and 8.3.8).

The new WHO DDD values from January 2019 apply to amoxicillin and amoxicillin with clavulanic acid, which changed the records of these notably and their share of the total consumption, when calculated in DID. Since there were no changes in DDD values for the other main penicillins, comparison of their use with other drug classes remained complicated, not reflecting the actual use of these. For comparison across drug classes, we therefore developed Danish adjusted DDD (DaDDD) for all main penicillins, (Figure 5.4b). The development was based on dosage recommendations from Danish treatment guidelines. These were then corrected for through comparison with the average doses actually given per treated patient, an information that was available through the elaborate data reported from the pharmacies each year. It is advisable to continue discussing the necessity of changes of the DDD also for these groups, for a more homonymous reporting of antimicrobial consumption in the future. Consumption measured in DID

Figure 5.4a Consumption of leading penicillins in the primary health care, DID, Denmark DANMAP 2018

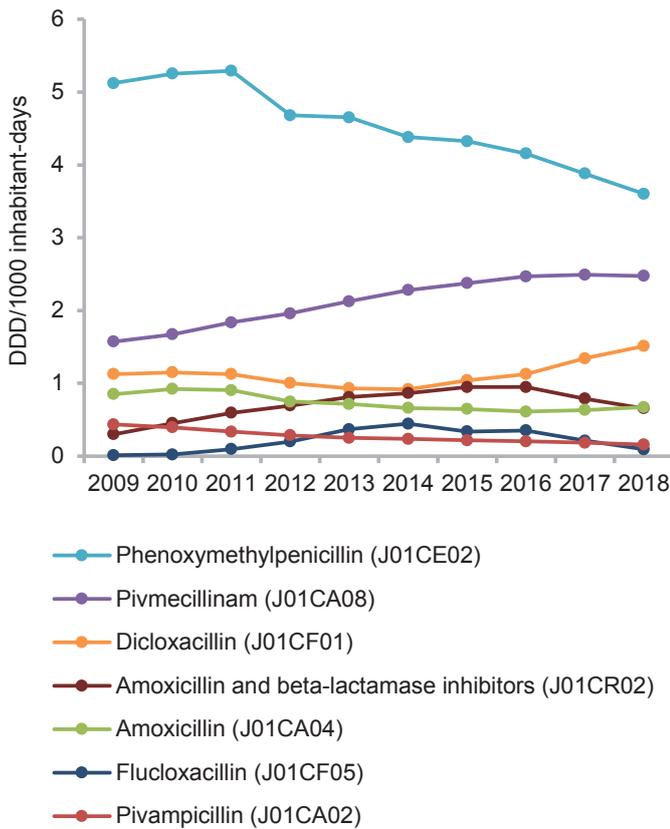
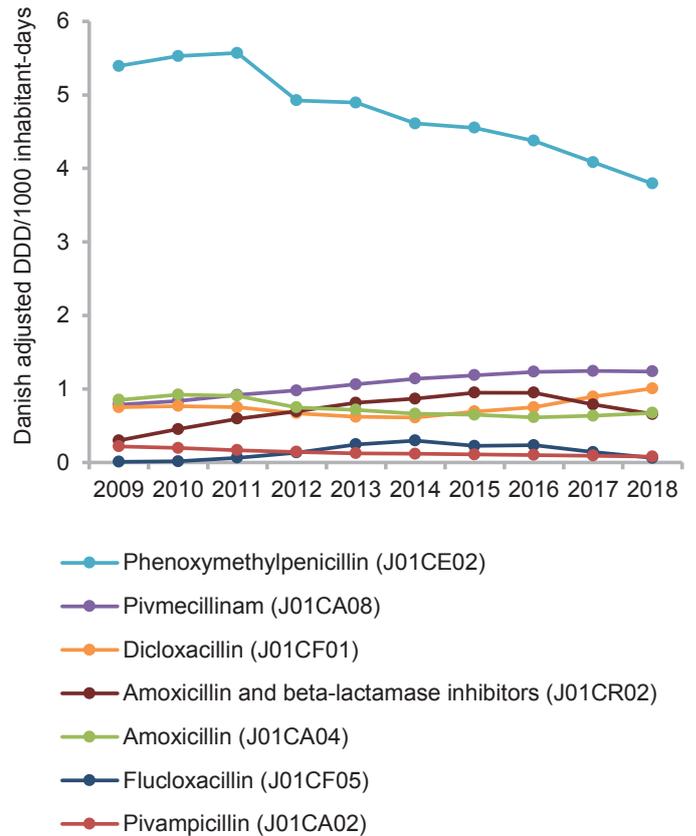


Figure 5.4b Consumption of leading penicillins in the primary sector, Danish adjusted DaDDD, Denmark DANMAP 2018



Data used for this figure is based on total sales in Denmark (individuals and clinics) ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

based on standard WHO DDD and DaDDD is presented in Figure 5.4a and b, respectively, for a visual comparison.

5.3.3 Consumption by age group

Initiatives aiming at reducing overuse and misuse of antibiotics often focus on consumption in the youngest and the elderly, since these are prone to infections due to either an immature immune system or aging. But inappropriate use is not restricted to overtreating children with fever conditions or elderly with unspecific urinary symptoms, for which there is the tendency to misinterpret the situation as being a bacterial infection needing treatment.

Figure 5.5 presents consumption in the different age groups based on different denominators: Figure 5.5a presents consumption in DID, Figure 5.5b in crude DDD, i.e. not corrected for population size, presenting the actual amount of antimicrobials consumed per age group. Figure 5.5c presents the number of patients treated and 5.5d the actual population sizes. All figures show data from 2009 to 2018. For children, WHO DDD values were used, although dosages given to children are based on bodyweight and therefore not directly comparable to adults. Children and adolescents are also presented in age groups of five years, while all others are clustered in 10-years groups.

5.3.4 Consumption of antimicrobials in children

The total consumption in children of all age groups continued the decreases observed for the last decade, regardless of the indicator used: In 2018, altogether 24.8 DID were consumed by children and young from 0 to 19 years. This corresponds to an average per age group of 6.2 DID, 187 treated patients per 1000 inhabitants (children) and 295 prescriptions redeemed per 1000 inhabitants (not shown). A decade ago, the corresponding numbers were: a total of 33.1 DID for all four age groups combined and corresponding averages of 8.3 DID, 271 treated patients and 462 prescriptions redeemed per 1000 children, (Figure 5.5a and 5.6).

As mentioned, measuring the consumption in children in defined daily doses is problematic, since the system of defined daily doses was developed based on the "maintenance dose per day for its main indication in adults" (https://www.whocc.no/ddd/definition_and_general_considera/). For children, different pharmacodynamics and -kinetics apply and especially dosing in the younger classes is based on doses per bodyweight in kg. Still, assuming that dosage regimens did not vary considerably within the last decade, it is possible to compare the consumption in each age group with itself over time. Thus, the consumption of DID in the different age groups show a clear tendency to reductions for especially penicillins and

macrolides, which decreased from an average of 2.9 DID and 1.3 DID in 2009 to 2.1 DID and 0.6 DID in 2018, respectively (data not shown). This trend can also be observed in the reduced number of treated patients, as presented for the main antimicrobials used in Figure 5.6.

From 2009 to 2018, the number of prescriptions redeemed for all young age groups (0-19 year olds) decreased from 462 to 295 prescriptions per 1000 inhabitants (-36%) and from 271 to 187 treated patients per 1000 inhabitants (-31%) for the decade, respectively. Differences in reduction varied

Figure 5.5a Consumption of all systemic antimicrobial agents in the primary sector in different age groups, DID, Denmark
DANMAP 2018

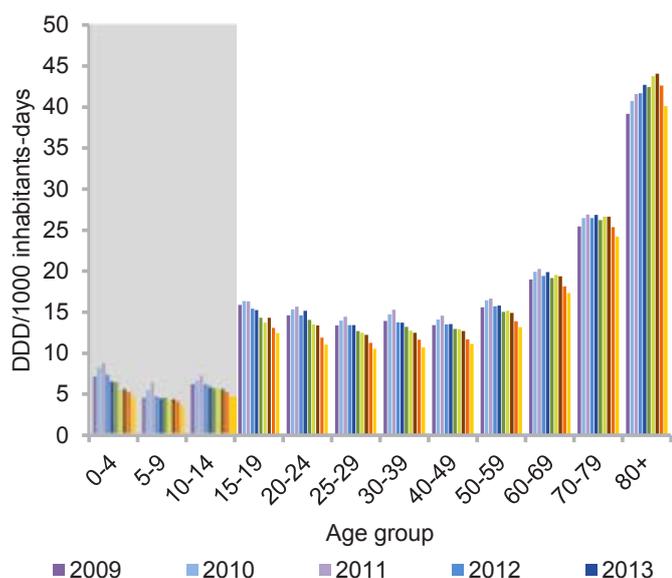


Figure 5.5b Consumption of all systemic antimicrobial agents in the primary sector in different age groups, DDD, Denmark
DANMAP 2018

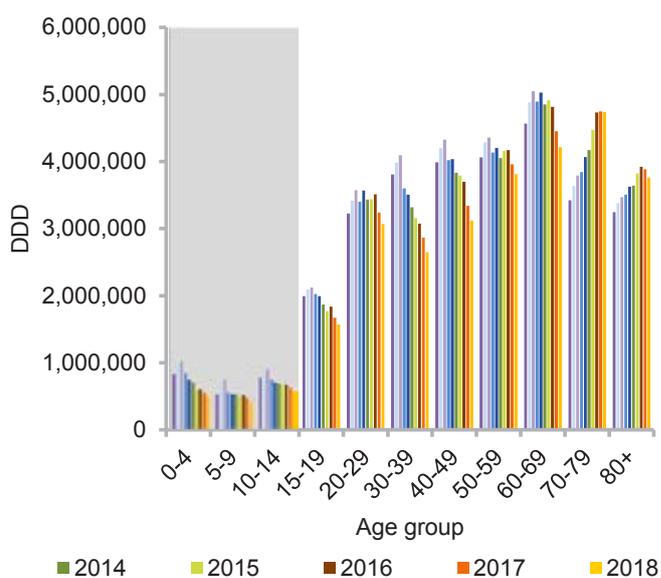


Figure 5.5c Treated patients per 1000 inhabitants in the primary sector in different age groups, Denmark
DANMAP 2018

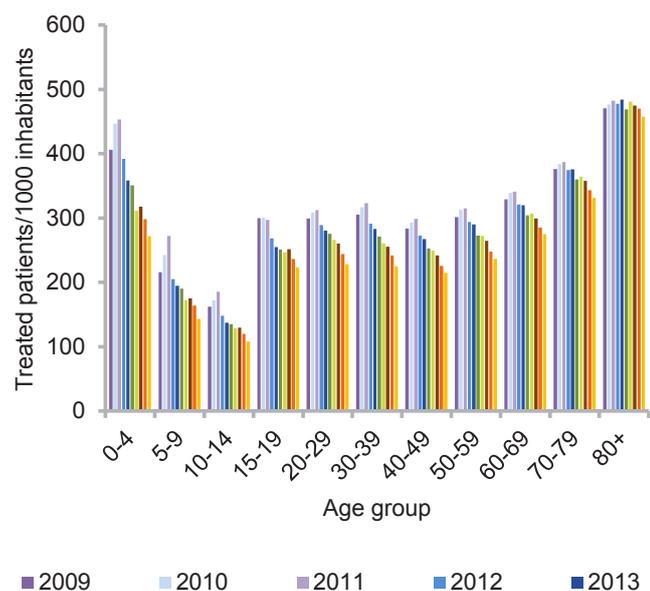
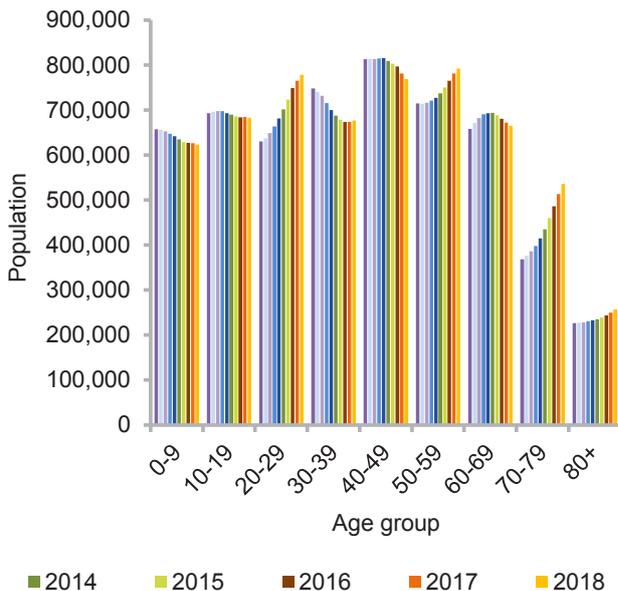


Figure 5.5d Population in different age groups, Denmark
DANMAP 2018



Data used in figure 5.5a-5.5c is based on registered sales to individuals
ATC numbers used in figure 5.5a-b stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Population size in Figure 5.d is based on data from Statistics Denmark at www.dst.dk

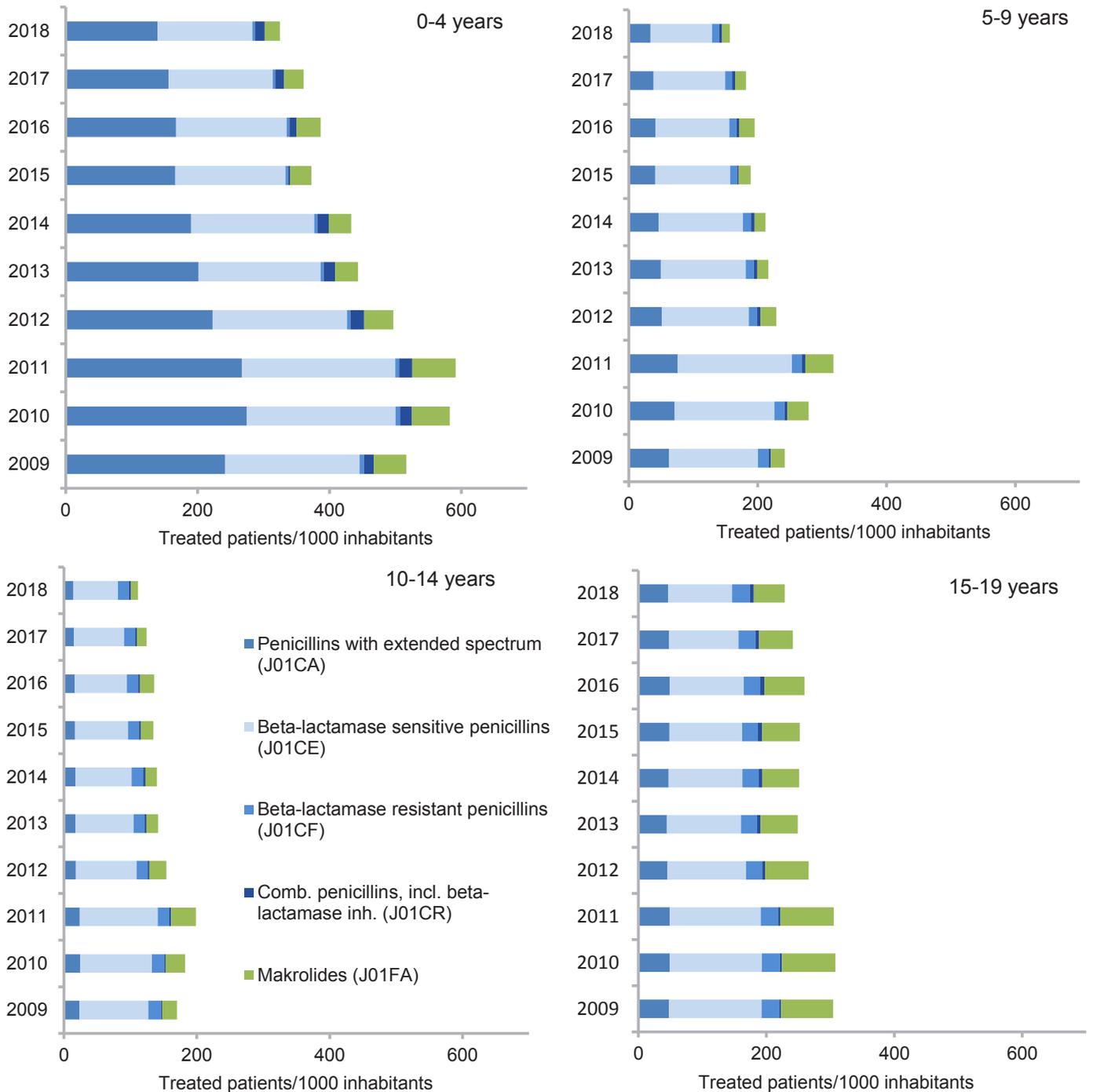
from a decrease of 41% in the number of prescriptions for the youngest (0-4 years old) to -30% for the oldest, (15-19 year olds). When measured in the number of treated patients, the decreases varied from -34% in the 5 to 9 years old to -26% in the adolescents (not shown).

In the youngest age group of 0 to 4 year olds, the boys

received on average 10% more prescriptions than the girls - a trend that has been quite stabile. Thus in 2009, they received 813 versus 711 prescriptions per 1000 inhabitants (0-4 years old) and in 2018, 479 versus 420 prescriptions per 1000 inhabitants, respectively (not shown). For the 5 to 19 year olds, opposite trends with girls receiving approximately 30% more prescriptions on average were observed (not shown).

Figure 5.6 Consumption of five antimicrobial agents children/adolecents age 0-19, DID, Denmark

DANMAP 2018



Data used in this figure is based on registered sales to individuals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

As for the general population, penicillins are the main antibiotics used in the treatment of bacterial infections in children. Beta-lactamase sensitive penicillins account for 30% to almost 50% of the consumption (depending on the indication) and the share of beta-lactamase sensitive penicillins compared to the other main antimicrobial classes used in children has increased in recent years. Thus, when measuring the number of treated patients per 1000 inhabitants from 2009 to 2018, the beta-lactamase sensitive penicillins decreased by 30 - 35% for all age groups (0 - 19 years old), while the beta-lactamase resistant penicillins, the penicillins with increased spectrum (primarily amoxicillin) and the macrolides decreased with 40 - 50%. Only for combination penicillins the number of treated patients increased over the decade, from 1% in the youngest to 139% in the adolescents.

Macrolides play an important role in the treatment of infections in children and the young. They are the drug of choice for respiratory tract infections with *Mycoplasma pneumoniae* and in pertussis, and in young school-aged children the consumption of macrolides will often mirror *Mycoplasma* epidemics, while consumption of macrolides in the youngest may to a certain extent mirror an epidemic with pertussis (but less pronounced due to generally fewer cases than with *Mycoplasma*). No epidemic occurred in the winter of 2016-2017 or 2017-2018. Macrolides are also used in the adolescents for the treatment of sexually acquired infections, e.g. *Chlamydia*. This (and acute pharyngitis) is probably the reason for the relatively high consumption of macrolides in the 15-19 year olds;

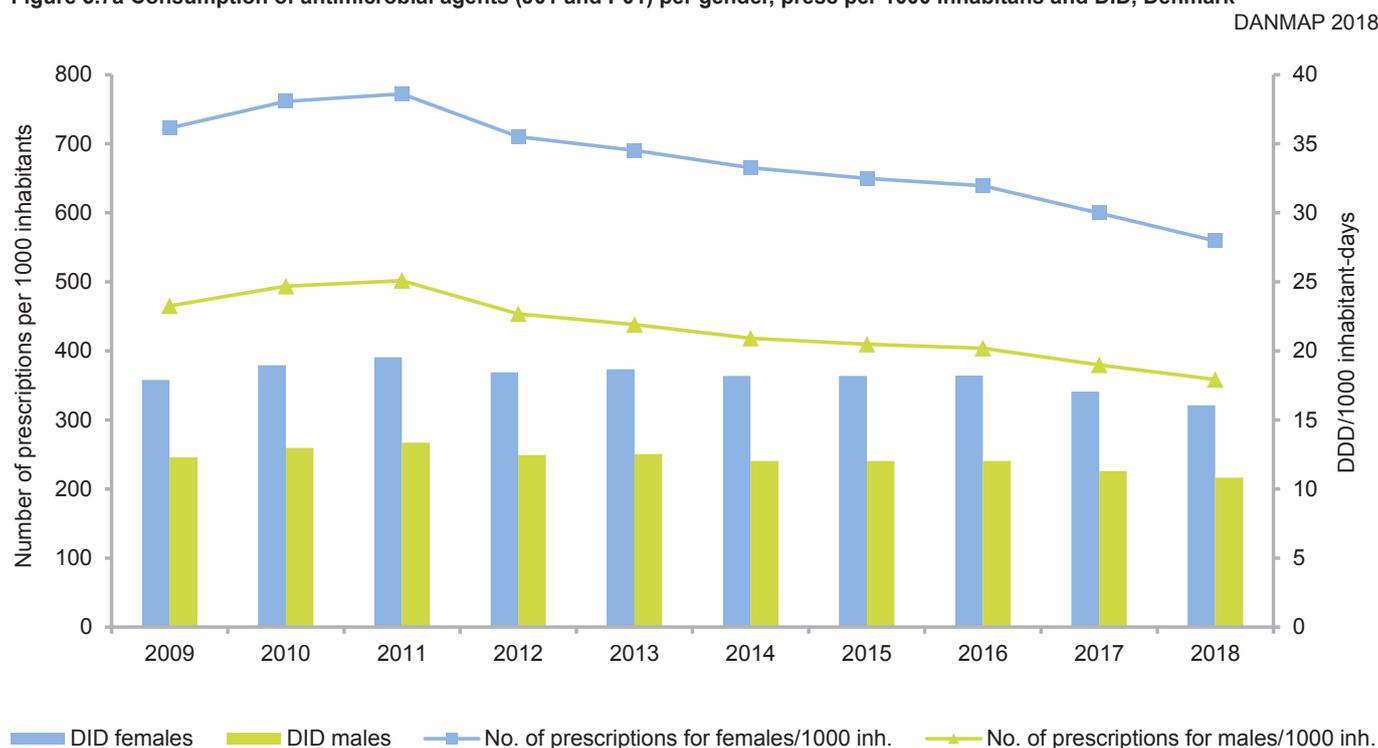
49 treated patients per 1000 inhabitants per year compared to 11 treated patients per 1000 for the 10-14 year olds, 12 treated patients per 1000 for the 5-9 year olds and 23 treated patients per 1000 for the 0-4 year olds for 2018, (Figure 5.6).

5.3.5 Consumption of antimicrobials according to gender

Differences between the genders regarding consumption of antimicrobials are well known, (Figure 5.7a). In general, women receive more treatment - a trend driven by a much higher incidence of urinary tract infections. Thus, the consumption of sulphonamides, trimethoprim and nitrofurantoin is three times higher for women than for men. Moreover, the consumption of pivmecillinam in women doubles the consumption in men. Also for beta-lactamase sensitive penicillins and macrolides the differences in consumption, especially when measured in DID, are substantial, (Figure 5.7b). For tetracyclines, there are less significant differences in gender and for the consumption of fluoroquinolones, no differences have been observed over the years, (not shown).

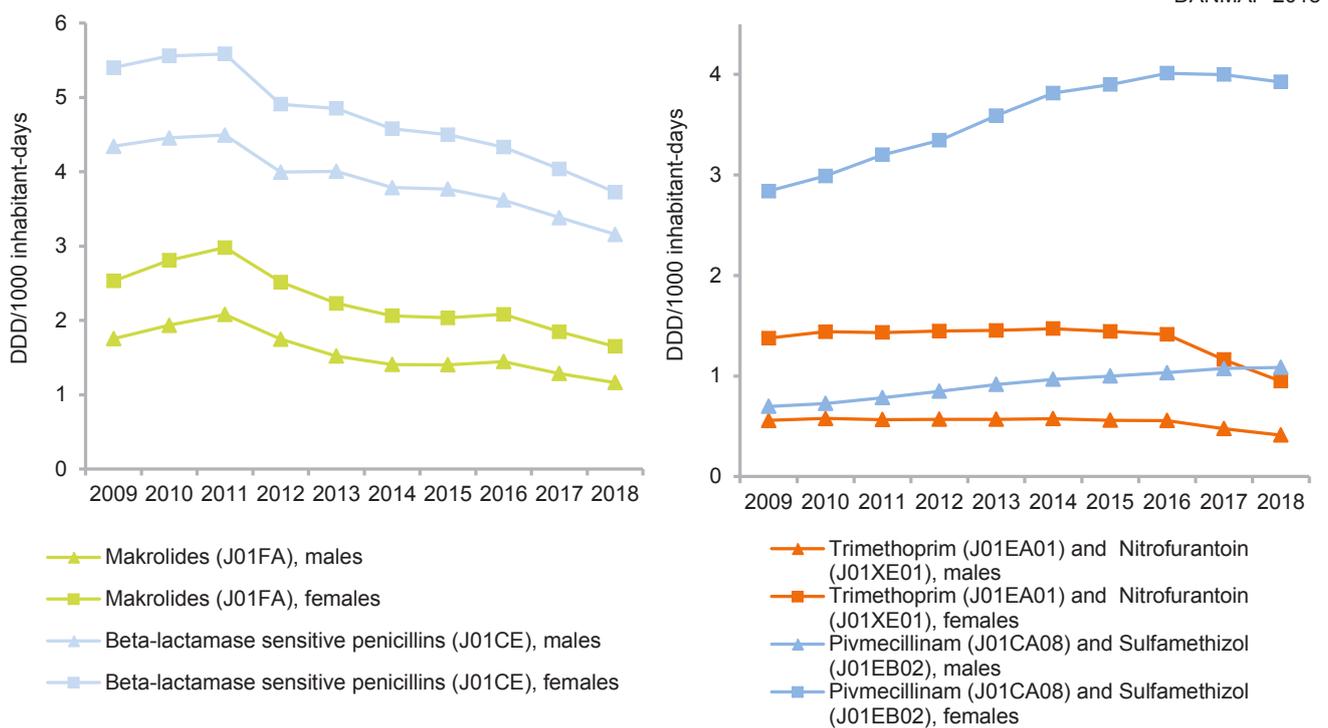
From 2009 to 2018, the number of treated women per 1000 inhabitants (all age groups) decreased from 356 to 287 (-19%) and the number of treated men per 1000 inhabitants from 256 to 198 (-23%). During the same period, the amount of DDD/prescription increased for women from 9.0 to 10.5 (17%), and for men from 9.6 to 11.0 (15%). Altogether the consumption in women decreased from 17.9 DID to 16.0 (-11%), and in men from 12.3 DID to 10.8 (-12%).

Figure 5.7a Consumption of antimicrobial agents (J01 and P01) per gender, presc per 1000 inhabitants and DID, Denmark



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.7b Consumption of most used antimicrobials against respiratory infections and urinary tract infections, Denmark



Data used in this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Drugs for the treatment of upper respiratory tract infections. For both women and men a decrease in the consumption of beta-lactamase sensitive penicillins and macrolides was observed. For women, the consumption decreased for the beta-lactamase sensitive penicillins from 5.4 DID in 2009 to 3.7 DID in 2018 and for the macrolides from 2.5 DID in 2009 to 1.6 DID in 2018. For men the changes were from 4.3 DID to 3.2 DID and from 1.8 DID to 1.2 DID, respectively, for the same decade, (Figure 5.7b).

Urinary drugs. From 2009 to 2017 the consumption of pivmecillinam in women increased continuously, from 2.4 DID to 3.8 DID and in men from 0.6 DID to 1.0 DID, while it remained almost unchanged in 2018 with 3.7 DID and 1.0 DID, respectively. In 2016, the national antibiotic campaign focused on reducing the amount of antimicrobials consumed in the treatment of UTIs in women using two different approaches: one broadcasting an educating movie on the social media targeted young women, the other directed at health personnel at nursing homes dealing with confused or dement elderly women with unspecific signs of UTI. In the beginning of 2017, recommendations regarding the use of nitrofurantoin were issued, advocating for caution in the use in elderly. This probably is the reason for the notable changes in the consumption of nitrofurantoin, from a stable 0.7 DID in women from 2009 to 2016 to 0.4 DID in 2017 and 0.2 DID in 2018. In men, the consumption increased from 0.2 DID in 2009 to 0.4 DID in 2017

and decreased to 0.1 DID in 2018. No significant changes were observed for pivmecillinam, sulfamethizol or trimethoprim for 2018, but the consumption seems to be levelling off since 2016. In Figure 5.7b the consumption of antimicrobial urinary drugs is grouped into pivmecillinam and sulfamethizol (against acute infections) and trimethoprim and nitrofurantoin (more often used in the prevention of UTI in elderly or in recurring infections).

5.3.6 Tetracyclines

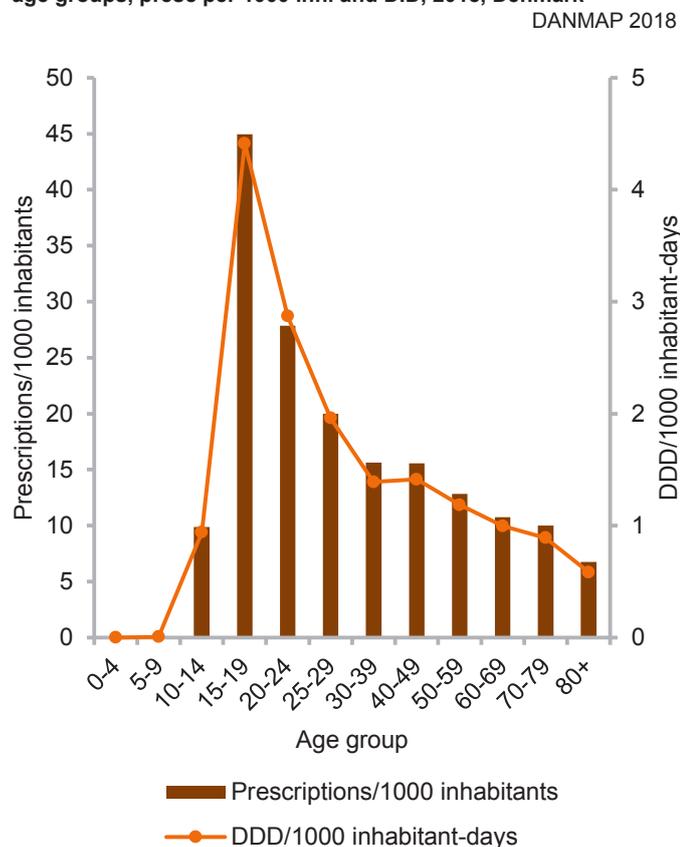
Tetracyclines are the fourth biggest group of antimicrobials consumed in Denmark. In 2018, they accounted for 1.40 DID, corresponding to 10% of the total consumption in primary health care. During the last decade, the consumption has decreased by 12.5% from 1.6 DID in 2009. In 2013, the consumption peaked unexpectedly at 1.96 DID but has since shown continuing decreases. Tetracyclines are used by all age groups above 12 years and by both genders, (Figure 5.8).

Tetracyclines account for a considerable part of the consumption of antimicrobials among adolescents due to the treatment of acne, (Table 5.2 on the distribution of indications among treated patients for 2016-2018). Treatment against acne lasts long (up to six months) and may even be repeated in a situation of relapse in patients, who may be suffering from the condition for years. Furthermore, within the same family/at the same family doctor, there may be the tendency to treat younger siblings if a

treatment course in an elder brother or sister has been of success. Both genders are affected, but there exist clear differences in prescription habits regarding boys and girls. Thus, among girls the treatment periods are longer and extend into the young adults of 20 to 24 years, while boys primarily are treated in shorter periods at the age of 15 to 19 years. In 2009, 15 to 19 year old boys received 76 prescriptions per 1000 inhabitants per year on average, whereas girls received 56, corresponding to 35 versus 30 patients treated per 1000 inhabitants. In 2018, the number of 15-19 year old boys receiving treatment had declined to 25 treated patients (corresponding to 42 prescriptions) per 1000 inhabitants, while the number of 15-19 year old girls remained unchanged with 30 treated patients (but corresponding to 48 prescriptions redeemed) per 1000 inhabitants. Thus in 2018, girls received 1.6 prescriptions per patient on average while it was 1.9 in 2009.

While the number of DIDs consumed did not change much in women (primarily used by young women from 15 to 24 years) from 1.7 DID in 2009 to 1.6 DID in 2018, it decreased more in men (primarily used by boys from 15 to 19 years) - from 1.5 DID in 2009 to 1.1 DID in 2018 (not shown). Increases in the occurrence of sexually transmitted infections and changes in the treatment recommendations for these may be challenges in the future, see chapter on the occurrence of *N. gonorrhoea* in Denmark 8.3.9.

Figure 5.8 Consumption of tetracyclines in different age groups, presc per 1000 inh. and DID, 2018, Denmark



Data used for this figure is based on registered sales to individuals ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.2 Percentage of total consumption of tetracyclines

Indication given on the prescription	DANMAP 2018		
	Year		
	2016	2017	2018
Against acne	44.9	50.7	54.9
Prevention of malaria	9.5	8.1	6.9
Against borrelia infection	2.0	2.6	4.1
Against pelvic inflammatory disease	1.3	1.8	1.9
Against skin and soft tissue infection	1.1	1.2	1.5
Unspecified indications	41.2	35.6	30.6

Data used for this table is based on registered sales to individuals ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.3.7 Measures at user level

In this and the following sections, the consumption of antibiotics is described at user level in either the number of prescriptions per 1000 inhabitants or the number of treated patients per 1000 inhabitants. The measures are thus based on all information available through the sales to individuals and do not include the approximately 4% of antibiotics, mainly penicillins, sold to clinics, dentists and doctors on call.

In 2018, the total number of prescriptions was 459 per 1000 inhabitants, a 6.3% reduction from the 490 prescriptions per 1000 inhabitants in 2017 and a 23% reduction compared to the 595 prescriptions per 1000 inhabitants in 2009 (Table 5.3). Decreases were observed for all antimicrobial drug classes apart from the penicillins with extended spectrum, which remained unchanged. In 2018, the average number of prescriptions redeemed per patient was 1.89. In 2009, the number was 1.94 (not shown). The number of treated patients in 2018 was 243 per 1000 inhabitants, a decrease of 21% compared to the 306 treated patients per 1000 inhabitants in 2009, (Table 5.4).

Trends in the number of prescriptions and treated patients for the different antimicrobial classes followed mainly the trends already described for the consumed DIDs. Most pronounced increases over the last decade in the number of prescriptions per 1000 inhabitants were seen for combination of penicillins, including betalactamase inhibitors (113%). Most pronounced decreases for the ten year period were in the number of prescriptions per 1000 inhabitants for the following: macrolides (-40%), beta-lactamase sensitive penicillins (-34%), sulphonamides (-33%), tetracyclines (-32%) and fluoroquinolones (-26%), (Table 5.3).

Similar decreases were noted for the decade, when measured in the number of patients treated: macrolides (-38%), beta-lactamase sensitive penicillins (-30%), sulphonamides (-38%), tetracyclines (-26%) and fluoroquinolones (-27%), (Table 5.4).

A comparison of the different indicators of consumption is presented in Figure 5.9. In 2018, the average DDD/prescription remained with 10.7 at the same level as in 2017, an increase of 15% compared to the 9.3 DDD/prescription in 2009.

Table 5.3 Number of prescriptions per 1000 inhabitants for leading antimicrobial agents in primary healthcare, Denmark

DANMAP 2018

ATC group	Therapeutic group	Year									
		2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	21.62	22.49	22.70	22.56	22.89	20.00	17.90	17.18	15.89	14.63
J01CA	Penicillins with extended spectrum	119.28	127.23	125.17	115.91	114.30	113.83	113.53	113.16	114.37	114.31
J01CE	Beta-lactamase sensitive penicillins	205.85	212.19	213.32	186.91	180.55	170.70	163.09	157.13	148.52	136.81
J01CF	Beta-lactamase resistant penicillins	42.10	42.32	42.75	40.42	41.25	41.04	40.81	41.87	41.87	43.35
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	11.15	16.53	21.11	24.71	28.01	29.02	30.73	31.13	27.09	23.71
J01E	Sulphonamides and trimethoprim	47.17	47.35	45.05	43.86	43.53	41.51	38.39	36.41	34.29	31.74
J01FA	Macrolides	87.24	97.34	104.22	85.89	74.51	68.01	68.00	68.85	60.00	52.64
J01MA	Fluoroquinolones	21.71	23.69	23.15	22.14	20.65	19.67	19.50	18.74	17.37	15.97
J01X	Other antibacterials (methenamine >99%)	17.93	17.49	18.24	18.03	17.41	16.73	16.28	15.82	10.18	6.76
P01AB01	Nitroimidazole derivatives (metronidazole)	19.02	19.67	19.69	19.68	19.26	19.06	19.15	18.63	17.26	16.31
J01 and P01AB01	Antibacterial agents for systemic use (total)	595.28	628.78	638.08	582.80	565.26	542.53	530.56	522.19	490.08	459.37

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Data used in this table is based on registered sales to individuals

Table 5.4 Number of treated patients per 1000 inhabitants for leading antimicrobial agents in primary healthcare, Denmark

DANMAP 2018

ATC group	Therapeutic group	Year									
		2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	13.02	13.44	13.66	13.53	13.86	12.20	11.32	11.04	10.35	9.69
J01CA	Penicillins with extended spectrum	81.07	85.04	84.19	77.31	76.10	75.32	74.87	74.05	74.04	73.56
J01CE	Beta-lactamase sensitive penicillins	158.72	162.81	164.34	145.53	142.19	134.79	130.06	125.69	119.32	110.89
J01CF	Beta-lactamase resistant penicillins	29.87	30.02	30.34	28.51	29.07	29.24	28.85	29.70	29.96	31.09
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	8.02	11.70	14.95	17.32	19.71	20.52	22.03	22.17	19.89	17.73
J01E	Sulphonamides and trimethoprim	29.51	29.31	27.63	26.48	26.16	24.65	22.45	21.17	19.87	18.42
J01FA	Macrolides	64.44	72.67	78.75	64.73	56.16	51.38	51.75	53.21	46.01	40.11
J01MA	Fluoroquinolones	16.87	18.45	18.10	17.25	16.04	15.30	15.04	14.37	13.36	12.26
J01X	Other antibacterials (methenamine >99%)	7.67	7.53	7.74	7.54	7.48	7.16	7.35	7.47	5.01	3.62
P01AB01	Nitroimidazole derivatives (metronidazole)	16.28	16.73	16.90	16.86	16.51	16.31	16.47	16.03	14.84	14.05
J01 and P01AB01	Antibacterial agents for systemic use (total)	306.41	318.69	324.91	296.40	289.54	278.62	273.49	269.72	255.72	242.55

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Data used in this table is based on registered sales to individuals

5.3.8 Prescribing activity in primary healthcare

Although Denmark has a very homogenous population with relatively small geographic and socioeconomic variations, considerable differences in the prescribing habits among medical doctors are frequently observed. In 2018, the Central Denmark Region had the lowest prescribing activity when compared to the other four regions, with 12.7 DID and 431 prescriptions per 1000 inhabitants, respectively (Table 5.5). The Region of Zealand had the highest prescribing activity with 14.7 DID and 501 prescriptions per 1000 inhabitants. For all regions, significant decreases in the DIDs and number of prescriptions redeemed were observed for the five years presented (on average 10% in DID and 15% in the number of prescriptions per 1000 inhabitants).

There may be several reasons to the differences in the number of prescriptions redeemed, fx variations in the density of the

population and number of general practitioners as well as the proportion of elderly or chronically ill in a given geographic area. Due to differing organisation of general practitioners and clinical practices across the country, comparison of prescribing habits based on the individual clinical praxis is difficult. A clinical praxis can be based on a single physician working solo but can also be a collaboration of up to seven physicians sharing facilities and staff. In addition, due to the lack of general practitioners in some areas, several new models of "health houses" served by physicians and other health staff are being established these years. General practitioners can follow their own prescription habits through the website www.ordiprax.dk, a closed IT system that collects all data on prescriptions and enables comparison with other praxis' on a regional level.

Support of the general practitioners regarding their prescribing habits is in general provided through regional medicine

consultants, who also have access to Ordiprax on clinic level, thus being able to monitor consumption and give individual advice. From 2018, the general practitioners in defined geographical areas have been joined in “quality clusters” for mutual support.

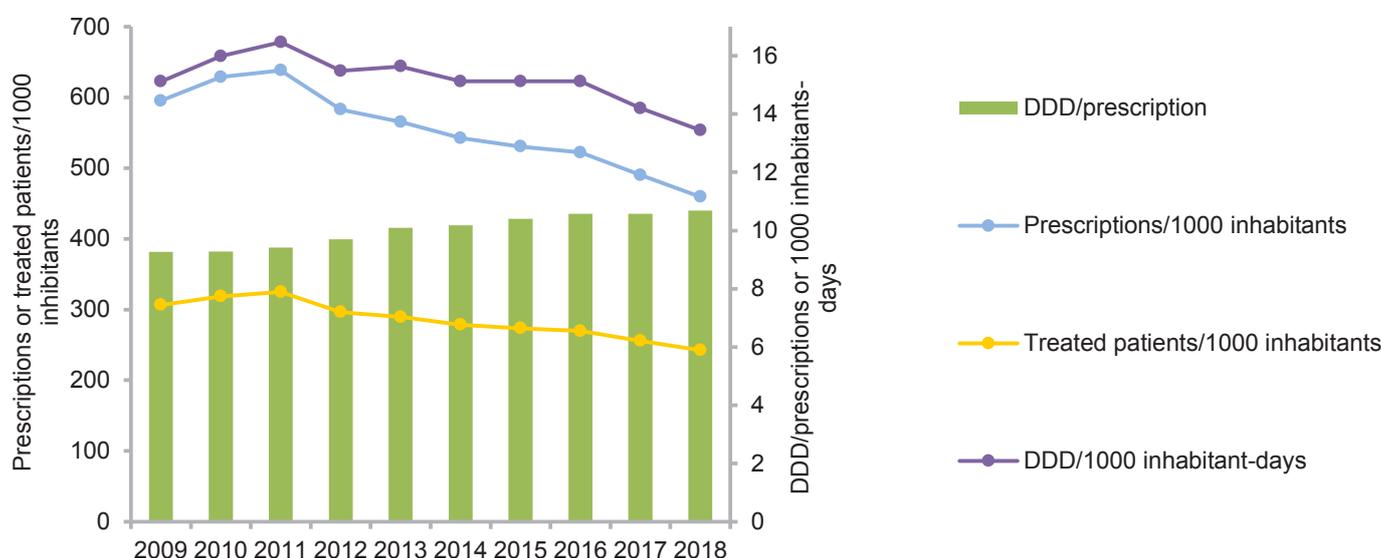
In Figure 5.10, the number of prescriptions on municipality level is shown, spanning from 368 to 614 prescriptions per 1000 inhabitants. In 2018, most municipalities lay within the range of 450 to 550 prescriptions per 1000 inhabitants. From the 98 municipalities in Denmark, four were excluded from the figure due to very small populations (typically islands).

As would be expected, prescribing habits of doctors with different specialties differ, fx are 60% of antimicrobial prescriptions from specialists in dermato-venerology for tetracyclines,

which is used to treat severe acne, (Figure 5.11). Out of all prescriptions issued by dentists, 59% were for beta-lactamase sensitive penicillins, (see textbox 5.2). An overview of the numbers of prescriptions issued by the different specialties can be found in Table 5.6.

As mentioned in the introduction, consumption in the primary sector includes prescriptions issued from hospital doctors upon discharge of a patient. In the past decade, the number of prescriptions issued through hospital doctors increased notably, probably due to changes in hospital work flow with shortening of bed days and increasing activity in ambulatory care. In 2018, hospital doctors accounted for 62.8 prescriptions per 1000 inhabitants (14% of the antimicrobials sold at pharmacies), (Table 5.6). In 2008, it was 38 prescriptions per 1000 inhabitants (corresponding to 6% of sales), (not shown).

Figure 5.9 Different indicators of antimicrobial consumption (J01, P01AB1) in primary health care, Denmark DANMAP 2018



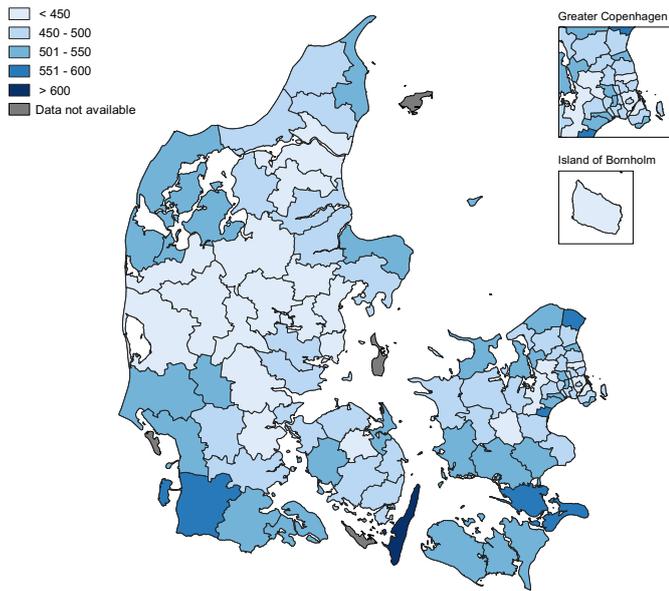
Data used for this figure is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.5 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark DANMAP 2018

Region	Indicator	Year				
		2014	2015	2016	2017	2018
Capital Region	DDD/1000 inhabitants/day	15.53	15.38	15.24	14.35	13.48
	Prescriptions/1000 inhabitants	549.13	533.22	519.10	489.50	453.55
Region Zealand	DDD/1000 inhabitants/day	15.78	16.12	16.42	15.52	14.67
	Prescriptions/1000 inhabitants	579.57	575.14	574.86	539.03	501.39
Region of Southern Denmark	DDD/1000 inhabitants/day	15.04	14.98	14.97	14.08	13.39
	Prescriptions/1000 inhabitants	556.21	539.98	530.16	496.77	470.58
Central Denmark Region	DDD/1000 inhabitants/day	14.40	14.43	14.39	13.45	12.74
	Prescriptions/1000 inhabitants	499.76	494.17	487.24	458.32	430.88
North Denmark Region	DDD/1000 inhabitants/day	14.42	14.44	14.63	13.53	13.06
	Prescriptions/1000 inhabitants	525.20	510.13	509.05	472.16	451.62
Denmark (total)	DDD/1000 inhabitants/day	15.12	15.12	15.13	14.19	13.44
	Prescriptions/1000 inhabitants	542.53	530.56	522.19	490.08	459.37

Data used in this table is based on registered sales to individuals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.10 Number of prescriptions from primary healthcare /1000 inhabitants in Danish municipalities DANMAP 2018



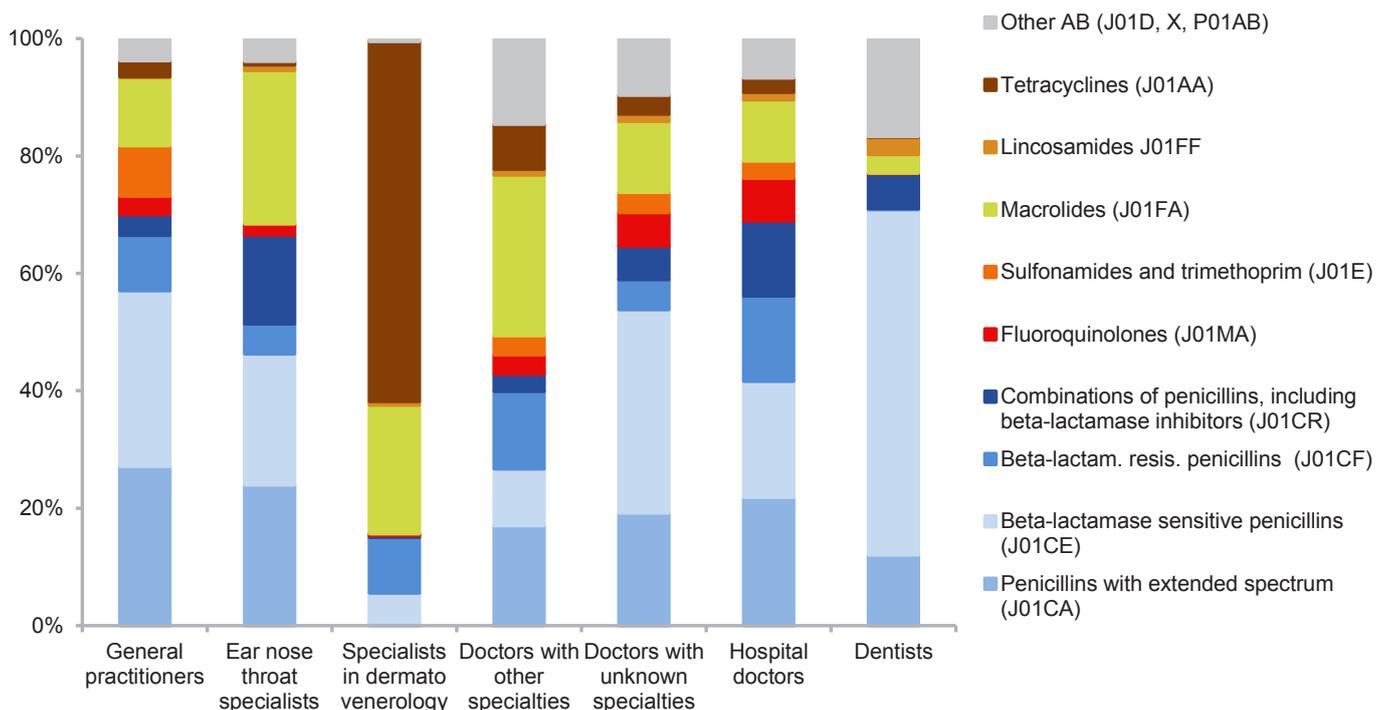
Data used for this figure is based on registered sales to individuals

Table 5.6 Number of prescriptions per 1000 inhabitants per prescribing specialty DANMAP 2018

Doctor type	Year		
	2016	2017	2018
General practitioners	390.7	368.6	341.5
Ear nose throat specialists	8.8	8.9	8.4
Specialists in dermato venerology	6.4	5.9	5.2
Doctors with other specialties	4.5	4.3	4.2
Doctors with unknown specialties	15.0	10.9	9.7
Hospital doctors	60.7	62.6	62.8
Dentists	36.5	29.1	27.8

Data used for this table is based on registered sales to individuals

Figure 5.11 Percentage of prescriptions issued through different medical specialties, primary sector, Denmark DANMAP 2018



Data used in this figure is based on registered sales to individuals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4 Hospital Care

5.4.1 Introduction

Antimicrobial consumption at hospitals is reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between the regions and the company Amgros. For more information see chapter 9.8, material and methods.

DANMAP 2018 covers the total sales on systemic antimicrobials (all ATC code J01 as well as ATC code P01AB01 and A07AA09) reported from all Danish hospitals. However, only Figure 5.1a on page 45 includes all consumption, (e.g. also including consumption at private hospitals and psychiatric departments), in 2018 accounting for approximately 2-3% of the total hospital consumption. In all other figures and calculations, only consumption at public hospitals with acute care functions is presented.

In DANMAP, data on hospital consumption is kept at a national or regional level. Data on hospital level can be supplied upon request.

Information on consumption at individual patient level is still lacking for the hospital sector. This information is expected to be available through the future national "Hospital Medicine Register", which is currently under development.

The consumption of antimicrobial agents in hospital care is presented as DDD per 100 occupied bed-days (DBD) and as DDD per 100 admissions (DAD) to account for hospital activity. Moreover, data are presented as DID to enable comparison with primary health care. The consumption is measured at a national and regional level.

As mentioned, during the past decade the hospitalisation patterns in Denmark changed notably. The shortening of bed days at hospitals and the increasing ambulatory care function, including increased surgical activity, causes increased pressure on the health system at municipality level, (Figure A5.2 and

A5.3 in web annex). Therefore demands arise for more rehabilitation beds for patients dismissed from hospital, but not yet ready for continuing treatment at home.

The increasing number of invasive infections and infections at other sites also induces pressure into the system, increasing the demand for antibiotic treatment (see section 8.1 introduction and Figure 8.3). Since selection pressure for the emergence of antimicrobial resistance follows with increasing hospital activity, the selection pressure has increased considerably from 2009 to 2018, (Figure A5.2 in web annex).

Table 5.7 presents data on regional and national hospital activity together with information on the size of population for 2009 and 2018. Denmark has a very high bed occupancy rate and overcrowding happens relatively often, especially during wintertime and in situations with influenza epidemics. In 2018, the number of admissions at somatic Danish hospitals was 1,354,248, while the number of bed-days was 3,976,635. Since 2009, the number of bed-days decreased with altogether 16%, while the number of admissions increased with 7.1% and the Danish population with 4.9%. During the decade, activity in ambulatory care increased from 6.454.112 patients treated to 7.984.223 patients treated, (24%). On average, the number of bed-days decreased with an annual 2.0%, while the number of admissions on average increased with 0.8% per year, (Figure A5.2 in web annex).

5.4.2 Somatic hospitals - DDD per 100 occupied bed days (DBD)

In 2018, the consumption of antimicrobial agents at somatic hospitals was 99.3 DBD, 3.5% higher than the observed 96.0 DBD in 2017 and 41% higher than the consumption measured a decade ago in 2009, (70.4 DBD). This is the highest consumption measured this decade, (Table 5.8).

The four penicillin groups accounted for altogether 52.4 DBD, corresponding to 53% of the total hospital consumption of antimicrobials (Figure 5.12, Table 5.8).

In 2018, combination penicillins accounted for 16.3 DBD, making it the largest group consumed in 2018 (16.4%). In 2017, a

Table 5.7 Activity at Danish hospitals

DANMAP 2018

Region	Number of bed-days in somatic hospitals		Number of admission to somatic hospitals		Population	
	2009	2018	2009	2018	2009	2018
Capital Region of Denmark	1,692,035	1,462,332	430,967	470,971	1,662,285	1,822,659
Zealand Region	680,125	592,449	189,736	232,554	821,252	835,024
Region of Southern Denmark	940,141	766,345	257,156	256,374	1,199,667	1,220,763
Central Denmark Region	944,486	786,970	267,367	283,670	1,247,732	1,313,596
North Denmark Region	493,817	368,539	118,969	110,679	580,515	589,148
Denmark	4,750,604	3,976,635	1,264,195	1,354,248	5,511,451	5,781,190

Data used in this table is based on the activity at acute care public somatic hospitals

Table 5.8 Consumption of antimicrobial agents for systemic use in somatic hospitals, DBD, Denmark

DANMAP 2018

ATC group	Therapeutic group	Year									
		2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	0.95	0.99	1.07	1.54	1.45	1.63	1.80	2.08	2.04	2.37
J01CA	Penicillins with extended spectrum	12.02	11.45	11.35	12.47	12.88	13.56	14.11	14.46	15.66	15.37
J01CE	Beta-lactamase sensitive penicillins	8.75	8.24	9.02	9.31	9.40	9.31	9.08	9.18	10.03	10.38
J01CF	Beta-lactamase resistant penicillins	6.69	6.88	7.69	7.96	8.69	8.96	9.22	8.75	8.81	10.39
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	3.83	4.83	6.48	9.30	10.92	12.76	14.40	15.00	13.48	16.26
J01DB	First-generation cephalosporins	0.12	0.12	0.12	0.12	0.11	0.06	0.04	0.04	0.04	0.04
J01DC	Second-generation cephalosporins	11.08	11.15	14.34	13.35	12.26	11.36	10.12	9.23	10.78	8.97
J01DD	Third-generation cephalosporins	1.17	1.01	1.07	1.03	1.08	1.00	1.04	1.03	1.33	1.20
J01DF	Monobactams	0.00	0.04	0.14	0.15	0.14	0.06	0.03	0.01	0.01	0.01
J01DH	Carbapenems	1.42	1.61	2.33	2.51	2.76	1.92	2.79	2.68	2.83	2.77
J01EA	Trimethoprim and derivatives	0.40	0.32	0.31	0.36	0.38	0.47	0.40	0.37	0.41	0.44
J01EB	Short-acting sulfonamides	0.36	0.28	0.21	0.18	0.16	0.14	0.12	0.10	0.10	0.10
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	2.22	1.86	2.91	3.26	4.28	4.68	5.06	5.20	5.40	5.80
J01FA	Macrolides	3.08	3.17	3.26	3.38	3.27	3.64	4.34	4.69	5.68	6.25
J01FF	Lincosamides	0.46	0.43	0.48	0.60	0.64	0.65	0.57	0.62	0.64	0.76
J01GB	Aminoglycosides	1.42	1.55	1.84	2.05	2.10	1.55	1.61	1.90	2.18	2.06
J01MA	Fluoroquinolones	8.40	8.24	8.39	8.37	8.60	8.51	8.09	7.26	6.98	6.81
J01XA	Glycopeptides	0.92	0.98	1.22	1.25	1.31	1.15	1.08	1.09	1.31	1.27
J01XB	Polymyxins	0.06	0.09	0.08	0.09	0.12	0.19	0.17	0.19	0.19	0.23
J01XC	Steroid antibacterials (fusidic acid)	0.29	0.32	0.25	0.23	0.22	0.23	0.16	0.11	0.07	0.06
J01XD	Imidazole derivatives	3.47	3.51	3.71	3.92	4.09	4.42	4.22	4.52	4.65	4.33
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.33	0.27	0.29	0.33	0.34	0.32	0.27	0.24	0.25	0.27
J01XX05	Methenamine	0.08	0.07	0.09	0.08	0.07	0.06	0.09	0.08	0.07	0.10
J01XX08	Linezolid	0.20	0.20	0.29	0.31	0.36	0.34	0.44	0.36	0.37	0.52
J01XX09	Daptomycin	0.02	0.02	0.01	0.02	0.02	0.03	0.04	0.05	0.08	0.14
P01AB01	Nitroimidazole derivatives (metronidazole)	2.43	2.39	2.34	2.29	2.25	1.98	2.01	2.18	2.03	1.94
A07AA09	Intestinal anti-infectives (vancomycin)	0.17	0.25	0.40	0.47	0.49	0.52	0.47	0.49	0.52	0.50
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	70.35	70.28	79.70	84.92	88.39	89.49	91.77	91.92	95.96	99.31

Data used in this table is based consumption at acute care public somatic hospitals

ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

shortage of piperacillin with tazobactam had been responsible for a drop in consumption that year. In 2018, delivery had been reestablished, resulting in an increase in the consumption of combination penicillins of altogether 7.7% since 2016.

Penicillins with extended spectrum are the second largest group consumed at Danish hospitals. In 2018, they accounted for 15.4 DBD (15.5%) of the consumption in somatic hospital, a 1.9% decrease from 2017 (15.7 DBD), the first decrease for this group since 2011. Beta-lactamase sensitive penicillins accounted for 10.38 DBD (10%) and beta-lactamase resistant penicillins for 10.39 DBD (11%).

Overall, the consumption of penicillins showed increasing trends for the decade. The combination penicillins increased steeply by 12.4 DBD (325%), the penicillins with extended spectrum and beta-lactamase resistant penicillins less markedly, but still continuously with 3.34 DBD (28%) and 3.70 DBD

(55%), respectively, (Figure 5.13a and 5.14). These trends are comparable to the trends observed for the primary sector, apart from changes for 2017 and 2018, where consumption in the primary sector decreased notably for the combination penicillins.

The consumption of beta-lactamase sensitive penicillins increased less continuously, presenting fluctuations with decreases for 2010 and 2014. In 2018, the consumption of beta-lactamase penicillins was 10.4 DBD.

Notable trends for other antimicrobials for 2009 to 2018 were increases observed for tetracyclines, for combinations of sulfonamides and trimethoprim and for macrolides. Although tetracyclines only account for a minor part of the antimicrobials consumed at hospitals, the drug class has been continuously increasing during the past decade; in 2009 they accounted for 0.95 DBD, while in 2018 the consumption had increased

to 2.37 DBD. Consumption of combinations of sulfonamides and trimethoprim, increased from 2.22 DBD in 2009 to 5.80 DBD in 2018, a total increase of 162% for the decade. A rise in macrolides was observed from 3.08 DBD in 2009 to 6.25 DBD in 2018 (103%), (Table 5.8, Figure 5.13a and 5.14).

Finally, for linezolid and daptomycin the consumption peaked in 2018 at 0.52 and 0.14 DBD, respectively, (Table 5.8). Although the consumption of both is only minor, these changes are noteworthy: for Linezolid due to the high risk of creating resistance, for daptomycin due to its use in the treatment of invasive vancomycin resistant enterococci (VRE), the number of which is still low but has increased dramatically (see chapter 8.2.5 on invasive enterococci and 8.3.3 on VRE). The consumption of linezolid increased with 157% since 2009 (0.76 DBD) and with 41% since 2017 (1.09 DBD). The Capital Region of Denmark accounted for 71% of the consumption of linezolid.

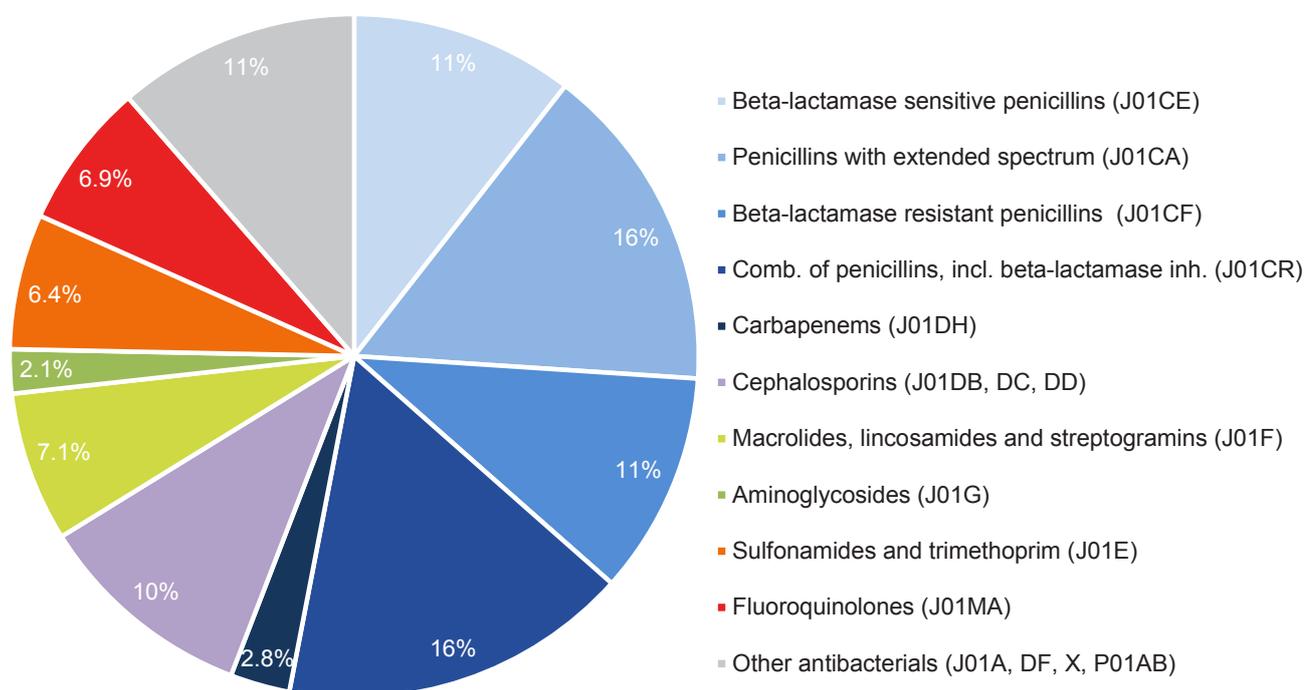
The consumption of Daptomycin increased from 0.06 DBD in 2009. Also for daptomycin the main use was in the Capital region of Denmark (89%), which coincides with the Capital region having the highest number of clinical cases of VRE in 2018, (section 8.3.3 and 8.3.4).

In 2018, the consumption of first line antimicrobials used in empirical treatment for main infections treated at hospitals continued its increases, a trend following the described increasing trends for the number of invasive isolates, (Figure 5.13a and Figure 8.1). In 2018, these leading antimicrobials constituted 70.1 DBD of the total consumption of 99.3 DBD (71%). In 2017, it was 69.0 DBD of a total of 96.0 DBD (72%).

Trends in the consumption at hospitals on a regional level, measured in DID and DBD, are presented in Figure 5.15, page 62 and 63.

Figure 5.12 Distribution of the total consumption of antimicrobial agents in somatic hospitals, DDD, Denmark

DANMAP 2018



Data used in this figure is based consumption at acute care public somatic hospitals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.13a consumption at acute care hospitals by leading groups of antimicrobial agents (J01), DBD, Denmark
DANMAP 2018

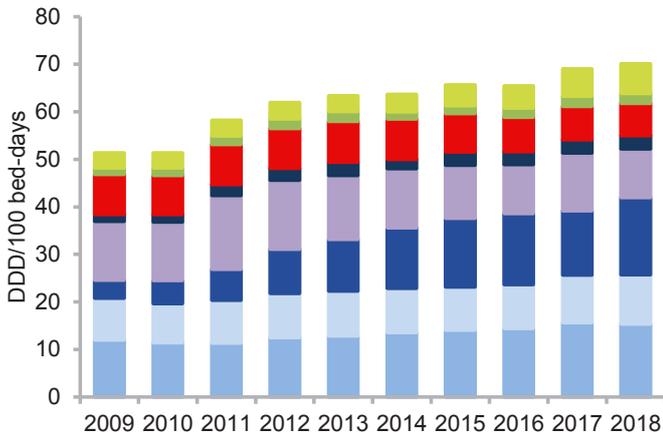
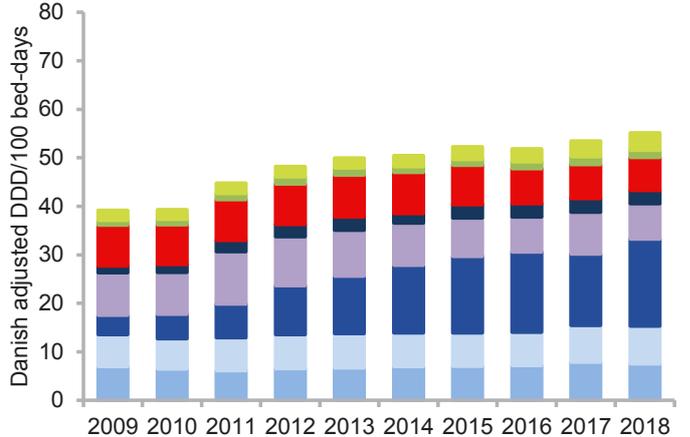


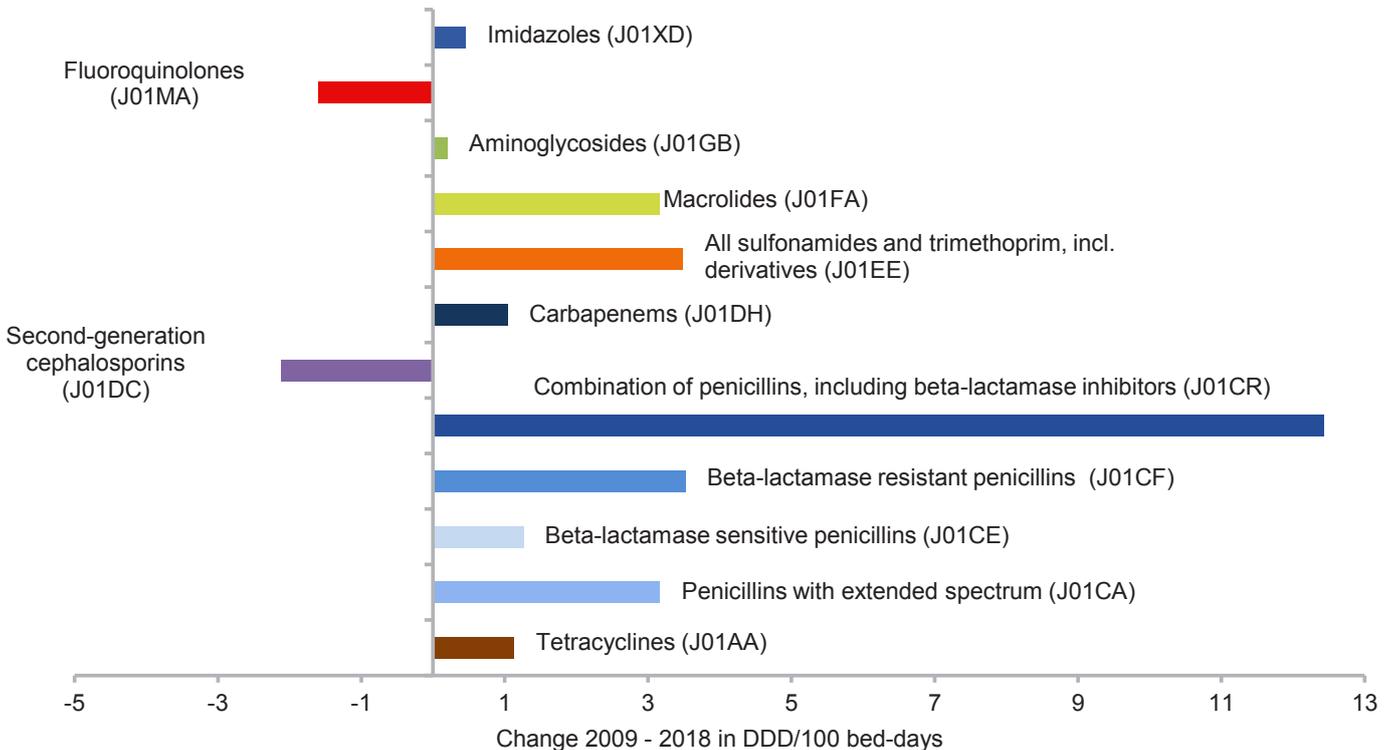
Figure 5.13b consumption at acute care hospitals by leading groups of antimicrobial agents (J01), measured in DaDDD/100 bed-days, Denmark
DANMAP 2018



- Macrolides (J01FA)
- Other aminoglycosides (J01GB)
- Fluoroquinolones (J01MA)
- Carbapenems (J01DH)
- Cephalosporins (J01DB, DC, DD)
- Combination penicillins, incl. beta-lactamase inhibitors (J01CR)
- Beta-lactamase sensitive penicillins (J01CE)
- Penicillins with extended spectrum (J01CA)

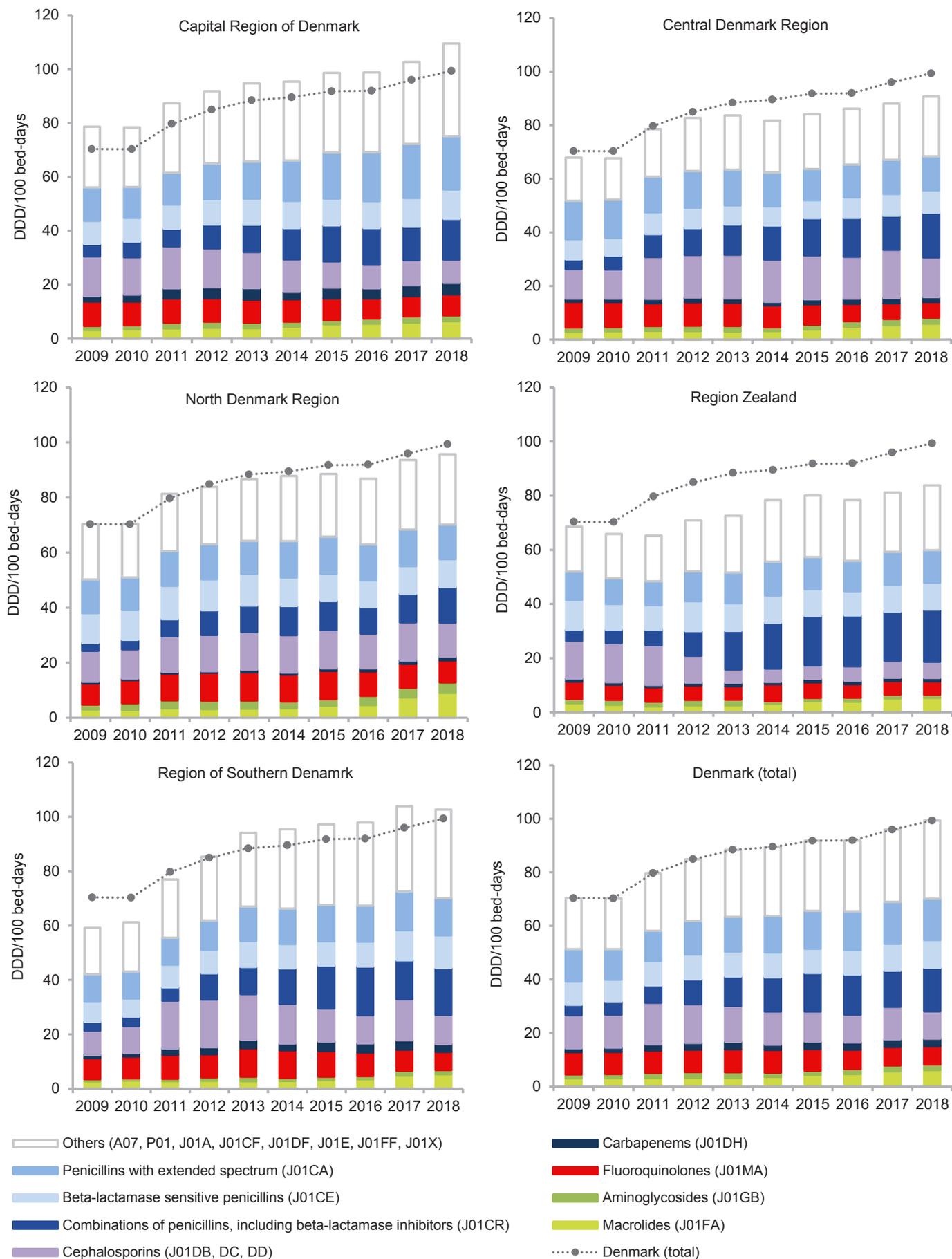
Data used in this figure is based on consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.14 Changes in the consumption by leading groups of antimicrobial agents in the hospital sector, DBD, Denmark
DANMAP 2018



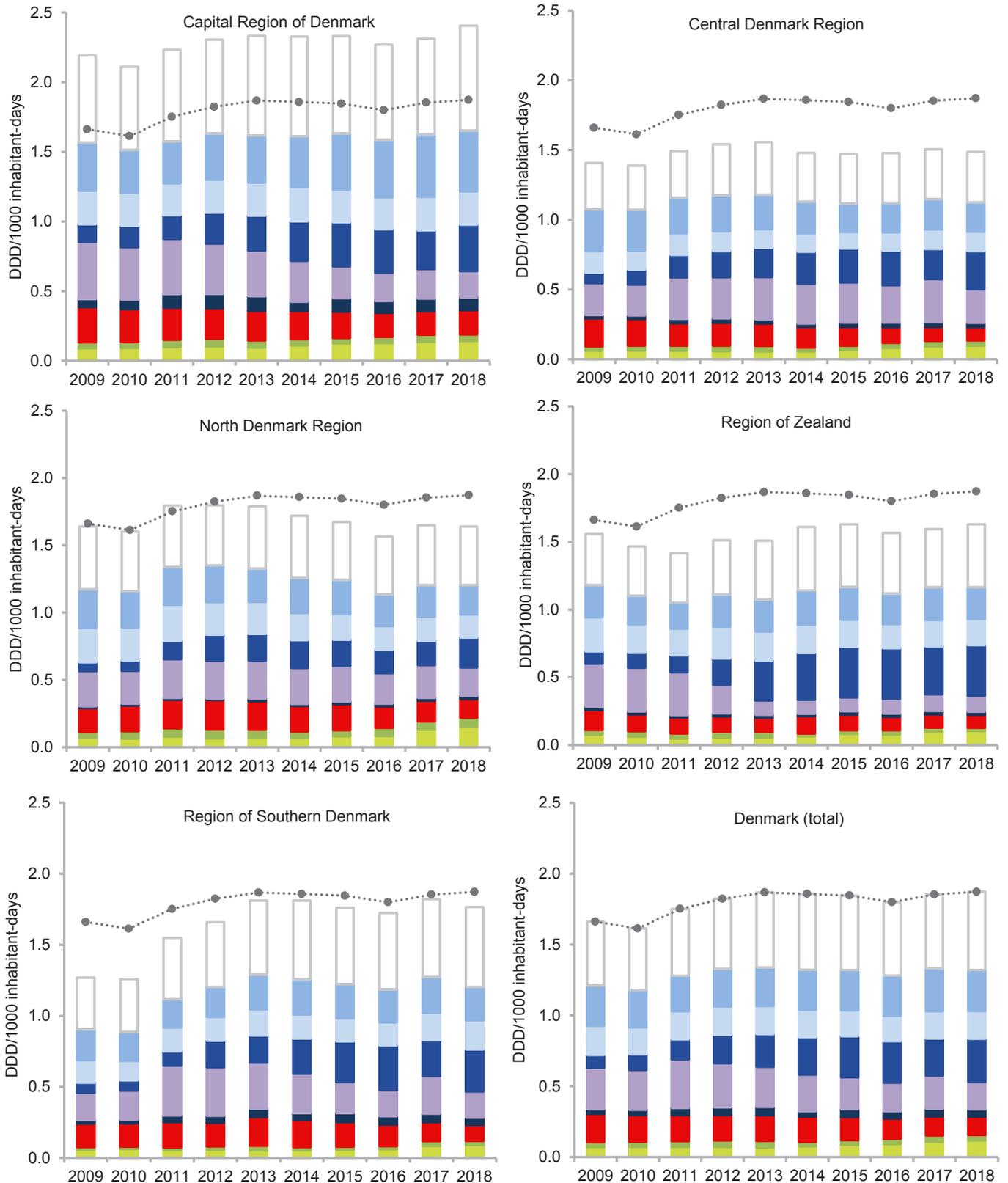
Data used in this figure is based consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.15a Consumption of antimicrobial agents for systemic use in the five health regions, DBD, Denmark DANMAP 2018



Data used in this figure is based consumption at acute care public somatic hospitals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.15b Consumption of antimicrobial agents for systemic use in the five health regions, DID, Denmark DANMAP 2018



- Others (A07, P01, J01A, J01CF, J01DF, J01E, J01FF, J01X)
- Penicillins with extended spectrum (J01CA)
- Beta-lactamase sensitive penicillins (J01CE)
- Combinations of penicillins, including beta-lactamase inhibitors (J01CR)
- Cephalosporins (J01DB, DC, DD)
- Carbapenems (J01DH)
- Fluoroquinolones (J01MA)
- Aminoglycosides (J01GB)
- Macrolides (J01FA)
- Denmark (total)

Data used in this figure is based consumption at acute care public somatic hospitals
 ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4.3 Other measures of consumption at somatic hospitals - DDD per 100 admissions (DAD)

The consumption of antimicrobials at hospitals can also be measured in relation to hospital activity calculated in number of patients "passing through", i.e. DDD per 100 admissions (DAD).

In 2018, the total consumption was 292 DAD, a 3.4% increase from the 282 DAD in 2017 and 10% increase from 264 DAD in 2009. The consumption measured in DAD has increased since 2015, in 2018 it reached the highest level ever measured, (Table 5.9). The trends in DAD reflect for most antimicrobials the trends observed in DBD. However, the observed rates of increases were more marked, when measured in DBD than in DAD for all antimicrobial classes, (Tables 5.8 and 5.9). This could be due to the change in hospital activity, as presented in Figure A5.2 in web annex.

At regional level, the hospital activity measured in admissions mirrors the density of the population, except for the Region of Southern Denmark and the North Denmark Region, where the population over the last decade has increased slightly, but the number of admissions in somatic hospital has decreased, (Table 5.7). For all five Danish regions, the number of bed-days in somatic hospitals decreased from 2009 to 2018. Trends in consumption measured in DID and DBD are presented in Figure 5.15a and b.

A comparison of the usage of antimicrobials for the treatment of animals and humans, respectively, measured in kg active substance is presented in Table A5.1 in web annex. For comparison of consumption at hospitals with the consumption in the primary sector measured in DID see Table 5.1 on page 46.

Table 5.9 Consumption of antimicrobial agents for systemic use in somatic hospitals, DAD,Denmark

DANMAP 2018

ATC group	Therapeutic group	Year									
		2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
J01AA	Tetracyclines	3.56	3.50	3.64	5.02	4.68	5.06	5.46	6.19	5.99	6.95
J01CA	Penicillins with extended spectrum	45.16	40.42	38.45	40.69	41.40	42.11	42.91	43.02	46.01	45.12
J01CE	Beta-lactamase sensitive penicillins	32.88	29.09	30.57	30.39	30.22	28.91	27.61	27.31	29.48	30.47
J01CF	Beta-lactamase resistant penicillins	25.14	24.28	26.05	25.96	27.93	27.81	28.05	26.02	25.90	30.52
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	14.38	17.03	21.97	30.35	35.11	39.63	43.81	44.62	39.61	47.74
J01DB	First-generation cephalosporins	0.45	0.43	0.39	0.39	0.36	0.19	0.13	0.13	0.12	0.11
J01DC	Second-generation cephalosporins	41.64	39.36	48.57	43.57	39.42	35.28	30.77	27.46	31.69	26.35
J01DD	Third-generation cephalosporins	4.40	3.56	3.64	3.37	3.48	3.10	3.16	3.07	3.91	3.52
J01DF	Monobactams	0.00	0.12	0.49	0.47	0.46	0.20	0.08	0.03	0.02	0.02
J01DH	Carbapenems	5.35	5.69	7.90	8.18	8.86	5.96	8.49	7.98	8.33	8.13
J01EA	Trimethoprim and derivatives	1.50	1.14	1.05	1.18	1.22	1.45	1.21	1.11	1.22	1.28
J01EB	Short-acting sulfonamides	1.36	1.00	0.72	0.58	0.52	0.44	0.36	0.30	0.30	0.29
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	8.33	6.56	9.85	10.62	13.75	14.53	15.40	15.48	15.85	17.03
J01FA	Macrolides	11.58	11.19	11.03	11.04	10.52	11.31	13.19	13.97	16.68	18.34
J01FF	Lincosamides	1.73	1.52	1.62	1.97	2.05	2.00	1.73	1.85	1.87	2.23
J01GB	Aminoglycosides	5.34	5.46	6.23	6.69	6.74	4.83	4.90	5.65	6.39	6.05
J01MA	Fluoroquinolones	31.57	29.07	28.43	27.31	27.63	26.43	24.61	21.60	20.52	20.00
J01XA	Glycopeptides	3.45	3.45	4.12	4.09	4.22	3.58	3.27	3.23	3.85	3.72
J01XB	Polymyxins	0.24	0.32	0.28	0.30	0.39	0.59	0.53	0.58	0.57	0.67
J01XC	Steroid antibacterials (fusidic acid)	1.08	1.11	0.85	0.74	0.71	0.71	0.50	0.34	0.20	0.18
J01XD	Imidazole derivatives	13.02	12.40	12.56	12.79	13.15	13.74	12.83	13.44	13.66	12.71
J01XE	Nitrofurans derivatives (nitrofurantoin)	1.24	0.97	0.97	1.06	1.09	0.98	0.82	0.71	0.74	0.79
J01XX05	Methenamine	0.28	0.26	0.31	0.26	0.23	0.17	0.27	0.24	0.21	0.31
J01XX08	Linezolid	0.76	0.72	0.99	1.01	1.14	1.05	1.33	1.08	1.09	1.53
J01XX09	Daptomycin	0.06	0.07	0.05	0.06	0.07	0.10	0.12	0.15	0.24	0.42
P01AB01	Nitroimidazole derivatives (metronidazole)	9.11	8.44	7.93	7.48	7.22	6.14	6.10	6.48	5.97	5.71
A07AA09	Intestinal anti-infectives (vancomycin)	0.65	0.89	1.36	1.52	1.59	1.62	1.43	1.45	1.53	1.45
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	264.29	248.06	270.02	277.09	284.18	277.93	279.09	273.49	281.94	291.63

Data used in this table is based consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4.4 Changes in the consumption of antimicrobials of critical interest

In Denmark, cephalosporins, fluoroquinolones and carbapenems have been collectively termed the antimicrobials of special critical interest due to their important role as first line drugs in the treatment of acutely ill patients suffering from bloodstream infections. Their use is also correlated to a marked risk of resistance, which makes a monitoring of the consumption of all three necessary. For many years, 2nd gen cephalosporins were the main drug in the treatment of patients with sepsis. In an attempt to reduce the consumption of these, the use of piperacillin with tazobactam, a combination penicillin, was recommended as sepsis treatment to all major hospitals during the period of 2005 to 2008. Within recent years, the recommendations on empirical treatment in patients with community-acquired sepsis have been further changed to the use of either beta-lactamase sensitive penicillins or penicillins with extended spectrum (in combination with gentamycin). Trends for the consumption of combination penicillins are shown in Figure 5.16. Due to a shortage of piperacillin with tazobactam in 2017, the overall consumption of the drug decreased markedly and was paralleled by a simultaneous increase in the consump-

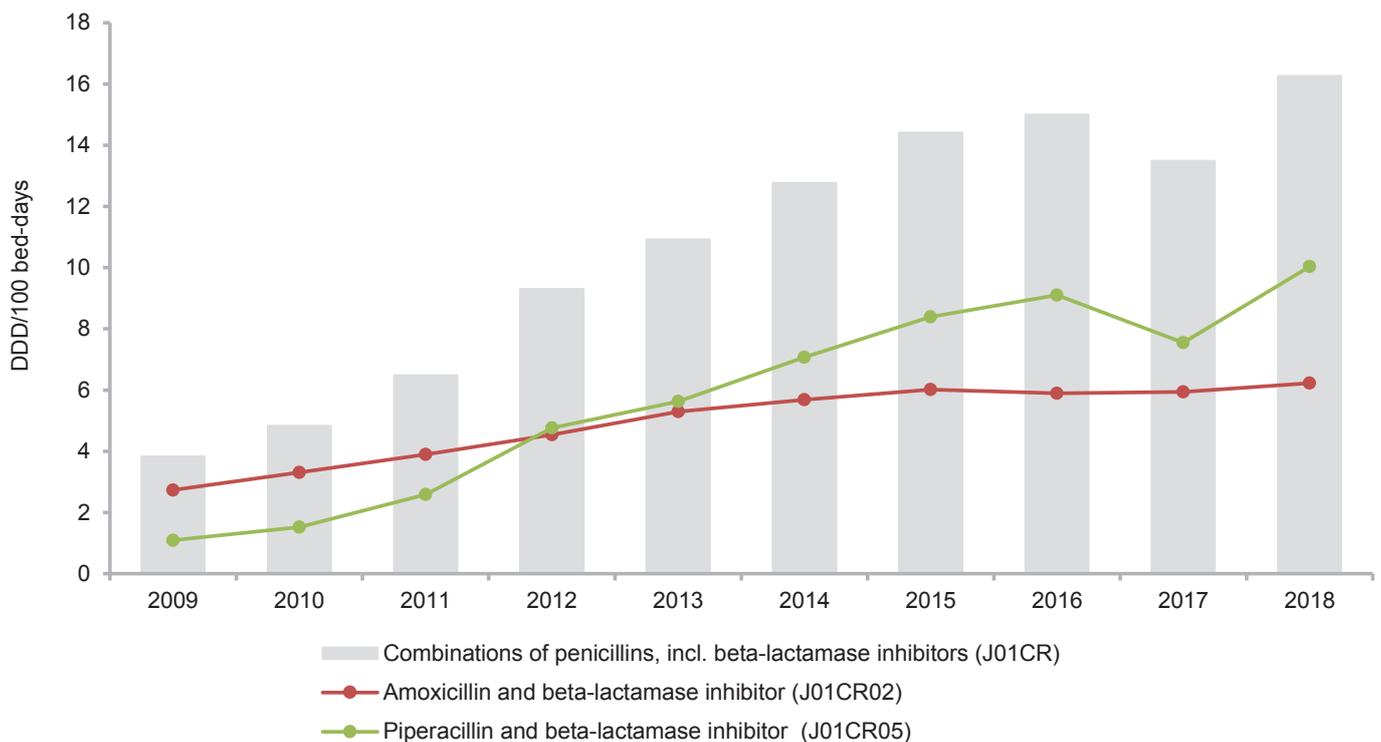
tion of cephalosporins the same year. This becomes obvious in the regional monitoring of the antimicrobials of critical interest for 2017, (Figure 5.17).

In 2018, the antimicrobials of special critical interest constituted together 20% of the total consumption at hospitals, measured in DBD. In 2017, it was 22% and ten years ago, in 2009, it was 32%. The trends in the consumption for the five healthcare regions and the average national level during 2009 to 2018 are presented in Figure 5.15 and as per cent of the consumption in DDD in Figure 5.17.

Cephalosporins accounted with altogether 10.2 DBD for 10% of the total consumption, a decrease of 16% from the 12.1 DBD in 2017, (Table 5.8). 2nd generation cephalosporins accounted for the biggest part, 8.97 DBD. Fluoroquinolones accounted for 6.81 DBD, a 2.5% reduction from 6.98 DBD in 2017. The consumption of fluoroquinolones peaked in the years of 2009 to 2013 and has since shown slight declines. Carbapenems accounted for 2.77 DBD in 2018, a 2.3% decrease from 2.83 DBD in 2017.

Figure 5.16 Consumption of combination penicillins in hospitals, DBD, Denmark

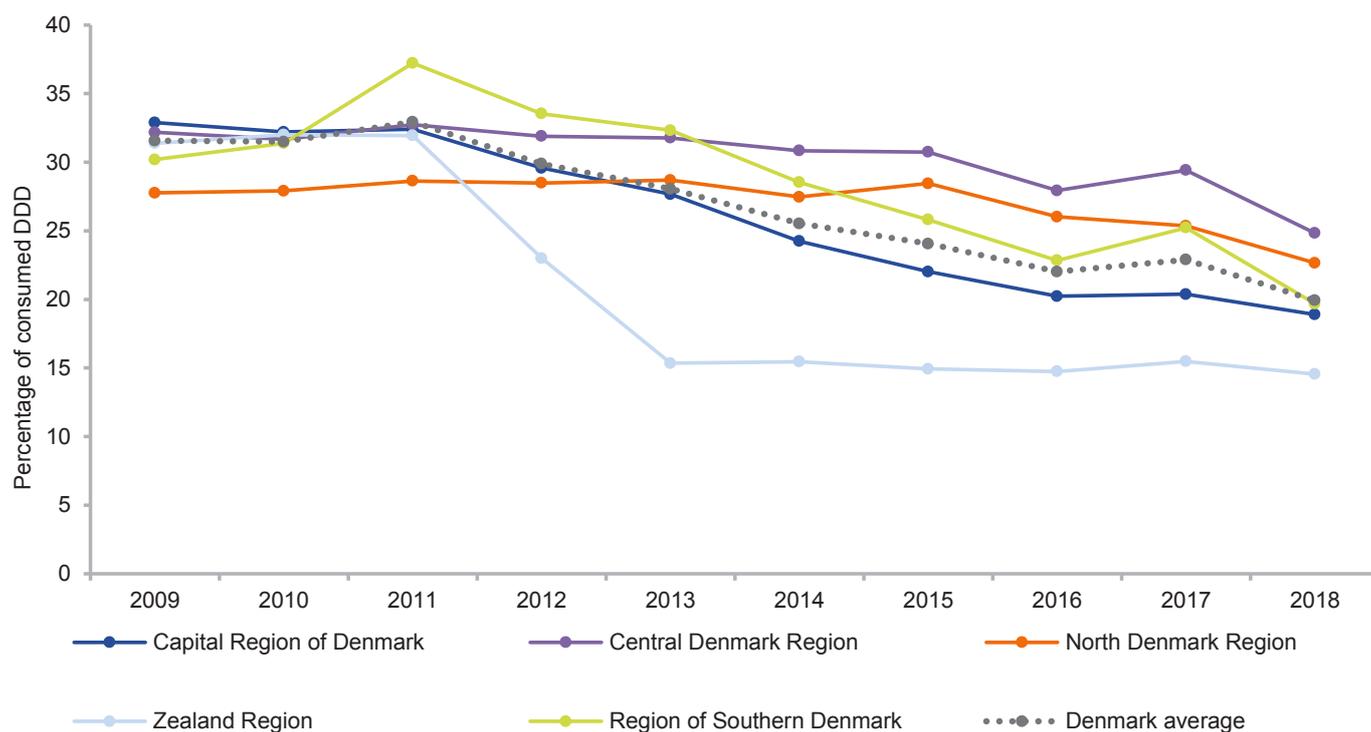
DANMAP 2018



Data used in this figure is based consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.17 Percentage of the consumption of antimicrobials of special critical interest combined, divided into regional consumption, based on DDD, Denmark

DANMAP 2018



Data used in this figure is based consumption at acute care public somatic hospitals
ATC numbers stem from the 2019 edition of the Anatomical Therapeutic Chemical (ATC) classification system

The consumption of the three antimicrobial groups of critical interest will be monitored closely also in the future. This is due to local, regional and national initiatives, the most important one being the goals developed by working groups under the "National Quality and Learning Teams", an initiative spanning all Danish regions for 2017-2019. Their aim is applying principles of antibiotic stewardship to the main acute care hospitals, primarily focusing on emergency departments and medical departments with a relatively high number of acute patients. For the monitoring of these initiatives the Group developed Danish adjusted DDD for the main antimicrobial classes used at hospitals. When these are applied to the antimicrobial sales reported to DANMAP, the trends in consumption present as shown in Figure 5.13b. For more information on the working group, monitoring and results please see <https://kvalitetsteams.dk/laerings-og-kvalitetsteams/lkt-rationelt-antibiotikaforbrug-paa-hospitaler/links-og-materialer> (only available in Danish).

The regional initiatives are supported by the implementation of the third measurable goal in the National Action Plan on antibiotics from 2017, aiming at a 10% reduction in the consumption of cephalosporins, fluoroquinolones and carbapenems from 2016 to 2020, when measured in DDD. For further information go to section 5.1, introduction to the human consumption. The mentioned shortages of piperacillin with tazobactam in 2017 may have caused reintroduction of cephalosporins in the treatment of acutely ill, septic patients at several hospitals. Therefore, extra attention will be paid to the consumption of cephalosporins also during next year.

We would like to acknowledge Maja Laursen from the National Health Data Authority in Denmark for data on all antibiotic consumption from primary and hospital care and help in proof reading of this chapter.

*Karoline Skjold Selle Pedersen and Ute Wolff Sönksen
For further information: Ute Wolff Sönksen, uws@ssi.dk*

Textbox 5.1

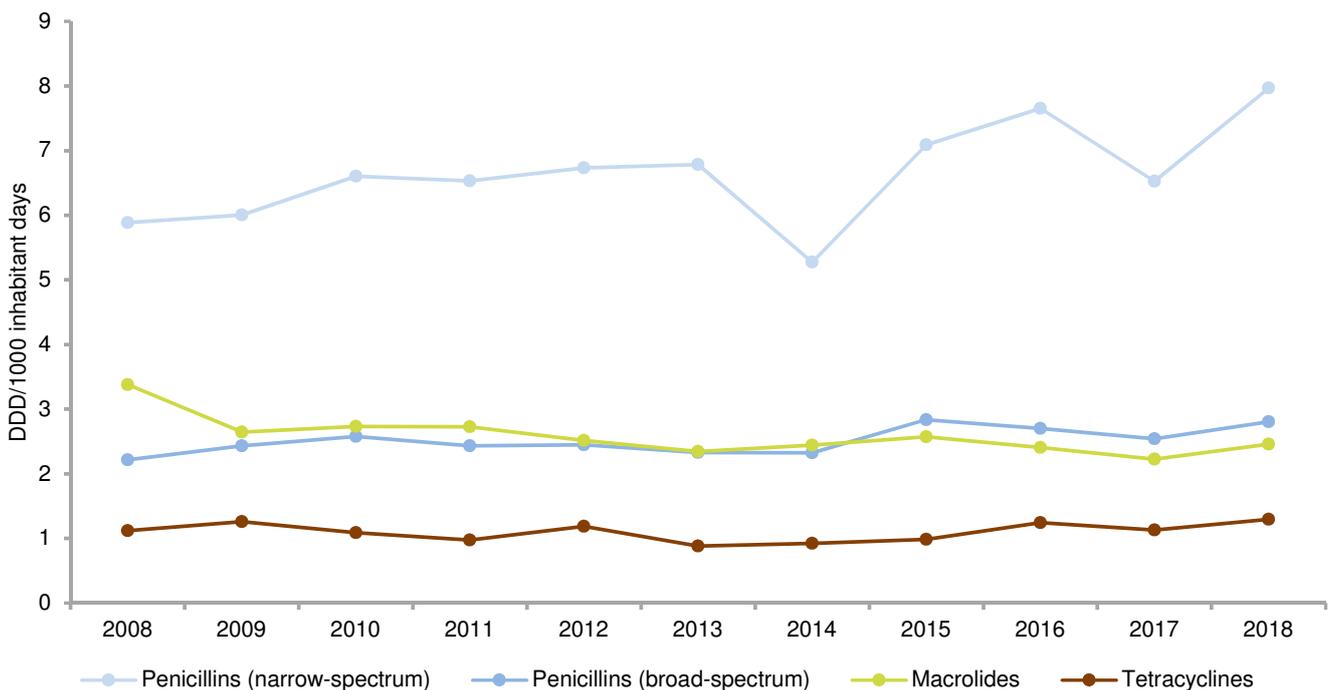
Incidence of multiresistant bacteria and consumption of antimicrobial agents in Greenland

Background. Greenland has a population of 55,877 inhabitants (January 2018) and Nuuk is the capital with around 18,000 inhabitants. Greenland is an autonomous administrative country of Denmark; it has its own Ministry of Health and the country is divided into five health regions. Although sparsely populated, due to its big geographic dispersion, there are five smaller hospitals, one national hospital and 11 health care centres in the five health regions. The national and largest hospital Dronning Ingrid's Hospital (182 beds), is situated in Nuuk. Around 15-16,000 persons are admitted to hospital at least once a year. Patients with specific or serious diseases that cannot be treated at Dronning Ingrid's Hospital (DIH) are transferred to Denmark or Iceland for further treatment e.g. haemodialysis, cancer treatment, brain surgery etc.

Resistant bacteria. From 2000 to 2018, 50 patients were diagnosed with methicillin resistant *Staphylococcus aureus* (MRSA), 104 patients with extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, three patients with vancomycin resistant enterococci (VRE), and 165 patients with *Clostridium difficile* infection among whom 54 had the O27 type.

Figure 1a Consumption of selected antimicrobial agents in humans in Greenland (DDD) 2008–2018: consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines

DANMAP 2018

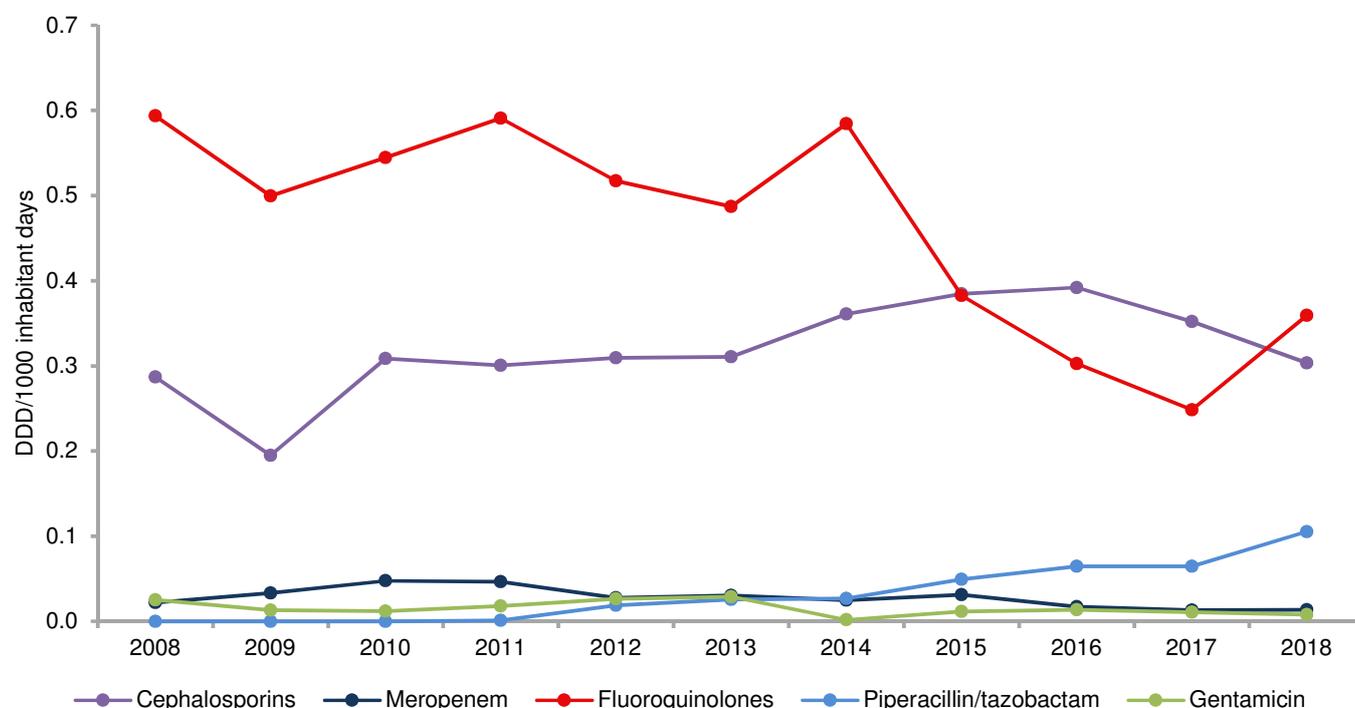


Note: Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin, and dicloxacillin. Broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor.

continued ... Textbox 5.1

Figure 1b Consumption of selected antimicrobial agents in humans in Greenland (DID) 2008–2018: consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin

DANMAP 2018



MRSA. Since 2015, a nearly four fold increase in incidence of MRSA has been observed. The largest increase was seen during 2017, and the main reason for this was an outbreak involving 12 persons in Tasiilaq at the East coast of Greenland (described in details in DANMAP 2017). In 2018, only four persons were reported with MRSA: three adults with infections (wound: 2; UTI: 1), and one premature child being MRSA-carrier in nose and throat. The child was colonised with MRSA while hospitalised in Denmark due to an MRSA-outbreak at the neonatal ward. The mother of the child was MRSA-negative at time of the detection, but in later samples (February 2019) she was also tested positive from the nose. There was no further transmission at DIH or among family members in Greenland.

VRE. In spite of ongoing VRE-outbreaks in Denmark, only three patients have been diagnosed with VRE in Greenland. Two patients were colonised with VRE in the rectum and one patient had pleurisy - in all three cases VRE occurred after hospitalisation in Denmark. No transmission was observed in the wards.

CPO. In recent years, an increase in incidence of carbapenemase-producing organisms (CPO) in Denmark has been observed but until now, no CPO has been reported in Greenland.

Other resistant bacteria: Most of the other resistant bacteria observed were imported from Denmark or abroad, but in some cases, especially in patients with ESBL-producing *Enterobacteriaceae*, treatment with broad-spectrum antimicrobial agents in Greenland probably selected for these bacteria. From 2012 to 2013, there were outbreaks with *C. difficile* type 027 in several hospitals and transmission within the country occurred. But due to a great effort in infection prevention and control from the hospital staff, these outbreaks were quickly stopped. Of the 18 new patients with *C. difficile* infection diagnosed in 2018, five were infected with the 027 type.

Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and distributed from the National Pharmacy. Figure 1a and b shows the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2008 to 2018. From 2008 to 2018 a larger increase in the consumption of narrow-spectrum (35.3%) and broad-spectrum penicillins (26.7%) was observed but only a minor increase of 4.1 and 3.7%, respectively, from 2016 to 2018. Increases in broad-spectrum antimicrobial agents such as macrolides (2%), tetracyclines (4.4%), and piperacillin-tazobactam (64.1%) were also observed from 2016 to 2018. Simultaneously, large decreases in consumption of gentamicin (42.9%), cephalosporins (22.7%), and meropenem (17.6%) were registered for the same period. From 2008 to 2018 an overall decrease of 39.6% was observed in consumption of fluoroquinolones with the largest decrease (57.4%) from 2014 to 2017. Unfortunately, from 2017 to 2018 a large increase of 44.2% was observed. The reason for this increase is not fully understood but will be on the agenda the next year.

Conclusion: The consumption data for antimicrobial agents are based on purchases and fluctuations are therefore seen from year to year. It is noteworthy that the increased focus on prescription of antibiotics due to teaching the doctors in the recent years has resulted in remarkable decreases in purchases of cephalosporins and meropenem, and in continued increase in purchase of piperacillin-tazobactam. It is - however - worrying that the purchase of fluoroquinolones has increased once again after the large decrease seen from 2014 to 2017.

Continued focus on the use of broad-spectrum antimicrobial agents, on the incidence of multiresistant bacteria, and on compliance to guidelines for infection prevention and control remains very important in Greenland also in the future.

*Anne Kjerulf, Jette Holt, Anne Birgitte Jensen, Peter Poulsen, Inge Mortensen
For further information: Anne Kjerulf (alf@ssi.dk)*

Textbox 5.2

Antibiotics in dentistry

According to the “National Action plan on the reduction of antibiotics in humans” issued in July 2017, dentists were responsible for approximately 7% of the total consumption of antibiotics in primary health care in 2016. Further, the number of prescriptions pr. 1000 inhabitants increased from 1999-2015 (1) and did not level off around 2011 as for general practitioners and other medical specialists. Based upon a 24% increase in the number of persons administered antibiotics by a dentist from 2005-2014, in 2015 the Danish Health Authority initiated drawing up a “National clinical guideline on use of antibiotics in dentistry” in order to identify focus areas for guidance of dentists in their use of antibiotics (2). The guideline with a key message of limiting the indications for use of antibiotics in both treatment and prophylaxis was issued in 2016, where the first decline in dental prescriptions was observed. Since then the decline in usage has been considerable, corresponding to a 31% decrease in 2018 from the peak of 39.4 prescriptions per 1000 inhabitants in 2015, (Figure 1).

Figure 1 Total annual number of dental prescriptions per 1000 inhabitants, Denmark

DANMAP 2018

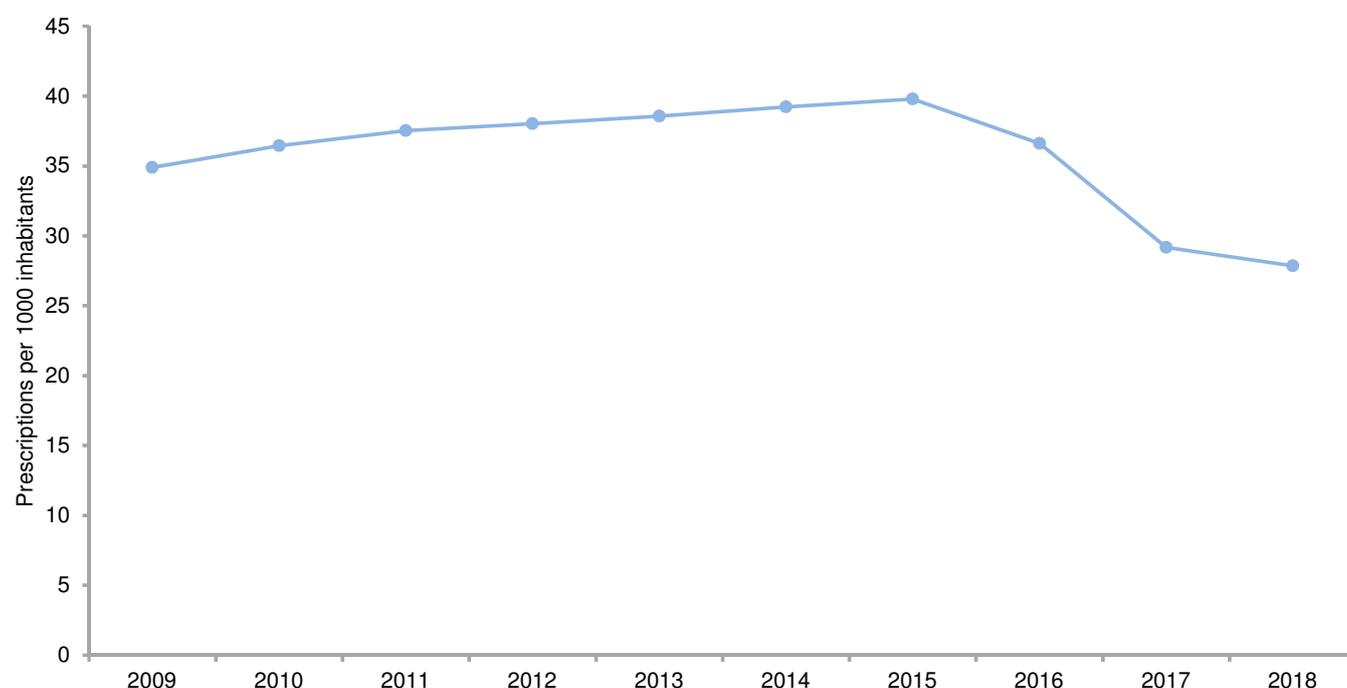
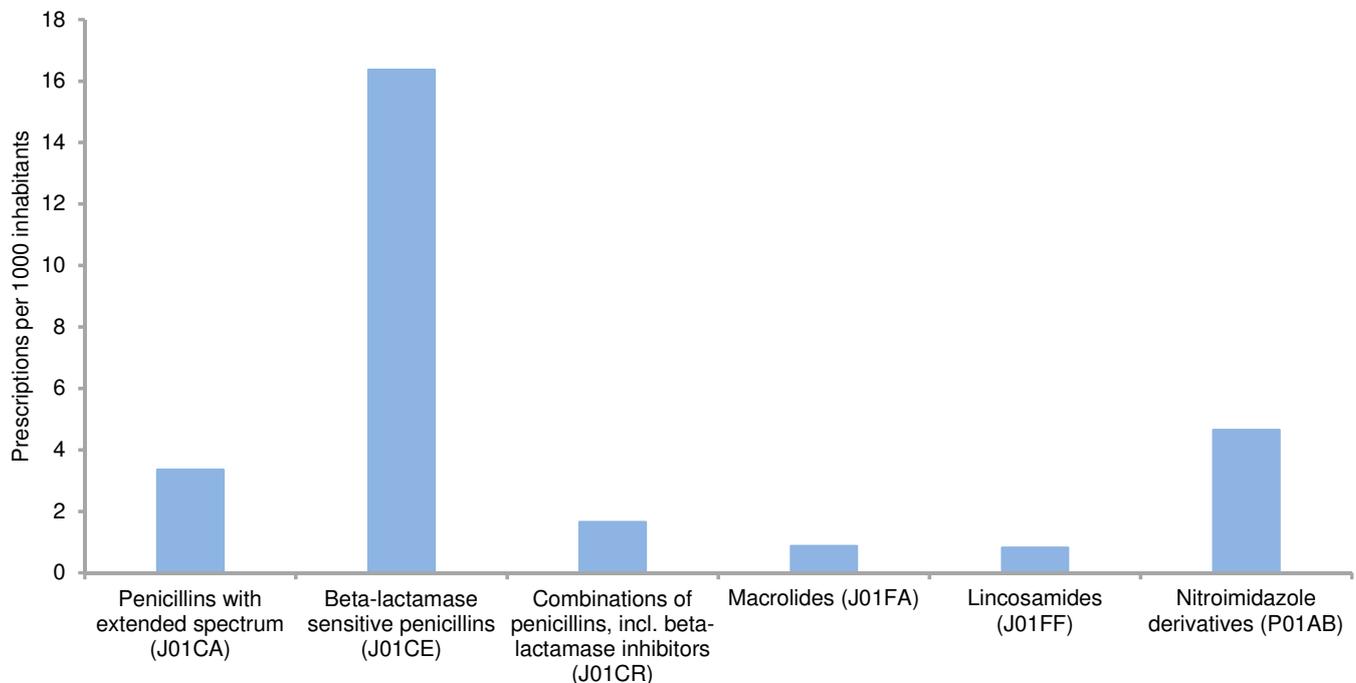


Figure 2 shows how the antibiotics prescribed by dentists are distributed among groups of antibiotics. Phenoxymethyl penicillin accounts for almost 60% of total prescriptions, which is far above the goal of 36% to be achieved in primary health care in 2020 (1). Metronidazole accounts for 17% followed by amoxicillin alone (11%) and in combination with beta-lactamase inhibitors (6%). Metronidazole in combination with phenoxymethyl penicillin is recommended for treatment of acute odontogenic infections (2), while amoxicillin is the drug of choice for prophylaxis.

The first goal of the “National Action plan on the reduction of antibiotics in humans”, i.e. a 25% reduction in the total number of prescriptions from 2016-2020 was achieved by dentists in 2018. However, given the rather high level of dental prescriptions in 2016, a continued reduction in total dental prescriptions may still be achievable. In addition, further reductions in use of broad spectrum antibiotics, especially amoxicillin with beta-lactamase inhibitors and possibly clindamycin may be attained. Adopting

Figure 2 Number of prescriptions per 1000 inhabitants of the antibiotics most frequently used by dentists in 2018, Denmark

DANMAP 2018



more detailed information on dental prescriptions regarding diagnosis and specific indications for use would allow access to gather more specific information on the patterns of antibiotic usage among dentists. This would create the base for informed decision making on where to take action in future efforts and guidelines in order to further strengthen the awareness of antibiotic prescribing habits among dentists.

*Karoline Skjold Selle Pedersen, Karen Geismar and Tove Larsen
For further information: Tove Larsen, tla@sund.ku.dk*

References

1. SUNDHEDS- OG ÆLDREMINISTERIET. National handlingsplan for antibiotika til mennesker. Tre målbare mål for en reduktion af antibiotikaforbruget frem mod 2020. 2017 https://sum.dk/~media/Filer%20-%20Publikationer_i_pdf/2017/Antibiotika-handlingsplan-frem-mod-2020/DK-Handlingsplan-05072017.pdf
2. National klinisk retningslinje (NKR) for brug af antibiotika ved tandlægebehandling. Sundhedsstyrelsen, Danmark. 2016. <https://www.sst.dk/da/udgivelser/2016/~media/F5344E45B2FC48699B5C9F4D918DAB9B.ashx>

*Tove Larsen, lektor, ph.d.
Odontologisk Institut
Det Sundhedsvidenskabelige Fakultet
Københavns Universitet
tla@sund.ku.dk*

Textbox 5.3

Infection Prevention and Control can Combat Antibiotic Resistance

Over- and misuse of antibiotics in the treatment of humans and animals alike has been well described as the main driver of antimicrobial resistance (AMR). Moreover, once resistant bacteria have been introduced into a given population it is crucial to detect their existence, since spreading them within the population will further worsen the situation. Thus, naturally there are two main ways of tackling AMR:

- To reduce and control consumption of antimicrobial agents in both human beings and animals
- To prevent the spread of antibiotic resistant microorganisms – especially among human beings.

Both ways have to go hand in hand in order to prevent the further development and escalation of AMR.

This textbox will focus on the infection prevention and control (IPC) aspects. Crucial for success is the establishment of an infection control program, which should contain the following four main elements:

- An organisation focusing on reducing healthcare-associated infections, and infections caused by antibiotic resistant bacteria
- Education of healthcare staff
- Guidelines for infection prevention and control
- Surveillance of healthcare-associated infections (HAI) and AMR

Of further importance is the establishment of these on both local and national level. In Denmark, all the above four elements have existed for many years.

On the central level, the National Center for Infection Control (NCIC) was established in 1977 at Statens Serum Institut (SSI). The purpose of the center is to provide national guidelines for infection prevention and control; to guide the Ministry of Health, the Danish Health Authority and other authorities in the management of IPC and AMR; to disseminate knowledge of IPC, e.g. by developing e-learning programs in hand hygiene; to evaluate disinfection products for use in the Danish healthcare system; to guide healthcare staff by email or phone consultations; and finally to support education of nurses and doctors within IPC. A new Nordic education at Gothenburg University will be starting in September 2019 and NCIC is assisting the university.

At the local level, there are infection control teams in all five regions with infection prevention nurses (May 2018: 108 nurses) and a few part time infection prevention doctors (clinical microbiologists). The local infection control teams develop local guidelines based on the national guidelines, perform audits in hand hygiene and universal precautions, and educate nurses and other healthcare professionals to become link staff. Most clinical departments at Danish hospitals have a link nurse or other link professionals dedicated to infection prevention and control.

National guidelines for infection prevention and control are developed by the NCIC in collaboration with infection prevention nurses and doctors from all five regions, clinical experts and other experts, depending on the subject. They are whenever possible evidence-based and mainly based on already existing national and foreign guidelines. The two essential national guidelines are on universal precautions and on isolation precautions. The latter has a more detailed description on AMR. All the guidelines are in Danish, free of charge and easy to download from the SSI website.



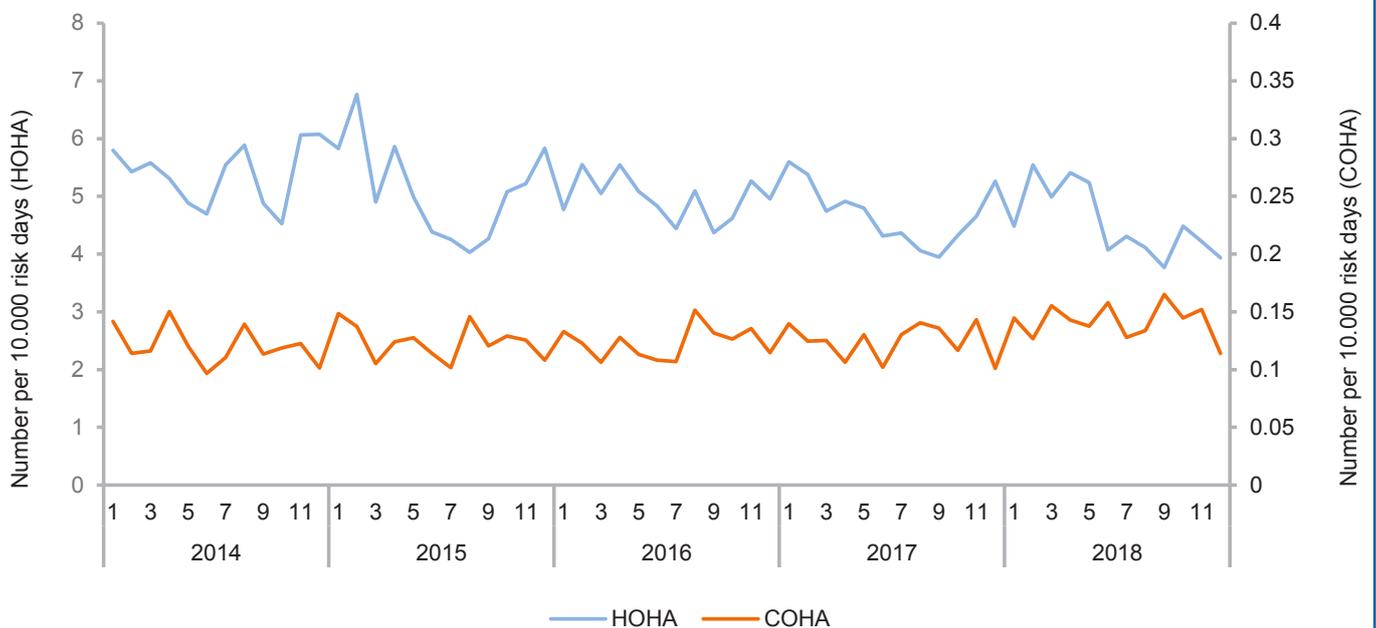
In addition, two detailed national guidelines exist on AMR from the Danish Health Authority - one on the prevention and tackling of methicillin resistant *Staphylococcus aureus* (MRSA) and one on carbapenemase-producing organisms (CPO). The purpose of both guidelines is to minimize the spread of these often highly resistant bacteria to the ill and weak patients at hospitals and in long term care facilities, simultaneously keeping the occurrence of these bacteria on a continued low level. The guidelines contain recommendations for active screening of patients on admission to hospital, based on assesment of certain risk situations, e.g. admission to a hospital abroad during the last six months. Both guidelines are free of charge and easy to download from the Danish Health Authority website www.sst.dk, see the MRSA guideline (available in English) and the CPO guideline (available in Danish),, respectively.

Appendices to the guidelines with detailed instructions on AMR infection prevention and control are published on the SSI site at www.ssi.dk, see the appendices for MRSA and CPO, respectively (at the moment only available in Danish).

Denmark has a long tradition concerning surveillance of HAI. Point prevalence surveys on HAI were performed twice a year for many years but were discontinued in 2014, with the simultaneous development and establishment of a new automated electronic surveillance system for HAI called HAIBA (for a detailed description see DANMAP 2014 and on the SSI website). Case definitions and surveillance have been established for the following HAIs: Bacteraemia, urinary tract infection, *Clostridium difficile* infection (CDI) and deep infection after planned total hip and knee replacement surgery. An example regarding the surveillance system is shown in Figure 1 for CDI: Surveillance of CDI during the last five years from 2014-2018 has shown a significant decrease in incidence of hospital onset hospital-acquired (HOHA) infections. The opposite is seen regarding the incidence of community onset hospital-acquired (COHA) infections with a significant increase.

Figure 1. Surveillance of *Clostridium difficile* infection (CDI) from 2014 to 2018

DANMAP 2018



Active national surveillance of human AMR has been done on a voluntary basis for many years by SSI and the DANMAP group. Notification is mandatory only for MRSA and CPO, since October 2006 and September 2018, respectively.

Anne Kjerulf, Elsebeth Tvenstrup Jensen, Sophie Gubbels and Brian Kristensen
For further information: Anne Kjerulf, alf@ssi.dk

Textbox 5.4, part 1

Candidaemia in Denmark: Epidemiology, antifungal consumption and resistance

Candidaemia is a serious and potentially life threatening condition which occurs mainly in hospitalised patients suffering from severe conditions. Risk factors for acquiring a candidaemia are among others stay at the intensive care unit (ICU), the presence of central vascular catheter, prior and particularly repeated or complicated abdominal surgery and exposure to broadspectrum antibiotics (1). The 30-day mortality is approximately 40%, being highest for patients in the ICU (2). *Candida albicans* is the most frequently found organism (1). However, over the past fifteen years an increase in *Candida glabrata* has been observed which is worrisome as this species is intrinsically less susceptible to fluconazole (3). Echinocandins are the drug of choice in the primary treatment of candidaemia with de-escalation to azoles once susceptibility is confirmed and the patient is stable (4). However echinocandin resistance is emerging especially in *C. glabrata* (3)(5).

A Danish nationwide surveillance has existed since 2004 involving all the Danish clinical microbiology departments. The departments culture and identify the *Candida* species from blood cultures and refer the isolate(s) to Statens Serum Institut (SSI) for confirmation of identification and susceptibility testing. In addition, *FKS* sequencing is done at SSI for isolates with reduced echinocandin susceptibilities.

The episode rate of candidaemia peaked in 2011 with an incidence rate of 10.1/100.000 inhabitants, but has otherwise remained stable (Figure 1) (6). As mentioned above the proportion of *C. albicans* has decreased and *C. glabrata* increased (Figure 1) (6). Due to this proportional change in species distribution the fluconazole susceptibility rate has also decreased from 69% in the period 2004-2007 to 60% in the period 2016-2018 (unpublished data). Echinocandin resistance was not observed during 2004-2007 (6). However, the latest published surveillance study by Astvad et al. in 2018 found an echinocandin resistance rate of 1.7% for the period 2012-2015 (6) and similarly, we found 1.4% echinocandin resistance rate for 2016-2018 (not yet published data).

Figure 1 Species distribution 2004-18 and episoderate

DANMAP 2018

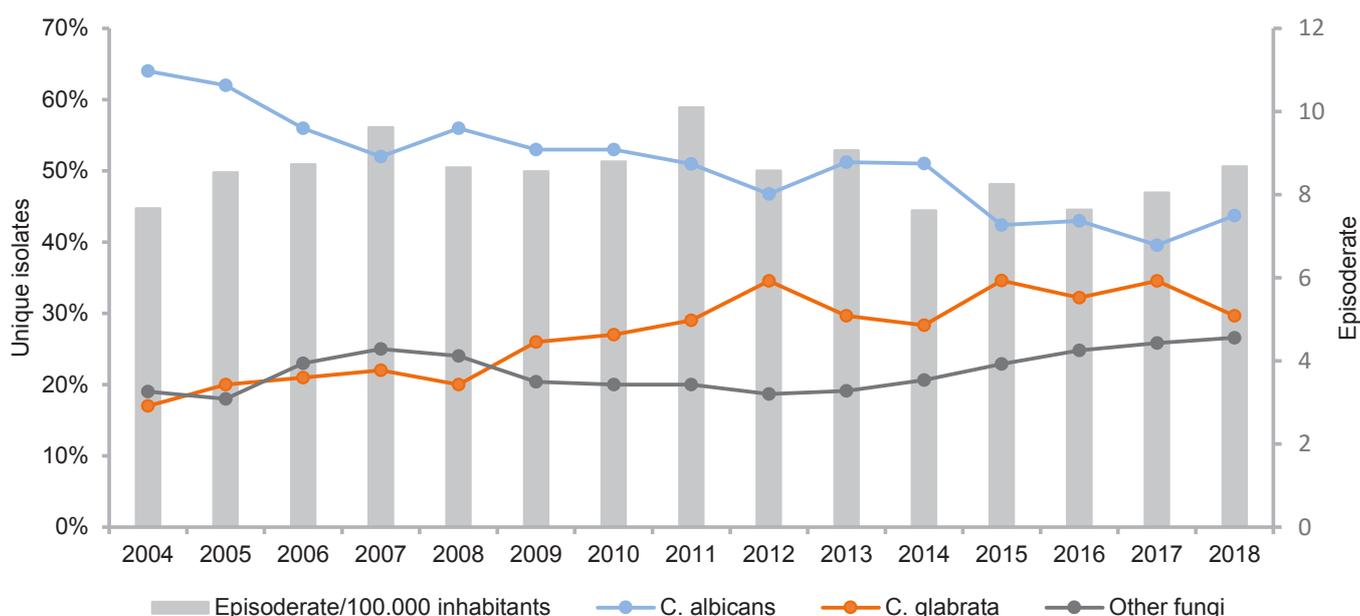


Figure 1. Episode rate and species distribution from the Danish nationwide candidemia surveillance 2004-18. A unique isolate is defined as: if found >21 days apart, confirmation of another species or different susceptibility pattern. Episode rate is defined as: one unique isolate or one polyfungal infection or one admission at one centre per 100.000 inhabitants. Other fungi are: Other *Candida* species, *Magnusiomyces*, *Saccharomyces*, *Cryptococcus*, *Rhodotorula* and other moulds.

In parallel with the increasing fluconazole and emerging echinocandin resistance rates the antifungal use in Denmark has changed. In 2016 and 2017 Denmark continued to have a higher consumption of fluconazole, itraconazole and posaconazole than the other Nordic countries. However, the use of amphotericin B and echinocandins were almost comparable across the Nordic countries (Figure 2). The consumption of the azoles in 2016 per 1000 inhabitants was: 225 DDDs for Denmark and 94, 114 and 152 DDD for Norway, Sweden and Finland, respectively. In 2017, consumption of azoles per 1000 inhabitants continued to be higher for Denmark than the other Nordic countries (212 DDD for Denmark and 89 and 144 DDD for Norway and Finland, respectively) despite a decrease in fluconazole consumption from 2016 to 2017 in Denmark for both hospital and primary health care sector (Figure 2). The decrease was greater for the primary health care sector. Caspofungin continued to be the main echinocandin used in 2016 and accounted for 62% of the echinocandin use. However, in 2017 anidulafungin accounted for 61% of the echinocandin use. These two compounds are regarded clinically equal and the choice between them is mainly driven by price.

In conclusion, although the episode rate has been stable over the past 15 years the species distribution has changed considerably. *C. glabrata* continues to rise while the rate of *C. albicans* continues to decrease at a much greater extent than in our neighbouring Nordic countries. This epidemiological change is worrisome since it explains the decrease in the fluconazole susceptibility rate and furthermore might be a result of the high antifungal use. Acquired resistance is still low, however, echinocandin resistance is of concern and will be closely monitored in the future since echinocandin is first line therapy in candidaemia and one of the few options for *C. glabrata* candidaemia.

Acknowledgements to the ten Danish Clinical Microbiology Departments at Danish hospitals for their interest and participation in the Danish *Candida* surveillance program including the submission of invasive *Candida* isolates of all types to the reference lab at SSI.

We would also like to thank the staff of the laboratory at the Mycology Unit at Statens Serum Institut.

Malene Risum, Raluca Datcu and Maiken Cavling Arendrup
For further information: Maiken Cavling Arendrup, maca@ssi.dk

References

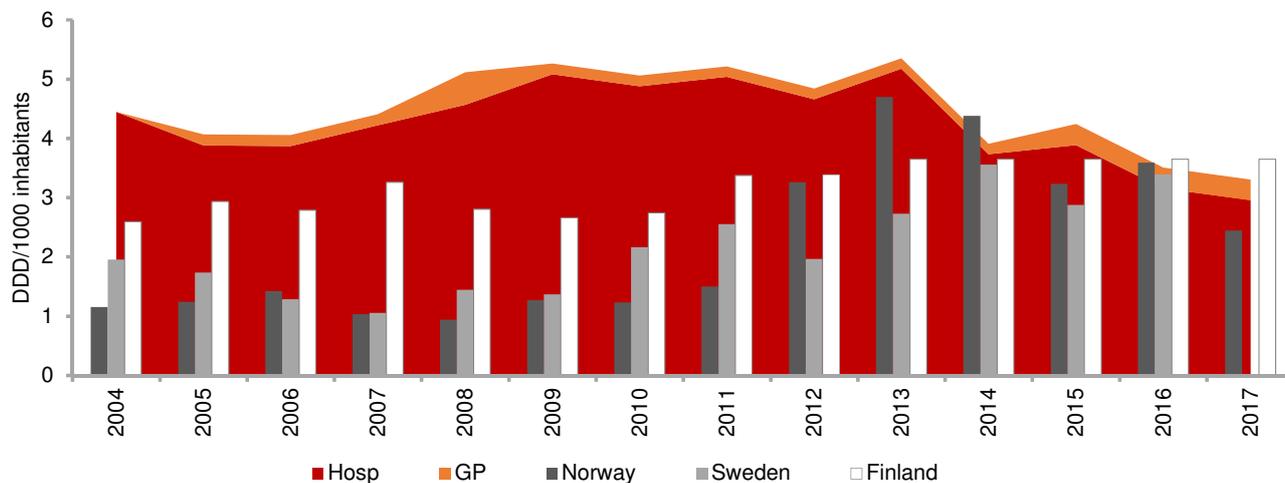
1. Kullberg BJ, Arendrup MC. Invasive Candidiasis. N Engl J Med. 2015 Oct 8;373(15):1445-56. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26444731>
2. Lausch KR, Søgaard M, Rosenvinge FS, Johansen HK, Boysen T, Røder B, et al. High incidence of candidaemia in a nationwide cohort: Underlying diseases, risk factors and mortality. Int J Infect Dis. 2018;
3. Arendrup MC, Perlin DS. Echinocandin resistance: An emerging clinical problem? Curr Opin Infect Dis. 2014;27(6):484-92.
4. Lausch KR, Søgaard M, Rosenvinge S, Røder BL, Mortensen KL, Dzajic E, et al. Treatment of candidemia in a nationwide setting : increased survival with primary echinocandin treatment. 2018;2449-59.
5. Arendrup MC, Perlin DS. Echinocandin resistance: an emerging clinical problem? Curr Opin Infect Dis [Internet]. 2014 Dec;27(6):484-92. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25304391>
6. Astvad KMT, Johansen HK, Røder BL, Rosenvinge FS, Knudsen JD, Lemming L, et al. Update from a 12-year nationwide fungemia surveillance: Increasing intrinsic and acquired resistance causes concern. J Clin Microbiol. 2018;

continued ... Textbox 5.4, part 1

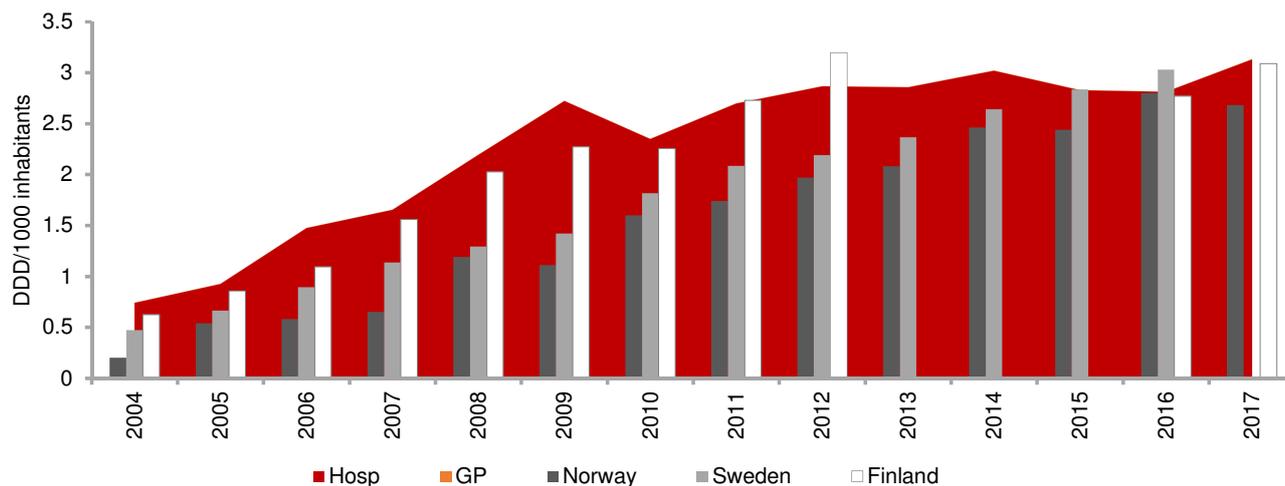
Figure 2 Annual consumption of amphotericin B (A), echinocandins (B) and fluconazole (C) in DDDs/1,000 inhabitants in 2004 to 2017

DANMAP 2018

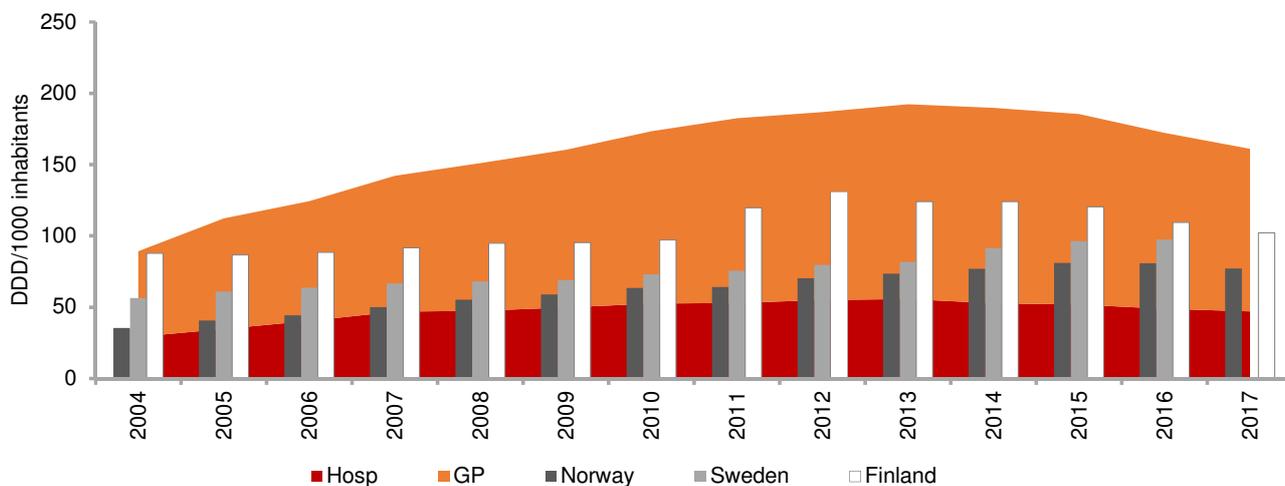
a) Amphotericin B 0.035 g/DDD



b) Echinocandins 0.5-1 g/DDD



c) Fluconazole 0.2 g/DDD



Annual consumption of amphotericin B (A), echinocandins (B) and fluconazole (C) in DDDs/1,000 inhabitants in 2004 to 2017. DDD: Defined daily dosage per 1000 inhabitants
Data on antifungal consumption was not available for Sweden in 2017

Textbox 5.4, part 2

Azole resistance in *Aspergillus* spp. Preliminary six months data from the newly established surveillance in Denmark

Azoles are the cornerstone in the treatment of aspergillosis due to superior efficacy and due to being the only antifungal drug class with oral formulations. Azole resistance (azole-R) in *Aspergillus* has been increasingly reported worldwide. The use of azole fungicides in the environment has been proposed to contribute to the emergence of azole-R *Aspergillus fumigatus*, and isolates harbouring the resistance mechanisms TR₃₄/L98H or TR₄₆/Y121F/T289A have been dominant among azole-R *A. fumigatus* from azole naïve patients as well as the environment (1). These two resistance genotypes have been sporadically found in clinical (2,3) as well as environmental samples in Denmark, although only TR₃₄/L98H from environmental samples has been previously published (4). The size of the problem remains unknown. It is noteworthy, however, that azole-R *A. fumigatus* isolate (TR₃₄/L98H) has previously been detected from soil outside Rigshospitalet in Copenhagen, and recently (June, 2019) a TR₄₆/Y121F/T289A isolate was discovered in a courtyard sample near intensive care unit at Aarhus University Hospital (AUH). This illustrates the ubiquitous presence of azole-R *A. fumigatus* underlining the severity of the problem. Therefore, a nationwide surveillance programme of azole-resistant *A. fumigatus* and underlying resistance mechanisms was established in 2018. Here we report data from the first six month's study period.

Unique *Aspergillus* isolates were included from all ten Danish clinical microbiological departments during the period October 2018 to March 2019. Isolates from same patients were defined as unique if 1) found >30 days apart, 2) confirmation of another species or 3) different susceptibility pattern. Inclusion criteria were: a) *Aspergillus* isolates regarded clinically significant, or b) any *Aspergillus* isolate detected on a Monday throughout the study period regardless of clinical significance to reflect the susceptibility pattern in general of circulating *A. fumigatus*. Included isolates originated from patients from both primary health sector and the hospital sector. Referral practices varied. Most laboratories referred all detected moulds or all *Aspergillus* isolates which then underwent species identification and susceptibility testing at the reference laboratory at Statens Serum Institut (SSI). One laboratory (AUH) performed species identification and susceptibility testing for most isolates locally, and the results were sent to SSI. Resistant isolates were sent to SSI for confirmation and molecular characterisation.

Table 1. Species distribution and susceptibility for *Aspergillus* complexes and *A. fumigatus*

DANMAP 2018

Species	Itraconazole			Posaconazole		Voriconazole		Isavuconazole	
	No.	Range	MIC50	Range	MIC50	Range	MIC50	Range	MIC50
<i>A. flavus</i>	21	≤0.03-1	0.125	0.125-0.25	0.125	0.5-1	1	0.5-4	1
<i>A. fumigatus</i>	411	0.06->16	0.25	0.03->4	0.06	0.25-8	0.5	0.5-16	1
<i>A. nidulans</i>	3	0.125	0.125	0.06-0.125	0.125	0.125-0.25	0.25	0.125-0.25	0.125
<i>A. niger</i>	61	0.25->16	1	0.125-0.5	0.5	0.5-2	1	1-8	2
<i>A. terreus</i>	18	0.06->16	1	0.03-0.5	0.5	0.25-4	1	0.25-16	2
Other <i>Aspergillus</i>	8	0.06->16	0.5	0.03->4	0.25	0.25-16	0.5	0.25-8	0.5

At SSI the referred *A. fumigatus* isolates underwent screening for azole-R following the EUCAST E.Def 10.1 method and using VIPcheck azole agar plates (Mediaproducs BV, Grönningen, NL). Screening positive *A. fumigatus* isolates and all non-*fumigatus* *Aspergillus* isolates underwent EUCAST E.Def 9.3.1 susceptibility testing. Isolates with azole MIC(s) above the EUCAST BP(s) underwent *cyp51A* gene sequencing.

A total of 695 *Aspergillus* isolates were included of which 503 were *A. fumigatus*. Susceptibility testing was performed for 411 *A. fumigatus* isolates from 319 patients at time of writing (Table 1). For *A. fumigatus*, 92.7% isolates were azole susceptible, 1.7% intermediate and 5.6% azole-R. From 19 out of 319 patients azole-R *A. fumigatus* isolates was recovered (6%). Among these, 18 out of the 19 patients (95%) harboured an isolate with a *cyp51A* mutation (Table 2). Fourteen out of 19 (73.7%) resistant isolates harboured a TR₃₄/L98H mutation derived from the environment. Furthermore, two patients harboured three azole resistant *Aspergillus terreus* isolates, each with a *cyp51A* mutation (Table 2).

continued ... Textbox 5.4, part 2

Table 2 Cyp51A profiles of resistant *A. fumigatus* and *A. terreus* isolates

DANMAP 2018

<i>A. fumigatus</i>		
No. of patients	Cyp51A profile	Azole resistance
13	TR ₃₄ /L98H	Pan-azole resistant
1	TR ₃₄ ³ /L98H*	Pan-azole resistant
2	G54R	Itraconazole and posaconazole resistant
1	G432S	Pan-azole resistant
1	M220K	Itraconazole and posaconazole resistant
<i>A. terreus</i>		
No. of patients	Cyp51A profile	Azole resistance
1**	M217I	Pan-azole resistant
1**	Y491H	Intermediate resistant
1	G51A	Itraconazole and posaconazole resistant

* Patient harboured both TR₃₄/L98H and TR₃₄³/L98H isolates, the latter potentially evolved in vivo

** Same patient with two different mutant isolates

We report a nationwide azole-R rate of 6% in *A. fumigatus* at the patient level from the first six months of the nationwide surveillance. The underlying resistance mechanisms was target gene mutations in all but one case and notably, the vast majority were of environmental origin linked to the use of azole fungicides. The fact that such isolates have increasingly been found in Denmark since 2009 is concerning and suggests that a one-health approach involving human and environmental azole management is necessary to limit further rise in azole-R *A. fumigatus*.

Acknowledgements to the ten Danish Departments of Clinical Microbiology, who have been close collaborators and contribute with isolates to the surveillance program.

We would also like to thank the staff of the laboratory at the Mycology Unit at Statens Serum Institut.

Malene Risum¹, Rasmus Krøger Hare¹, Nissrine Abou-Chakra¹, Jan Berg Gertsen², Lise Kristensen² and Maiken Cavling Arendrup¹
¹Mycology Unit, Statens Serum Institut
²Department of Clinical Microbiology Aarhus University Hospital

References

1. Stensvold CR, Jorgensen LN, Arendrup MC. Azole-Resistant invasive aspergillosis: Relationship to agriculture. *Curr Fungal Infect Rep.* 2012;
2. Astvad KMT, Jensen RH, Hassan TM, Mathiasen EG, Thomsen GM, Pedersen UG, et al. First detection of TR46/Y121F/T289A and TR₃₄/L98H alterations in *Aspergillus fumigatus* isolates from azole-naive patients in Denmark despite negative findings in the environment. *Antimicrob Agents Chemother.* 2014;
3. Jensen RH, Hagen F, Astvad KMT, Tyron A, Meis JF, Arendrup MC. Azole-resistant *Aspergillus fumigatus* in Denmark: a laboratory-based study on resistance mechanisms and genotypes. *Clin Microbiol Infect.* 2016;
4. Mortensen KL, Mellado E, Lass-Flörl C, Rodriguez-Tudela JL, Johansen HK, Arendrup MC. Environmental study of azole-resistant *Aspergillus fumigatus* and other aspergilli in Austria, Denmark, and Spain. *Antimicrob Agents Chemother.* 2010;



6

RESISTANCE IN ZOOONOTIC BACTERIA

6. Resistance in zoonotic bacteria



Highlights: In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea in humans including salmonellosis and campylobacteriosis. This is due to the self-limiting nature of the diseases. If needed, it is recommended that patients are treated with macrolides (azithromycin and erythromycin).

Isolates from animals and meat for susceptibility testing are mainly collected by repeated, representative, national surveys conducted at Danish slaughter houses.

In 2018, the level of azithromycin resistance in *Salmonella* Typhimurium isolates from humans was less than 1%, and 5% in Danish pork. No erythromycin resistance was found in *Campylobacter jejuni* isolates from animals or human patients in 2018.

Resistance to quinolones remained the most common resistance type found in *C. jejuni* from all populations: broilers, cattle and humans. Around one third of all isolates of animal origin and domestically acquired human cases were resistant to ciprofloxacin, whereas 83% of the travel associated human isolates were ciprofloxacin resistant. The majority of ciprofloxacin resistant isolates from poultry and humans were also resistant to tetracycline.

Tetracycline resistance in *C. jejuni* from broilers increased significantly from 16% in 2017 to 32% in 2018 and this increase coincided with an increase among the human isolates of domestic origin from 22% in 2017 to 34% in 2018. Resistance to both tetracycline and ciprofloxacin also increased in isolates from broilers.

Salmonella Typhimurium and *S. Derby* were the two most prevalent serotypes isolated from Danish pigs and pork. About two thirds of the *S. Typhimurium* isolates were monophasic and a similar pattern was observed among the human *S. Typhimurium* isolates. The dominance of clonally related monophasic *S. Typhimurium* isolates influences the resistance patterns in all populations causing high levels of resistance to tetracycline, ampicillin and sulfonamide. About half of all *Salmonella* isolated from Danish pigs and pork were *S. Derby*, where lower levels of resistance were observed.

Of the antimicrobials of critical importance in Denmark, resistance to colistin, 3rd generation cephalosporins and carbapenems remained low or absent in isolates from all populations and fluoroquinolone resistance has not been identified in *S. Typhimurium* from Danish pigs and pork since 2010 and 2007, respectively.

Among human cases resistance to fluoroquinolones remained high among *S. Typhimurium* isolates from travel 25% versus 4% in isolates from cases acquired in Denmark. Resistance to 3rd generation cephalosporins and carbapenems remained very low, less than 1%, in *S. Typhimurium* from human cases and was not found in the *Salmonella* isolates from Danish pigs and pork.

6.1 Introduction

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food, water, vectors or the environment. A detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2018 [www.food.dtu.dk].

Campylobacter and *Salmonella* surveillance has been part of the DANMAP programme since 1995, where isolates from broilers, cattle and pigs as well as from human cases were susceptibility tested. Isolates from samples of fresh meat were included from 1997. Since 2014, sampling and testing of *Campylobacter* and *Salmonella* in animals and foods have been done in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU] and supplemented by additional surveillance addressing national objectives.

Zoonotic bacteria resistant to antimicrobials used for treatment of human patients have consequences for the patient and society by prolonging and/or increasing the severity of the disease. Resistance to antimicrobials not used for treatment of the given disease has less direct importance, but may impact public health either by: i) horizontal transfer of resistance genes to other pathogens resulting in treatment failure of these or ii) by facilitating selection of strains harbouring resistance genes of public health importance, if resistance to both (e.g. co-resistance) are present. The relative importance of the indirect consequences are unknown.

In Denmark, antimicrobials are not recommended for treatment of diarrhoea in patients unless there is prolonged duration or the patient is severely ill. Macrolides (azithromycin and erythromycin) are recommended as drug of choice if treatment is required, regardless of the nature of the disease. [Promedicin: <http://pro.medicin.dk>].

6.2 *Campylobacter*

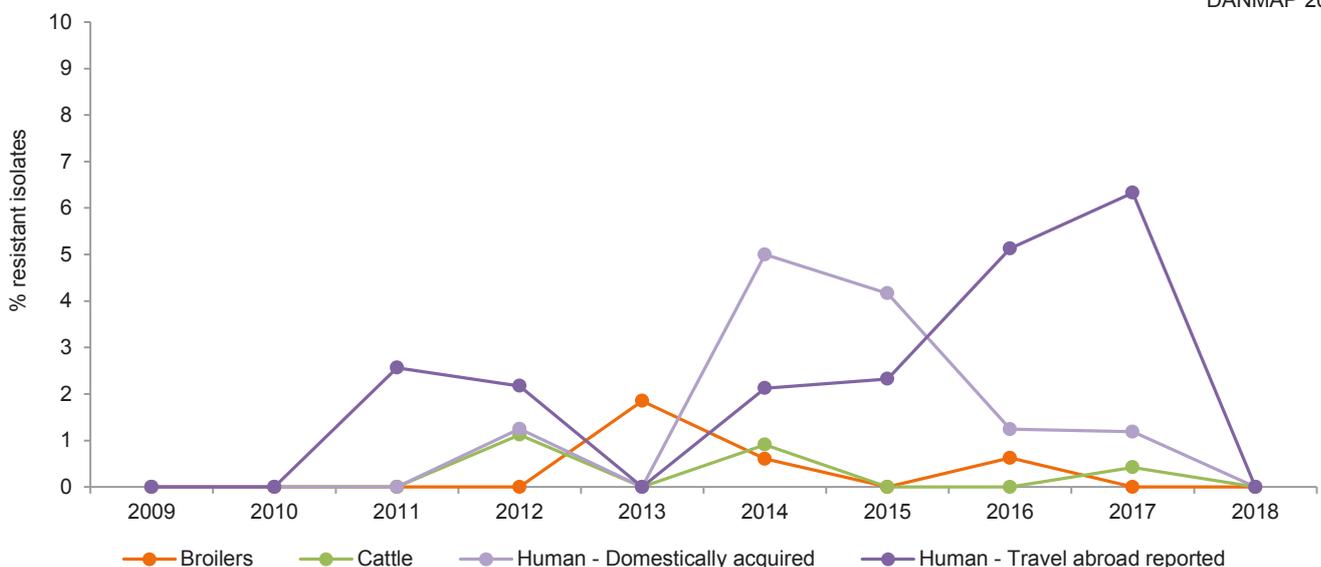
Thermotolerant *Campylobacter* spp. are the most commonly reported cause of gastrointestinal bacterial infections in humans in the EU [ECDC/EFSA 2018. EFSA journal 16(12):5500]. In Denmark, campylobacteriosis is also the most common cause of bacterial gastroenteritis with an estimated 40-60,000 food borne cases per year. Around 7% of these patients seek health care leading to approximately 4,000-5,000 laboratory confirmed cases per year [Pires 2014. DTU report, ISBN: 978-87-93109-31-5]. In 2018, a total of 4,546 human laboratory confirmed cases of campylobacteriosis were reported (78.5 per 100,000 inhabitants) [Annual Report on Zoonoses in Denmark 2018].

Around one third of human *Campylobacter* cases are travel associated. The most common source of domestically acquired cases is poultry meat. Cattle is also an important source and transmission from cattle happens through meat, unpasteurized milk, the environment, and direct contact. Dogs, other food sources and the environment are also sources of *Campylobacter* infections [Kuhn et al. 2018. Clinical Epidemiology 10:1695; Pires 2017. DTU report ISBN: 978-87-93565-10-4].

Macrolides are used to treat infections in animals. In 2018, 12,951 kg of macrolide was prescribed for animals. The major-

Figure 6.1 Erythromycin resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

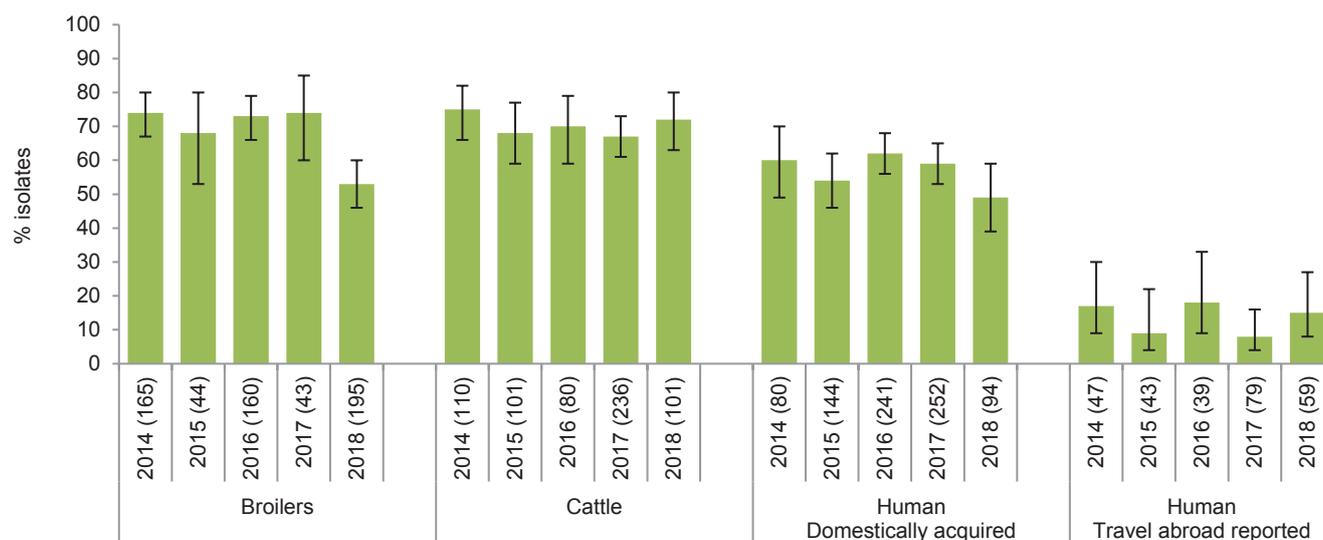
DANMAP 2018



Note: A human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Figure 6.2 Distribution (%) of fully sensitive *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

DANMAP 2018



Note: The number of isolates included each year is shown in parentheses, confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals. An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel

ity (93%) of this was used in pigs, whereas cattle and poultry used 245 kg and 162 kg, respectively. Fluoroquinolones are not used in food production animals and are only prescribed to pets and humans in Denmark.

In 2018, the animal *Campylobacter* isolates for DANMAP were obtained by sampling of randomly selected broiler caeca and cattle caeca at slaughter. In 2018, 836 broiler flocks and 154 cattle <1 year of age were sampled at slaughter. One isolate per farm was susceptibility tested. Sampling, isolation and susceptibility methods followed EFSA's recommendations for animal and food isolates.

In humans, campylobacteriosis is a notifiable disease. For the purpose of DANMAP a selection of isolates from reported human *C. jejuni* cases are susceptibility tested. In 2018, *Campylobacter* isolates were submitted to Statens Serum Institut (SSI) by three clinical microbiological laboratories. The isolates were geographically dispersed and representative of both urban and rural areas of Denmark. Travel history of the patient was collected by the general practitioner when possible, and reported to the diagnostic laboratory with submission of patient samples. The isolates were species typed at SSI and a total of 193 *C. jejuni* isolates were susceptibility tested in accordance with the ECDC recommendations. Only one isolate per patient was tested.

MIC distributions for *C. jejuni* from broilers, cattle and humans are available in the web annex (Tables A6.1- A6.2). For further details on methodology, see chapter 9.

6.2.1 Resistance in *Campylobacter jejuni*

Macrolide resistance in *Campylobacter* is monitored using erythromycin. No erythromycin resistance was observed in *C. jejuni* from animals or humans in 2018 (Table 6.1).

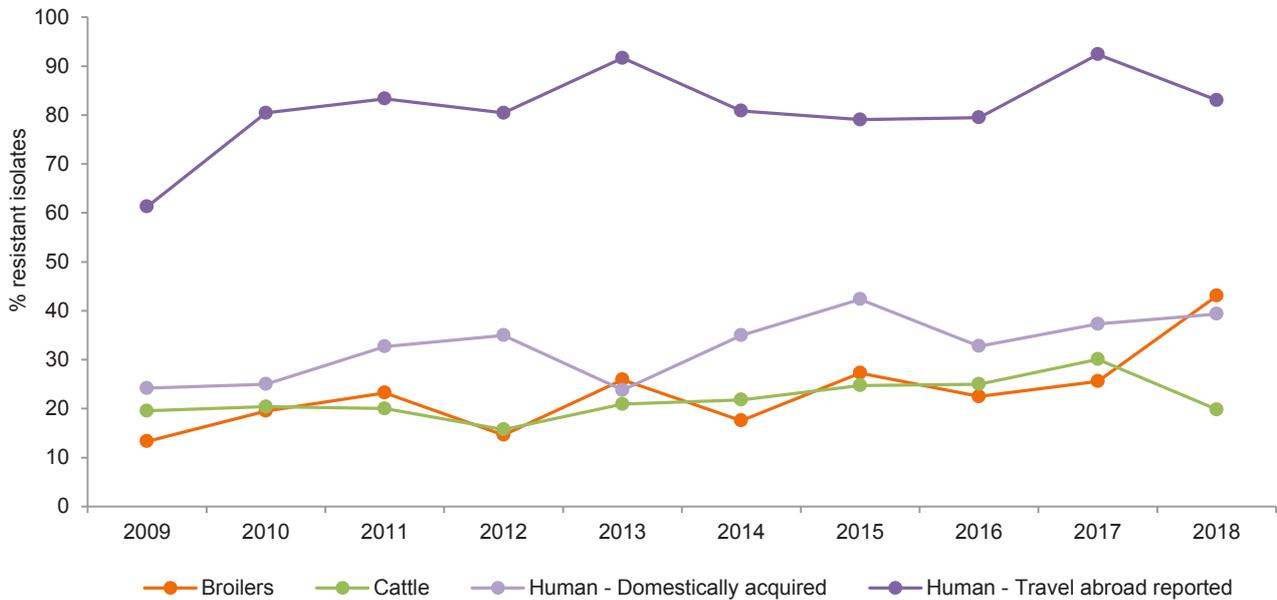
Over the last decade, macrolide resistance was slightly more common in isolates from humans than in animals, but only in low levels. Macrolide resistance never exceeded 7% in any year and only a few erythromycin resistant *C. jejuni* isolates were identified in poultry and cattle in the last decade, varying between zero and two resistant isolates per year (Figure 6.1). This indicates that the actual prevalence of macrolide resistance is very close to the limit of detection by the current sampling scheme. Based on the available number of isolates, we are 95% confident that macrolide resistance is not exhibited by more than 1.5% and 3% *C. jejuni* from broilers and cattle, respectively (see section 9.7).

The proportions of fully sensitive *C. jejuni* from broilers, cattle and humans are presented in Figure 6.2.

Among the domestically acquired human infections, 49% were fully susceptible to all antimicrobials tested (Table 6.1 and Figure 6.2). This is the lowest number of fully sensitive *C. jejuni* reported within the last five years, mainly due to a significant increase in tetracycline resistance, from 22% in 2017 to 34% in 2018 (Table 6.1). The number of fully susceptible strains from patients with a known history of travel was significantly lower than the corresponding number of domestically acquired cases. Similar to previous years, the occurrence of resistance to ciprofloxacin and tetracycline was significantly higher in

Figure 6.3 Ciprofloxacin resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

DANMAP 2018



Note: An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

travel associated *C. jejuni* isolates (83% and 63%, respectively) compared to isolates from domestically acquired infections (39% and 34%, respectively).

Among the human isolates, the most frequent resistance profile was resistance to quinolones only (30/193) or quinolones in combination with tetracycline (64/193), see the AMR profile distributions in web annex Table A6.3.

In broilers, the level of fully susceptible isolates also decreased in 2018 reflecting an increase in isolates with co-resistance to ciprofloxacin and tetracycline; from 16% in 2017 to 28% in 2018. Tetracycline resistance in *C. jejuni* from broilers increased significantly from 16% in 2017 to 32% in 2018. This increase coincided with an increase among the human isolates.

A significant increase was observed in ciprofloxacin resistance in *C. jejuni* from broilers this year and a slow, but statistically significant increasing trend has been observed over the last 10 years (Figure 6.3). An increase in ciprofloxacin resistance is similar to what is observed internationally [EFSA/ECDC 2019. EFSA journal 17(2):5598]. Ciprofloxacin resistance levels were similar in domestically acquired human cases and the main *Campylobacter* sources: broilers and cattle.

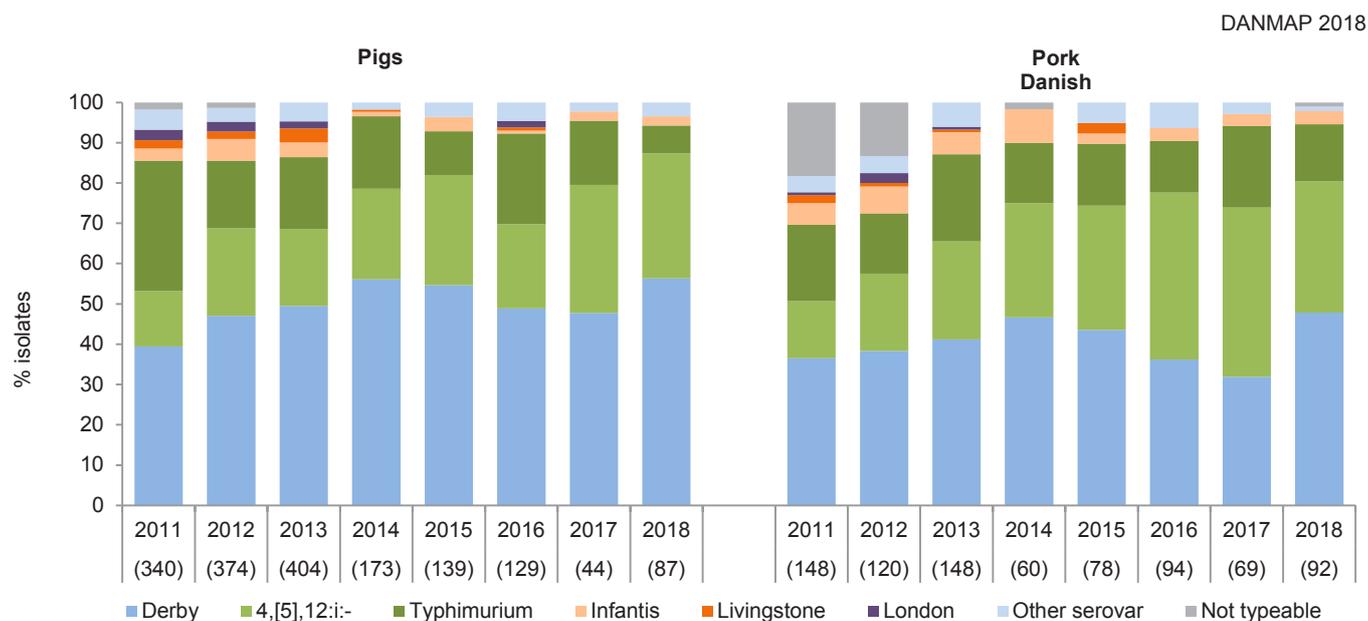
In cattle, the level of fully susceptible isolates was slightly higher in 2018 than in 2016 and 2017 (Figure 6.2). The level of resistance to ciprofloxacin dropped significantly from 30% to 20%. This will be monitored in the coming years to establish whether this positive development continues. Tetracycline resistance level remained the same as in 2017 (Table 6.1).

Fluoroquinolones are not used in food production animals in Denmark - indicating that the continued increase in ciprofloxacin resistance observed in Denmark is driven by something other than the direct usage of fluoroquinolones. In general, the Danish poultry sector uses very little antimicrobials, but tetracycline is the most common antimicrobial used in poultry (Table 4.1). The high level of *C. jejuni* isolates with both ciprofloxacin and tetracycline resistance suggests the pos-

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2018

Antimicrobial agent	Broilers		Cattle		Human		Total
	Danish	Danish	Domestically acquired	Travel abroad reported	Domestically acquired	Travel abroad reported	
Ciprofloxacin	43	20	39	83	39	83	55
Erythromycin	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0
Nalidixic acid	43	20	38	83	38	83	54
Streptomycin	5	4	4	12	4	12	8
Tetracycline	32	8	34	63	34	63	45
Fully sensitive (%)	53	72	49	15	49	15	38
Number of isolates	195	101	94	59	94	59	193

Note: An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin

Figure 6.4 Relative distribution (%) of *Salmonella* serotypes from pigs and pork, Denmark

Note: Number of isolates included each year is presented in the parenthesis. Isolates from pigs originate from caecum samples collected at slaughter and isolates from pork originate from carcass swabs collected after slaughter for the national control programme

sibility of co-selection of ciprofloxacin resistance by the use of tetracycline in poultry. Whether that is what happens in reality, warrants further investigation.

As the previous years, no resistance to gentamicin was observed in 2018 (Table 6.1), providing 95% confidence that resistance to the antimicrobial is only present in 1.5% or less of the *C. jejuni* isolates from broilers. Gentamicin resistance was not observed in any of the human isolates.

6.3 *Salmonella*

Salmonella is the second most frequent zoonotic bacterial pathogen in humans in Denmark and can have a severe impact on both animal and human health [Annual Report on Zoonoses in Denmark 2018].

In Denmark, *S. Typhimurium* and *S. Enteritidis* are the serotypes that most frequently are associated with human illness. Human cases caused by *S. Enteritidis* are frequently associated with consumption of contaminated eggs, whereas *S. Typhimurium* cases often are associated with contaminated pork. Many *Salmonella* cases are travel associated and in 2018, 45% of cases reported travel history in association with their illness.

Salmonella isolates for DANMAP 2018 were obtained from national surveillance and control programmes. Pig isolates originated from slaughterhouses, where representative samples from healthy pigs (caecum) and pork (carcass swabs) are collected. Salmonellosis is a notifiable disease in humans

and isolates from the reported *S. Typhimurium* cases are susceptibility tested and included in the DANMAP report. Only one isolate per farm, meat sample or human case was included in this report. For further details see chapter 9.

The occurrence of *Salmonella* in broilers, layers and cattle are monitored in Denmark each year. However, these isolates are not included in DANMAP 2018, as only few isolates were found and thus, fall below the inclusion threshold for DANMAP of 15 isolates per population. The occurrence of resistance in human *Salmonella* isolates other than *Typhimurium* is also monitored, but the results are not included in the DANMAP report. However, the data are reported to EFSA and ECDC, and are included in the European Union summary report on antimicrobial resistance, 2018.

The DANMAP report focuses on the resistance in *S. Typhimurium* and its monophasic variants. However, resistance in other *Salmonella* serotypes from pigs and pork is also monitored from 2011 and onwards, which is the year Denmark started to susceptibility test all serotypes according to EU legislation.

In DANMAP, *S. Typhimurium* includes the monophasic variants with antigenic formulas *S. 4, [5],12:i:-*, unless otherwise stated.

The antimicrobials recommended by EFSA were used for susceptibility testing. MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.4 - A6.8).

6.3.1 *Salmonella* in Danish pigs and pork - all serotypes

From 553 representative pig caeca and 18,994 pig carcass's (pork) sampled at Danish slaughterhouses, 179 *Salmonella* isolates were obtained. A total of 161 of these were tested for antimicrobial resistance. As in the previous years, *S. Typhimurium* and *S. Derby* were the most common serotypes, representing 94% of all the isolates (Figure 6.4). In recent years, the relative occurrence of *S. Derby* has decreased, and in 2015 *S. Typhimurium* replaced *S. Derby* as the most prevalent serotype from domestically produced pork. In 2018, *S. Derby* was again the most dominant serovar, albeit with a small majority only.

A total of 32% of the *Salmonella* isolates from pigs were multidrug-resistant (defined as resistance to 3 or more of the 12 antimicrobial classes in the test panel, see Table 9.5). As 16% of the pig caeca was *Salmonella* positive, this indicates that approximately 900,000 of the 18 million pigs slaughtered in Denmark during 2018 carried multidrug-resistant *Salmonella*.

6.3.2 *S. Typhimurium* in Danish pigs and pork

S. Typhimurium remains the most important zoonotic serotype originating from pigs in Denmark. A total of 76 *S. Typhimurium* including 19 diphasic and 57 monophasic variants, were isolated from Danish pigs and pork in 2018. Most of the susceptibility-tested isolates were resistant to one or several antimicrobials and only 11% and 13% of the isolates from pigs and pork were fully susceptible to all antimicrobial agents in the test panel (Table 6.2).

As in the previous years, resistance to ampicillin, tetracycline and sulfonamide were common in the *S. Typhimurium* isolates from pigs and pork. Over the last five years, resistance to trimethoprim and chloramphenicol have increased in isolates from pork and in 2018, they were more frequent in isolates from pork than from pigs. Resistance to ampicillin and sulfonamide increased significantly in *S. Typhimurium* from pigs from 2017 to 2018 (48% vs. 75% and 52% vs. 82%, respectively). This follows the trend seen over the last 10 years where statistically significant increases in resistance to ampicillin, sulfonamide and tetracycline have occurred in isolates from both pigs and pork (Figure 6.5).

This year, 57% of the *S. Typhimurium* isolates from pigs and pork carried the ASuT resistance profile and 63% were multidrug-resistant (Figure 6.6), see the AMR profile distributions in web annex Table A6.9. One diphasic isolate was resistant to tigecycline (Table 6.2) with a MIC value of 4 µg/ml. Tigecycline resistance is rare in Denmark and was only previously detected in DANMAP samples from pigs in 2014, where two isolates were resistant (MIC = 2 µg/ml). The 2018 isolate was multidrug-resistant and further resistant to: ampicillin, chloramphenicol, sulfonamide, tetracycline and trimethoprim.

Two multidrug-resistant monophasic isolates from pork were resistant to azithromycin. They were both also resistant to

ampicillin, sulfonamide, tetracycline and trimethoprim and one isolate further to chloramphenicol. Azithromycin is an important antimicrobial for treatment requiring diarrhoea in humans in Denmark.

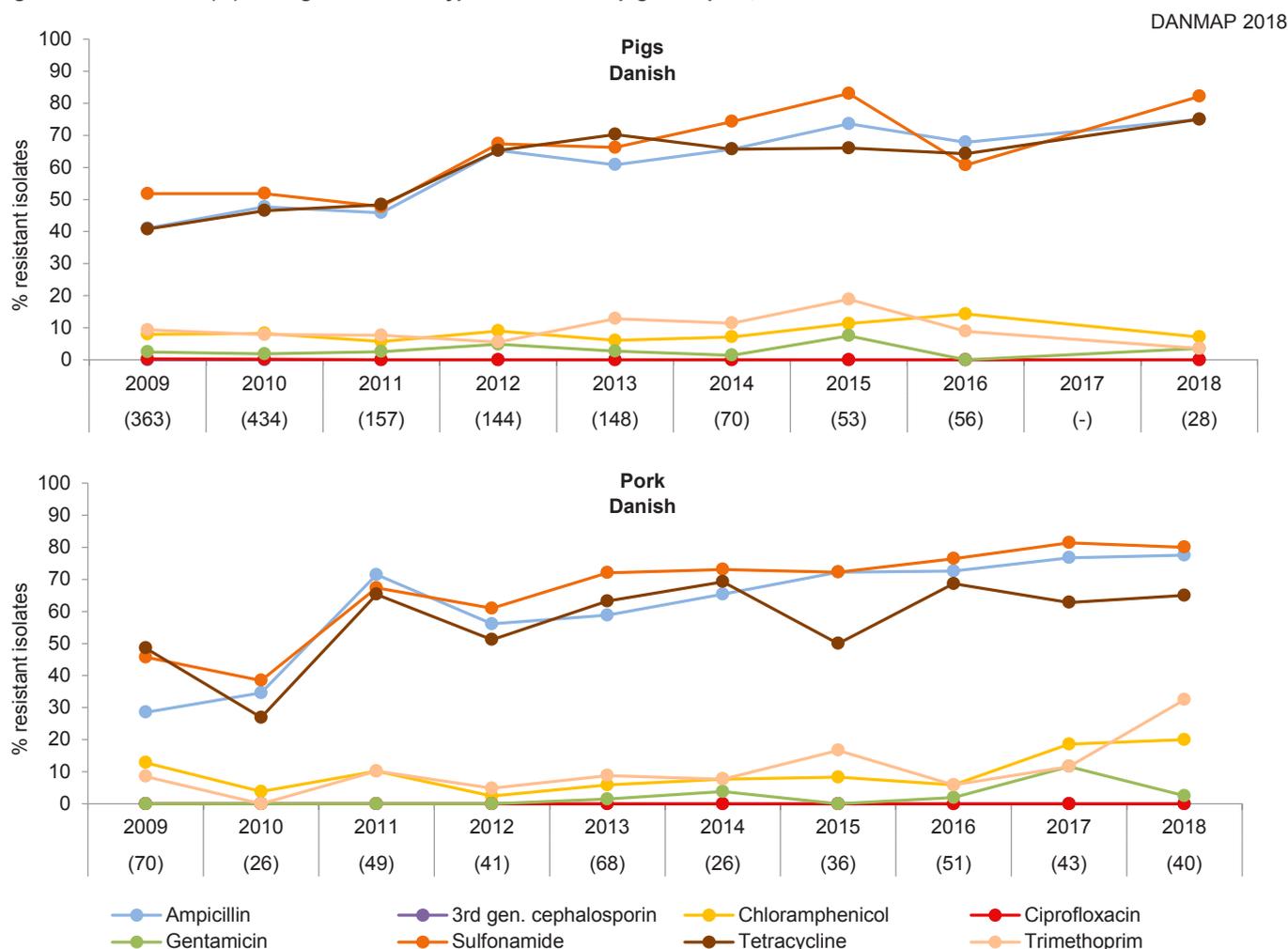
None of the *S. Typhimurium* isolates from pigs or domestic pork were resistant to quinolones, cephalosporins, colistin or carbapenems. Based on the available number of isolates, we are 95% confident that these resistances are not present in more than 10% of *S. Typhimurium* isolates from pigs and 7% *S. Typhimurium* from pork (see section 9.7).

Tetracyclines, macrolides, pleuromutilins and beta-lactamase sensitive penicillins are the main antimicrobial agents used in pigs in Denmark (Figure 4.4). The distinct reduction in usage of tetracycline over the last 4-5 years in pigs were still not reflected in the 2018 levels of resistance in *S. Typhimurium* from Danish pigs and pork.

Table 6.2 Resistance (%) in *Salmonella Typhimurium* isolates from pigs, pork and human cases, Denmark DANMAP 2018

Antimicrobial agent	Pigs		Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
	%	%	%	%	%
Ampicillin	75	78	67	69	64
Azithromycin	0	5	<1	0	<1
Cefotaxime	0	0	<1	3	<1
Ceftazidime	0	0	<1	2	<1
Chloramphenicol	7	20	8	23	10
Ciprofloxacin	0	0	4	25	8
Colistin	0	0	1	2	2
Gentamicin	4	3	1	6	2
Meropenem	0	0	0	0	0
Nalidixic acid	0	0	2	11	4
Sulfonamide	82	80	66	63	62
Tetracycline	75	65	70	72	71
Tigecycline ^{a)}	0	3	1	3	2
Trimethoprim	4	33	10	22	10
Fully sensitive (%)	11	13	22	8	22
Number of isolates	28	40	146	65	305

Note. Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-. An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin
a) All human isolates classified as tigecycline resistant had a MIC value that was one dilution step higher than the ECOFF

Figure 6.5 Resistance (%) among *Salmonella* Typhimurium from pigs and pork, Denmark

Note: Number of isolates included each year is presented in the parenthesis and '-' indicate data that less than 25 isolates ($n = 21$) from pigs were included for 2017. Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-. Isolates from pigs originate from caecum samples collected at slaughter (2011-2018) and boot swabs collected at farms (2009-2013). Isolates from pork originate from carcass swabs collected after slaughter for the national control programme

The 2018 increase in the use of macrolides for weaner and finisher pigs did not result in increased resistance to azithromycin (Figure 4.4). However, this will be monitored in the coming years.

6.3.3 Resistance in other relevant *Salmonella* serotypes in Danish pigs and pork

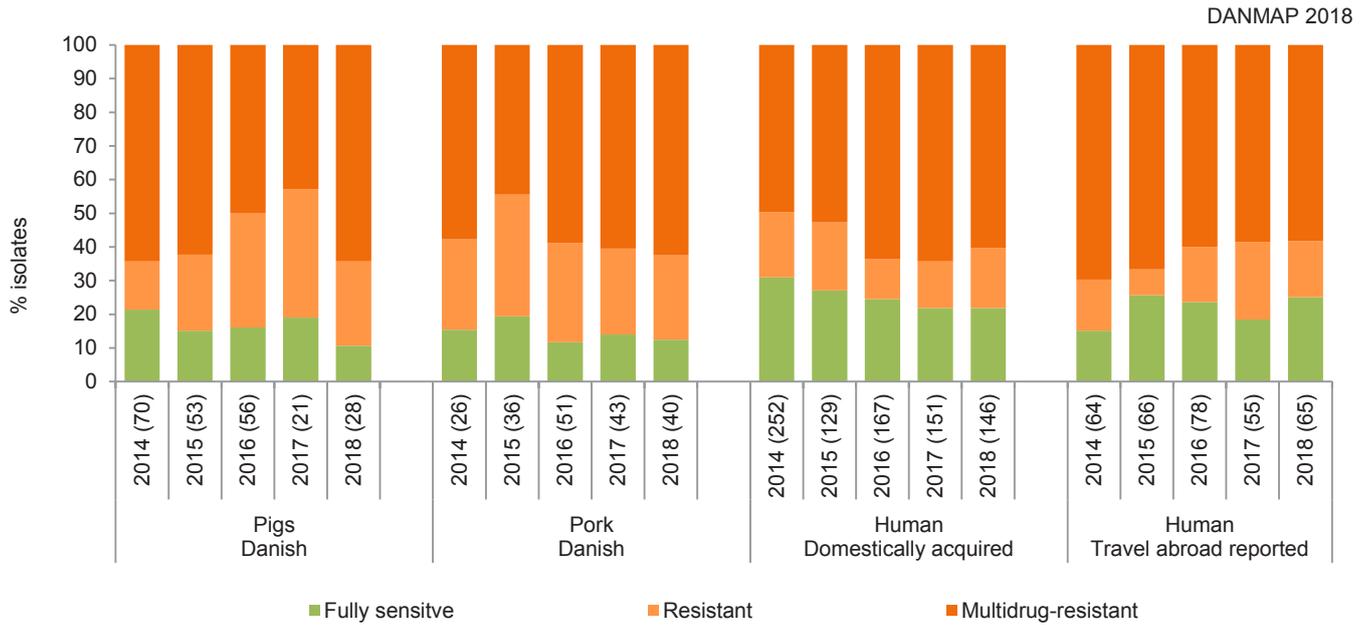
S. Derby was isolated from 49 slaughter pigs and from 44 pork samples. *S. Derby* is common among pigs, but only 12 human cases were reported in Denmark in 2018. In the last couple of years, a declining trend of fully susceptible *S. Derby* isolates has been observed. In 2015, 72% of *S. Derby* isolates were susceptible to all antimicrobials tested, in 2016 the proportion was 63%, and in 2017 58% were fully susceptible. This year, 70% of *S. Derby* isolates were again fully susceptible to all antimicrobials tested, bouncing back to 2015 levels. Resistance to tetracycline, sulfonamides, trimethoprim and ampicillin were most common, either alone or in combination. Only 13% of isolates were multidrug-resistant, one of these was further resistant to gentamicin and four to chloramphenicol.

Resistance to ciprofloxacin without simultaneous resistance to nalidixic acid was found in one isolate, suggesting plasmid-mediated quinolone resistance (PMQR). WGS of the isolate was examined through ResFinder 4.0, confirming the presence of the PMQR-gene *qnrS1*. This is the first registered case of PMQR in *Salmonella* from Danish pigs. The isolate was further resistant to chloramphenicol and ampicillin, introducing a possible risk of spread through co-selection, as ampicillin especially is commonly used in the pig production.

6.3.4 *Salmonella* in humans

A total of 1,168 human laboratory-confirmed cases of salmonellosis were reported (20.2 cases per 100,000 inhabitants). The most common serotypes were *S. Typhimurium* (including the monophasic variants) and *S. Enteritidis* with 5.3 and 4.6 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2018].

Figure 6.6 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Salmonella* Typhimurium from pigs, pork and human cases, Denmark



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:-. An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included (Table 9.3)

6.3.5 *S. Typhimurium* in humans

The serotypes of *Salmonella* were derived from whole genome DNA sequence data. *S. Typhimurium*, including the monophasic variants, were most commonly identified among the human cases (306 cases) and MIC data from 305 of these isolates were included in this report. The monophasic variants represented 64% of the *S. Typhimurium* cases (196 monophasic and 109 diphasic). Information on patient travel history was available for 69% of the 305 cases, 21% of the cases were categorised as travel associated, 48% were acquired in Denmark, and the remaining cases had unknown travel status (Table 6.2). A total of 89 human cases were considered 'outbreak-related' of which 65 cases were associated with the monophasic variant. Two large outbreaks caused by the monophasic variant were recorded compromising 43 (ASuT) and 17 (fully sensitive) patients respectively. The other outbreak related cases compromised less than eight cases. All outbreaks, except one affecting six patients, were domestic.

As in the previous years, high levels (62-71%) of resistance to sulfonamide, ampicillin and tetracycline were observed (Table 6.2). Isolates that exclusively exhibited resistance towards these three antimicrobials were observed in 126 isolates of which 120 were monophasic. The large number of monophasic isolates exhibiting the ASuT phenotype is associated with a successful clone that has emerged worldwide over the last few years. See the AMR profile distribution in the web annex Table A6.9.

The levels of resistance in isolates from domestically acquired cases are overall at the same levels as in the previous years (Figure 6.7). The level of trimethoprim resistance increased from 1% in 2017 to 10% in 2018, a level that is in accordance with the levels observed in 2015 and 2016.

The level of resistance in isolates from human cases associated with travel were also in line with the observed levels in the previous years and were significantly higher than the level observed among isolates from domestic cases (Figure 6.7). Fluoroquinolone resistance increased from 11% to 25% from 2017 to 2018 in isolates from travel associated cases.

Resistance to colistin was observed in isolates from both domestically acquired (1%), and travel associated human cases (2%) and gentamicin resistance was also found in both domestic (1%) and travel associated isolates (6%).

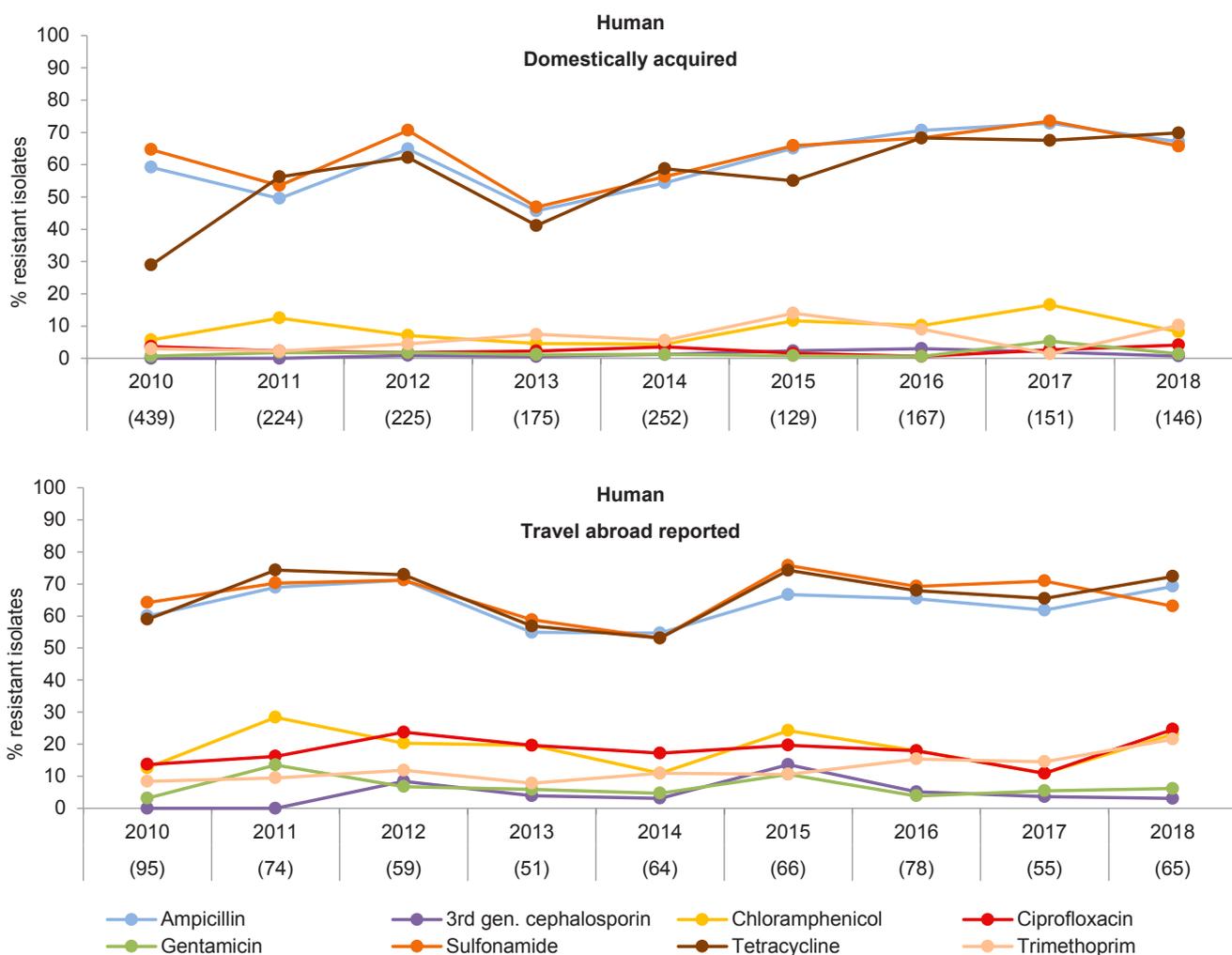
The level of cephalosporin resistance was overall <1% for both cefotaxime and ceftazidime. Carbapenem resistance was not observed in any of the tested strains.

*Johanne Ellis-Iversen, Helle Korsgaard,
Channie Kahl Petersen and Jeppe Boel*

*For further information: Johanne Ellis-Iversen,
joell@food.dtu.dk or Jeppe Boel, jeb1@ssi.dk*

Figure 6.7 Resistance (%) among *Salmonella* Typhimurium from human cases, Denmark

DANMAP 2018



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-. An isolate is categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

Textbox 6.1

Antimicrobial resistance in *Salmonella* from the national control programme in sow, multiplier and breeder pig herds

Background: Serological surveillance of *Salmonella* in Danish pig herds has been performed since 1995 (Order no. 1426 of 30/11/2018). Pigs from breeding and multiplying herds are blood sampled monthly and slaughter pig herds are monitored continuously by serologic testing of "meat juice" collected at slaughter. The number of samples and frequency of sampling are determined by the size of the herd and previous levels of sero-positive samples (see Annual Report on Zoonoses in Denmark for details). If the level of sero-positive samples exceeds the specified cut-off level, the herd and its supplier herds will be considered for on-farm bacteriologic confirmatory testing according to the criteria specified in the legislation. All *Salmonella* isolates recovered from these visits are susceptibility tested as part of the surveillance of critical resistance (Order no. 1426 of 30/11/2018). The farm derived isolates were included in DANMAP until 2014, when confirmatory testing of slaughter pig herds was discontinued. This and other changes to the legislation resulted in a non-representative sample of the remaining herds and the isolates were excluded from DANMAP.

The level of *Salmonella* is decreasing or remains at low levels in all animals in Denmark. This is a positive development, but it reduces the sensitivity of the *Salmonella* AMR surveillance. The number of isolates available for MIC testing from the annual surveys of slaughter pigs and meat is decreasing, hampering the ability to detect especially low level, new and emerging resistance.

The aim of this study was to investigate, whether the *Salmonella* isolates from sow, multiplier and breeder herds would enhance the current national surveillance of antimicrobial resistance in *Salmonella* from finisher pigs, if included as an additional surveillance component. To assess the surveillance value, we investigated the representativeness of the herds and AMR patterns for new information not already captured by the DANMAP slaughterhouse survey.

Data and data sources: The pen-faecal samples collected for the bacteriologic confirmatory testing were analysed at private industry laboratories and isolates were sent for serotyping and susceptibility testing at DTU National Food Institute (2014-2016) and the DVFA laboratory (2017-2018). For further details, see the Materials and methods in DANMAP 2014 and 2018.

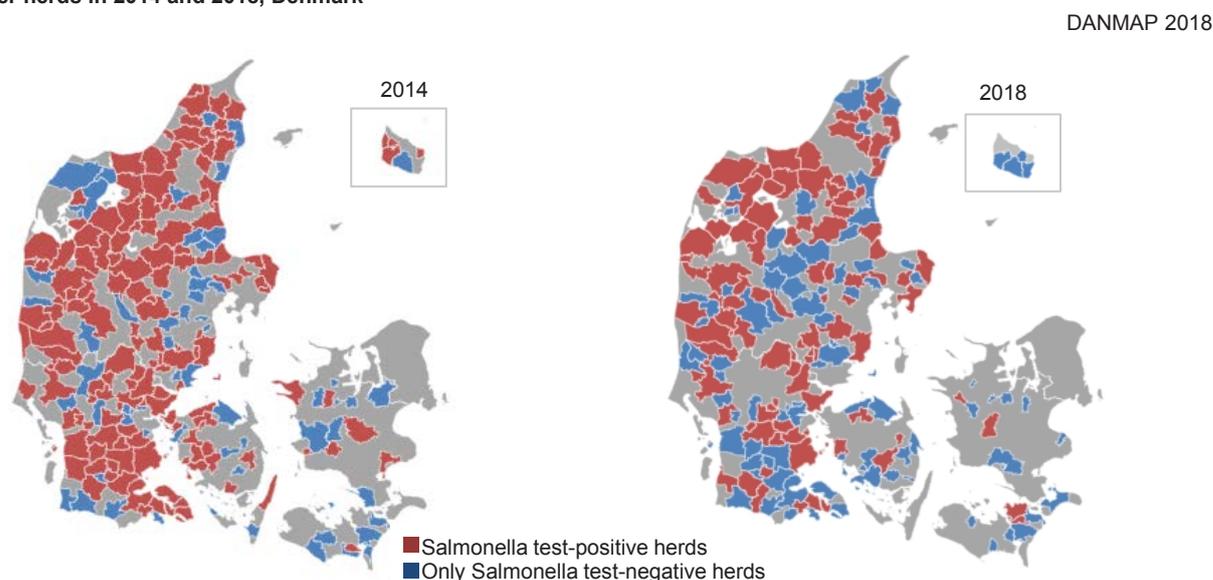
The official Zoonosis Register (Zoor) stores information on sampling and test results from the National *Salmonella* surveillance in the Danish pig production, and provided information on the herds where on-farm tests were performed during 2014-2018 (Accessed, 9. April 2019). From the DTU and DVFA Laboratory databases, the first isolate per serovar per year per herd from confirmatory testing in sow, multiplier and breeder herds during 2014-2018 was selected for inclusion. The test panel used in 2014 was slightly different than the panel used during 2015-2018 [DANMAP 2014, Table 9.1].

Results and discussion: Data from the official Zoonosis Register show that the annual number of performed confirmation tests in herds with sows, weaners and breeder pigs decreased during the five year period; from 625 tests (in 431 herds) during 2014 to 392 tests (in 298 herds) during 2018. The main reason for this decrease is that if herds are positive for *S. Typhimurium* (incl. monophasic variants), *S. Derby*, *S. Infantis* or *S. Choleraesuis*, they are considered positive for 60 months. As re-testing within this period is not performed, the size of the susceptible population is gradually reduced. Furthermore, testing is not performed in herds with recent sero-negative test results from pigs at slaughter or in herds found *Salmonella* positive with other serovars during the previous six months. These comprehensive exemption rules are implemented to optimise the use of resources and minimise cost for the private industry. However, it significantly reduces the number of available isolates for AMR testing and the continuously changing susceptible population makes it almost impossible to assess the representativeness of the isolates for AMR surveillance.

The geographical distribution of the tested herds visually reflected the general distribution of pig producers in Denmark. Most of the tested herds were located in Jutland, but herds from all regions were tested each year during the five year period. Figure 1 presents the regional distribution of the *Salmonella* tested sow, multiplier and breeder herds in 2014 and 2018. It shows zip-code areas without performed tests (grey), with only test-negative herds (blue) and with at least one *Salmonella* test-positive herd (red).

continued ... Textbox 6.1

Figure 1 Regional distribution (zip-code areas) of performed confirmatory tests for detection of *Salmonella* in sow, multiplier and breeder herds in 2014 and 2018, Denmark



Note. Confirmatory testing were conducted in 431 herds in 2014 and 298 herds in 2018, where some herds were tested more than once. Zip-code areas without performed tests are coloured grey

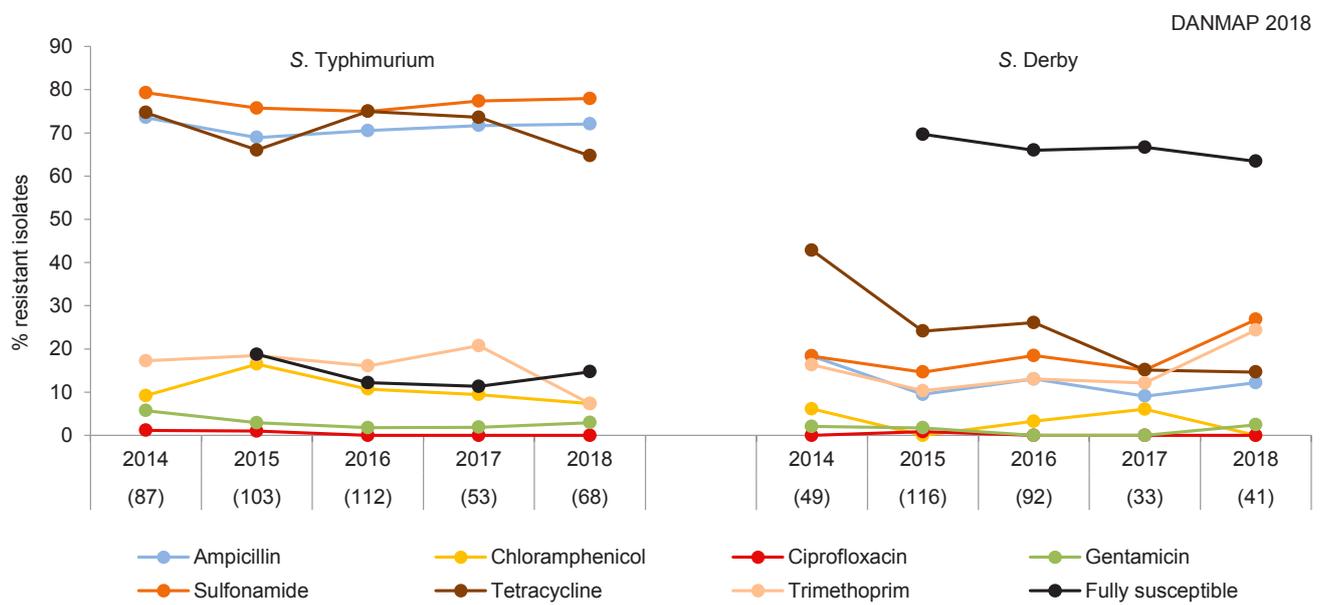
When including only one serovar per herd per year, MIC data from a total of 955 isolates were available from the period 2014-2018. Only few herds are represented in more than one year for each serovar (4% for *S. Typhimurium* and 1% for *S. Derby*). Very few of these isolates were resistant to antimicrobial agents considered of critical importance for treatment of human infections. During the five years, azithromycin resistance was observed in 15 of 736 tested isolates (2%), and very few isolates were resistant to 3rd generation cephalosporins (1 of 945 isolates), fluoroquinolones (3 of 945 isolates) and colistin (4 of 955 isolates). Meropenem resistance was not observed in the 770 tested isolates.

The levels of resistance in *S. Typhimurium* incl. monophasic variants (473 isolates) and *S. Derby* (347 isolates) in the farm samples (Figure 2) are very similar to those of the slaughter pig caeca (Figure 6.5) during the five year period. In *S. Typhimurium*, stable and high levels of resistance to ampicillin, sulfonamide and tetracycline, low to moderate levels of resistance to trimethoprim and chloramphenicol and very low or no resistance towards the other compounds in the test panel were observed. Among the *S. Derby* isolates, the proportion of tetracycline resistance decreased significantly over the five years period, whereas resistance to the other compounds remained at the same low to moderate levels. No significant change in proportions of fully susceptible isolates were observed during 2015-2018 (Figure 2), but in all years the proportion of fully susceptible isolates was significantly higher among *S. Derby* compared to *S. Typhimurium* (63% vs. 15% in 2018, respectively).

Conclusions: The temporal and spatial patterns of sow, multiplier and breeder herds with *Salmonella* confirmatory tests in 2014-2018 are widely distributed at the national level. This allows consideration of the AMR results inclusion in DANMAP. However, as the rules for exemption from testing are very comprehensive and both affect herds that are presumed *Salmonella* negative as well as *Salmonella* positive, these data are not robust as evidence for changes in observed numbers of test-positive herds, changes in serovar distribution or changes in occurrence of antimicrobial resistance in *Salmonella* spp. over time. Nonetheless, the observed levels of resistance in *S. Typhimurium* and *S. Derby* from sow, multiplier and breeder herds from 2014-2018 supported the reported AMR levels in finisher pigs. Due to the findings and the uncertainty around denominator data, the component will not have sufficient surveillance value to be reported annually, but will be summarised every 3 to 5 years.

Helle Korsgaard, Johanne Ellis-Iversen
For further information: Helle Korsgaard, hkor@food.dtu.dk

Figure 2 Resistance (%) among *Salmonella* Typhimurium and *Salmonella* Derby from sow, multiplier and breeder herds, Denmark



Note: Number of isolates included each year is presented in the parenthesis. Isolates originate from boot swabs collected at the farms. *S. Typhimurium* includes isolates verified as monophasic variants with antigenic formulas S. 4,[5],12:i:-

Textbox 6.2

Resistance in bacteria from diagnostic submissions from pigs

Background and data source: Data on antimicrobial susceptibility of three important veterinary pathogens *Escherichia coli* O149, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae* were obtained from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup during 2018. The number of isolates belonging to other bacterial species was too low to follow annual trends.

The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Internationally approved clinical breakpoints are not available for most of the drug-bacterium combinations, so the occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Note that in 2019, the clinical breakpoint for colistin resistance was adjusted according to EUCAST clinical breakpoints for enterobacteriaceae (>2 µg/ml; however >8 µg/ml was maintained for *Salmonella*), and these breakpoints are used retrospectively here.

MIC distributions and occurrence of resistance are presented in the web annex (Tables A6.10 - A6.12).

Table 1 Resistance (%) among bacteria from diagnostic submissions from pigs, Denmark

DANMAP 2018

Antimicrobial agent	<i>Actinobacillus pleuropneumoniae</i> %	Haemolytic <i>Escherichia coli</i> %	<i>Streptococcus suis</i> %
Amoxicillin/clavulanic acid	-	2	-
Ampicillin	0	61	-
Apramycin	-	15	-
Cefotaxime	-	2	16
Ceftiofur	0	0	-
Chloramphenicol	-	21	0
Ciprofloxacin	0	0	2
Colistin	-	0	-
Erythromycin	100	-	49
Florfenicol	0	13	0
Gentamicin	-	13	0
Nalidixic acid	-	7	-
Neomycin	-	16	-
Penicillin	0	-	0
Spectinomycin	0	47	16
Streptomycin	-	74	36
Sulfametoxazol	-	-	70
Sulfonamide	-	67	-
Sulfonamide/trimethoprim	0	-	4
Tetracycline	3	61	48
Tiamulin	1	-	12
Tilmicosin	0	-	-
Trimethoprim	-	55	5
Tulathromycin	4	-	-
Number of isolates	102	282	111

Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.10 - A6.12)

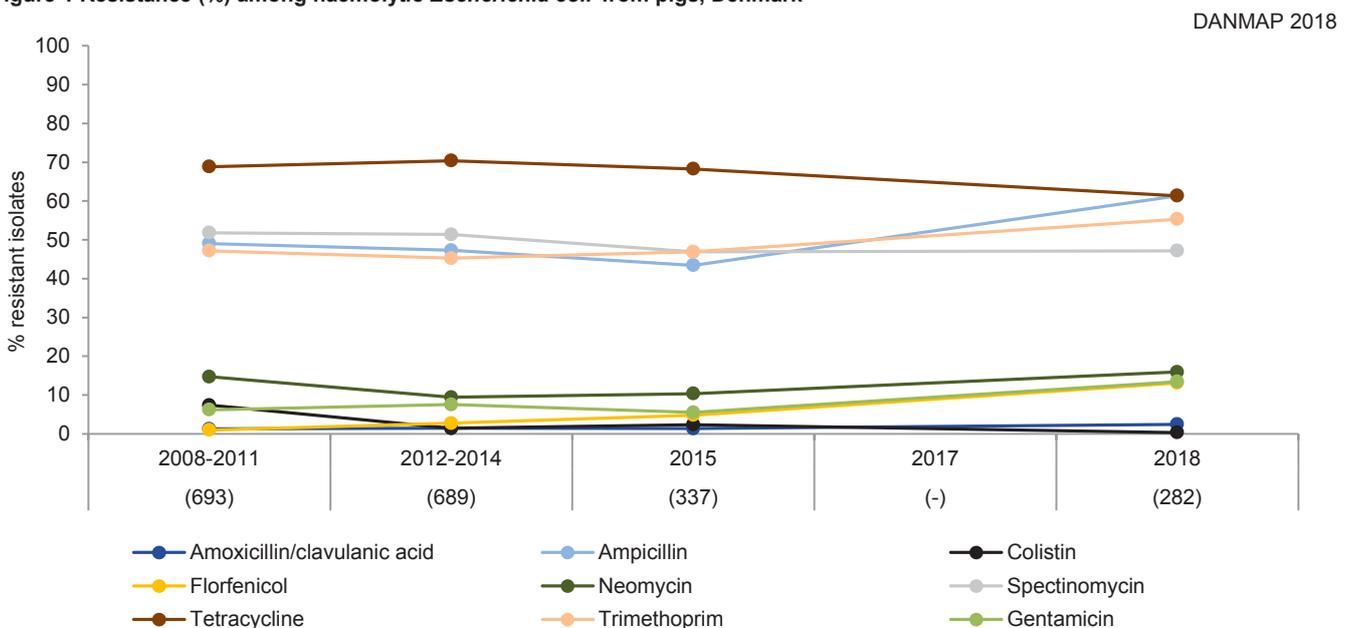
E. coli - haemolytic pathogenic strains

Enterotoxigenic *E. coli* (ETEC), *Brachyspira pilosicoli* and *Lawsonia intracellularis* are the most prevalent causes of bacterial diarrhoea in Danish pigs. Also, these bacteria are often found in combination. In previous years, the *E. coli* isolates have been identified by serotyping, with the most virulent ETEC strains belonging to serovars O138, O139, O141, and O149, which are haemolytic and mostly positive for enterotoxin, and/or verotoxin 2e. Only a minor part of the haemolytic strains, 13% in 2018, were from edema disease cases (O139, F18⁺ VT2e⁺, enterotoxin negative). These strains are also mostly positive for F4 or F18 fimbrial adhesins, which are used for attachment to the intestinal mucosa. The haemolytic *E. coli* reported here originates almost exclusively from porcine enteritis or edema disease.

Since 2014, PCR identification has been the most frequent method for identification of the diarrhoeal pathogens in Denmark, including identification of *E. coli* F4 and *E. coli* F18. Since 2018, almost all haemolytic *E. coli* strains sent for susceptibility testing have been typed for fimbrial adhesins and the strains are now rarely serotyped. In general, the F18 positive strains belong to the serovars O138, O139 and O141, while serovar O149 carry the F4 fimbriae. However, this is not a clear cut correlation. Furthermore, the fimbriae types are not available in the MIC dataset. Consequently, the pattern of antimicrobial resistance in *E. coli* in 2018, cannot be compared on serotype level to the occurrence of resistance in previous years. In Figure 1, we have chosen to compare with data for all haemolytic *E. coli* isolated from pigs in 2015 and previous years.

As in previous years, high resistance levels were recorded in 2018 for ampicillin, streptomycin, sulphonamides, tetracyclines, trimethoprim, and spectinomycin (Figure 1). The level of resistance to tetracycline appeared (not statistically significant) lower in 2018 compared to 2015 (61% vs. 68%), suggesting a decreasing trend in parallel with the trend in tetracycline resistance in *E. coli* O149 reported in DANMAP 2017. Most cases of porcine diarrhoea that require treatment occur during the weaning period and tetracyclines, neomycin, or aminopenicillins are the compounds of choice in case of *E. coli* infection. However, the use of

Figure 1 Resistance (%) among haemolytic *Escherichia coli* from pigs, Denmark



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.10)

continued ... Textbox 6.2

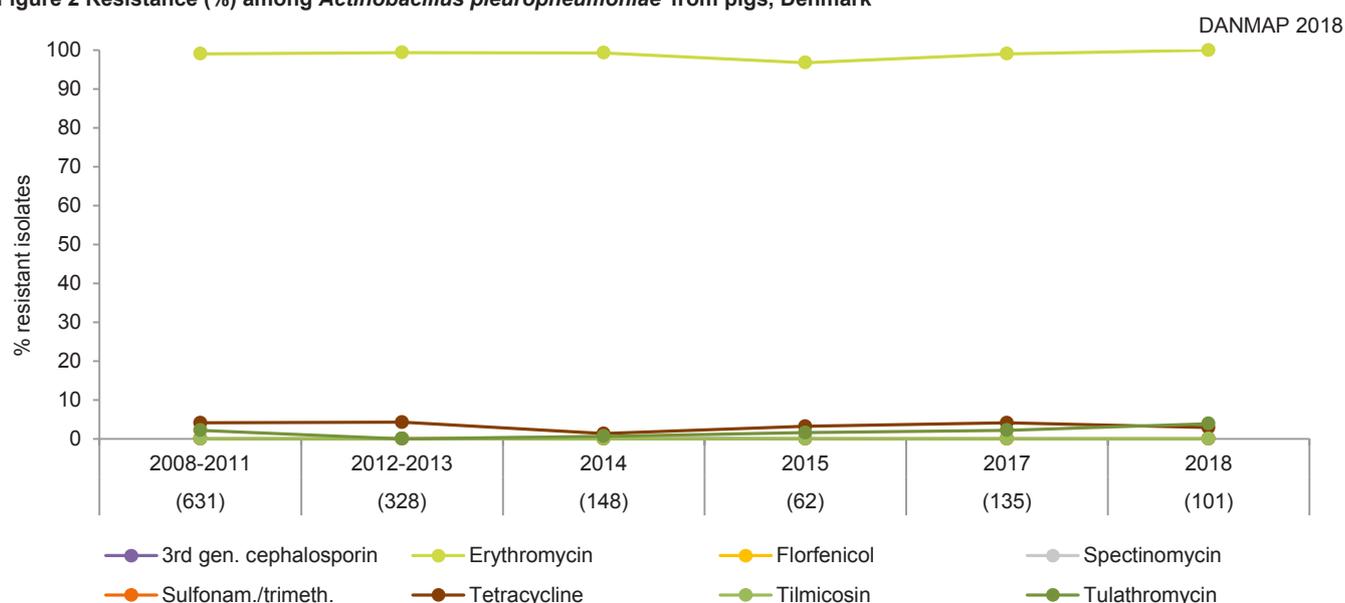
tetracyclines has decreased significantly from 2016 and onwards, most likely due to changes in the Yellow Card legislation (putting higher weights to tetracyclines, see Textbox 4.1). In 2018, a significant increase was observed in the level of resistance to ampicillin, neomycin, gentamicin and apramycin (Figure 1). The parallel increase in resistance among the two latter were most likely a result of cross resistance, as 78% of the apramycin resistant isolates were also resistant to gentamicin (Table 1). In pigs, the aminoglycosides are only used for gastrointestinal infections, and particularly the use of neomycin has been increasing in recent years, after a reintroduction in 2017. It is uncertain whether the increasing level of resistance in these compounds and the apparent decrease in tetracycline resistance is due to natural variation or whether it is a trend following changes in the pattern of antimicrobial usage. A significant increase was also noted for florfenicol, as part of a gradual increase during the last decade, with 13% in 2018 (Figure 1). The reasons for this apparent steady increase need further investigation. Isolates that were resistant to florfenicol were also resistant to chloramphenicol, but resistance levels to chloramphenicol did not increase significantly.

For *E. coli*, the breakpoint for colistin was adjusted from $>8 \mu\text{g/ml}$ to $>2 \mu\text{g/ml}$. This revealed that colistin resistance has decreased significantly in recent years from a very low level to absent in 2018. Colistin was never among the most frequently used compounds, but the usage of colistin declined close to zero during 2017, due to changes in official guidelines and the Yellow Card. The relatively high resistance levels to many compounds increase the benefits of susceptibility testing before treatment.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae causes pleuropneumonia in pigs. It is a severe infection although severity varies with serotype. Therefore, outbreaks require rapid onset of treatment to minimise losses. Fortunately, *A. pleuropneumoniae* has a predictable resistance pattern, with very low occurrence of resistance to most compounds. Almost all isolates are resistant to erythromycin but have very low occurrence of resistance to other macrolides like tilmicosin, which are often used for treatment (Figure 2).

Figure 2 Resistance (%) among *Actinobacillus pleuropneumoniae* from pigs, Denmark



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.11)

Tulathromycin is also frequently used, but the occurrence of resistance has increased numerically (not statistically significantly) in recent years from around 1-2% during 2008-2017 to 4% in 2018 (Table 1). Although the resistance is still at a low level, alternatives should be considered for treatment, due to the risk of treatment failure, and because of the risk of continued increases in tulathromycin resistance. Multiple alternatives are available: No resistance to florfenicol, sulphonamide-trimethoprim and tilmicosin has been observed for the last decade, and the occurrence of resistance remains absent or very low to penicillin, spectinomycin and tiamulin (Table 1). It is also worth noting that no resistance to ciprofloxacin has been observed in Danish isolates for more than 10 years.

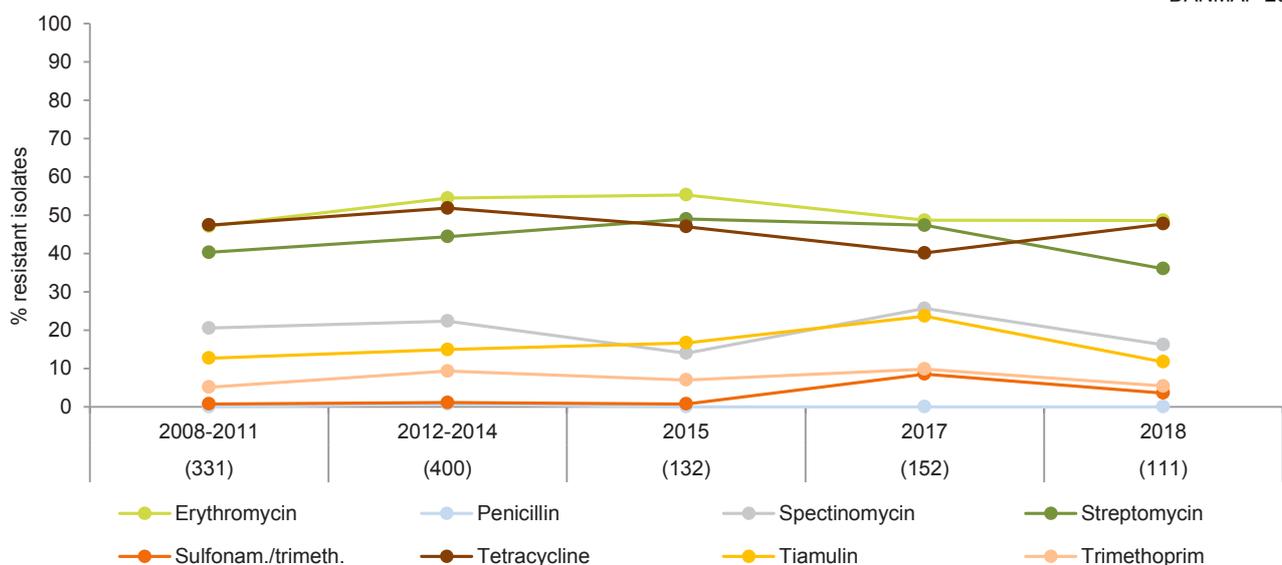
Streptococcus suis

Streptococcus suis may cause several different infectious conditions in pigs, such as meningitis, otitis media, arthritis, pneumonia, and septicaemia, and causes losses to the farmers due to increased mortality and veterinary costs. As in previous years, resistance was highest to macrolides (erythromycin), streptomycin, and tetracyclines (Figure 3). The observed fluctuations in tetracycline resistance were non-significant. However, there are several treatment options using compounds with very low levels of resistance (Table 1). As in 2017, all isolates were susceptible to both penicillin and florfenicol in 2018 and both of these are recommended first choice antimicrobials in the official guidelines. There was a high occurrence of resistance to sulphonamides, but a low level of resistance to trimethoprim, and even slightly lower resistance level for sulfonamide and trimethoprim in combination. The relative number of isolates resistant to sulfonamide-trimethoprim decreased (near significant) in 2018, with 3.6% resistant isolates, as compared to 8.6% in 2017. The occurrence of resistance to pleuromutilins (tiamulin) and spectinomycin is at a moderate level, but was significantly lower in the isolates tested in 2018, as compared to previous years (Figure 3).

Vibeke Frøkjær Jensen and Sven Erik Jorsal, National Veterinary Institute, DTU
Charlotte Mark Salomonsen, Laboratory for Pig Diseases, SEGES
For further information: vffe@vet.dtu.dk

Figure 3 Resistance (%) among *Streptococcus suis* from pigs, Denmark

DANMAP 2018



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.12)



7

RESISTANCE IN
INDICATOR BACTERIA

7. Resistance in indicator bacteria



Highlights: In 2018, the most prominent changes in resistance in indicator *E. coli* from food-producing animals were a reduced occurrence of multidrug-resistance in isolates from broilers and pigs and an increase in fully susceptible isolates from pigs compared to previous years.

Resistance patterns and levels in indicator *E. coli* from poultry, pigs and cattle were overall similar to previous years. No resistance to cefotaxime and ceftazidime was detected when using the non-selective isolation method. Such phenotypes were detected using selective isolation methods in samples from broilers, thus indicating that they are present in a relatively small proportion of commensal *E. coli* of broilers. Furthermore, no colistin, meropenem and tigecycline resistance was detected. Currently, the zoonotic risk linked to transfer of resistance to critically important antimicrobials from animals to humans appears to be very limited in Denmark.

Imported chicken meat was more likely to contain *E. coli* producing ESBL/AmpC than Danish broilers and broiler meat. The levels were comparable to those of 2016. The most common ESBL/AmpC enzymes identified across the broiler sources were again the AmpC enzyme, CMY-2 and the ESBL enzyme, CTX-M-1. As previously, all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

7.1 Introduction

Escherichia coli are included in the DANMAP programme to monitor occurrence of antimicrobial resistance in different reservoirs through the food chain for the following reasons: i) they are present as commensals in the gut microbiota of healthy animals and humans, ii) they can acquire antimicrobial resistance both via mutations in chromosomal genes and horizontal transfer of antimicrobial resistance genes, and iii) they have the potential to cause infections in both animals and humans and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

E. coli exhibiting resistance to third-generation cephalosporins via production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) are one of the fastest spreading antimicrobial resistance problems in both humans and production animals worldwide. Several studies report similar ESBL/AmpC genes, plasmids and/or clones of *E. coli* isolates in animals, meat and human infections, which suggests a zoonotic link [Roer et al 2019. J Antimicrob Chemother. 74(3):557; Valcek et al 2019. J Antimicrob Chemother. 74(8):2171].

Carbapenemase-producing Enterobacteriaceae (CPE) are a great threat to human health, because carbapenems are last-line antimicrobial agents for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Currently, CPE have been detected sporadically in production animals in EU but never in Denmark [EFSA/ECDC 2019. EFSA journal 17(2):5598].

Since 2014, isolation and antimicrobial susceptibility testing of indicator *E. coli* and ESBL/AmpC-producing *E. coli* have been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU].

7.2 Indicator *Escherichia coli*

All isolates originated from caecal samples randomly collected from healthy pigs, broilers and cattle at slaughter. Only one isolate per farm was included. Susceptibility to the antimicrobials recommended by EFSA was measured by broth microdilution to determine minimal inhibitory concentrations (MIC). MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.1). These results

were obtained using the non-selective isolation procedure. Results obtained by using selective procedures for detection of cefotaxime-resistant *E. coli* are reported in section 7.3.

7.2.1 Indicator *E. coli* from broilers

From 184 representative pools of broiler caeca collected at Danish slaughterhouses, 174 *E. coli* isolates were obtained. A total of 166 of these were tested for antimicrobial resistance (Table 7.1). More than half (60%) of the isolates were susceptible to all antimicrobials tested (Figure 7.1). Moderate (13-20%) resistance to ampicillin, nalidixic acid, ciprofloxacin, sulfonamide, tetracycline and trimethoprim was observed. Low (2%) and very low (1%) chloramphenicol and gentamicin resistance was observed, respectively. No resistance to the remaining compounds tested, including antimicrobials that are critically important for human medicine (azithromycin, cefotaxime, ceftazidime, meropenem, colistin and tigecycline), was observed (Table 7.1).

Resistance to ampicillin, sulphonamide and trimethoprim was significantly higher in indicator *E. coli* from broilers than in those from cattle.

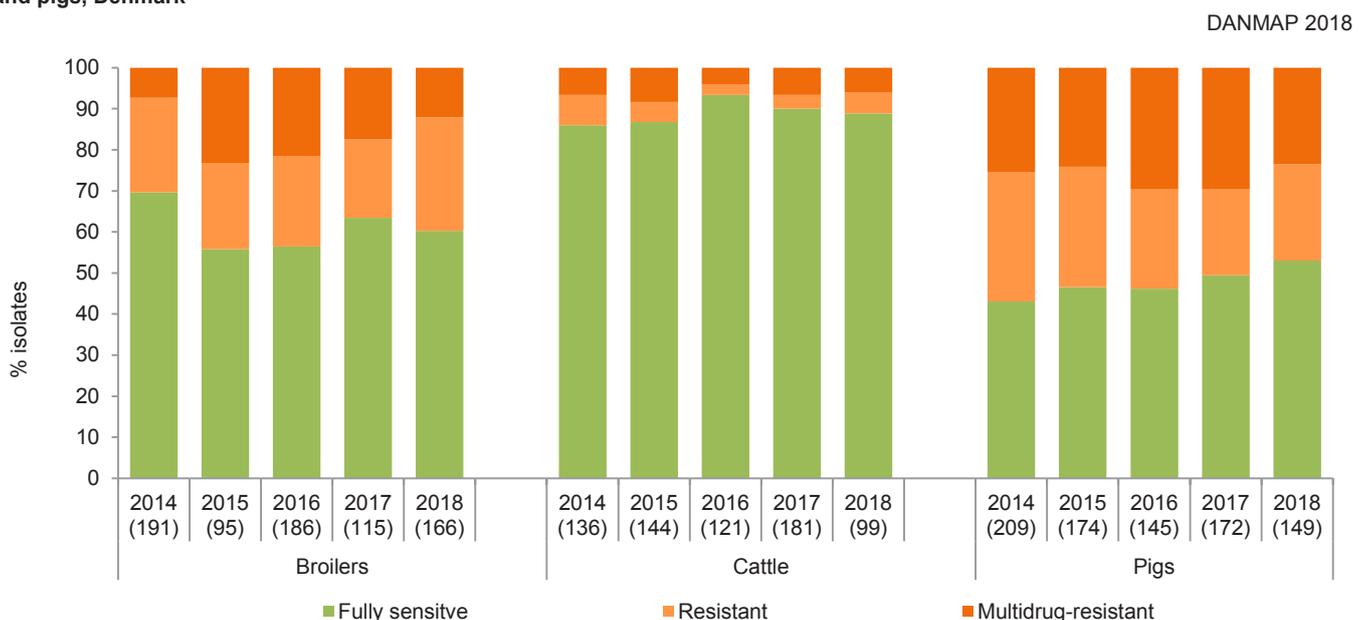
A total of 16 resistance profiles were detected among the 66 resistant isolates indicating relatively high diversity.

Table 7.1. Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2018

Antimicrobial agent	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Ampicillin	17	7	27
Azithromycin	0	0	4
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Chloramphenicol	2	5	7
Ciprofloxacin	13	0	1
Colistin	0	0	0
Gentamicin	1	0	<1
Meropenem	0	0	0
Nalidixic acid	13	0	1
Sulfonamide	20	7	30
Tetracycline	13	11	33
Tigecycline	0	0	0
Trimethoprim	13	1	23
Fully sensitive (%)	60	89	53
Number of isolates	166	99	149

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel

Figure 7.1 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark



Note: The number of isolates included each year is shown in parentheses. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included (Table 9.3)

The AMR profiles are listed in web annex Table A7.2. Most resistant isolates (53%) displayed resistance to one antimicrobial only or to two antimicrobials of the same class (see definition of classes in Table 9.5). Thus, 24%, 9%, 9%, 8% and 3% of resistant isolates showed resistance to quinolone (nalidixic acid and ciprofloxacin), sulfonamide, tetracycline, ampicillin and trimethoprim, respectively.

Co-resistance occurred in the remaining 31 (47%) resistant isolates. In some of the isolates ($n = 11$), resistance to compounds belonging to two antimicrobial classes was observed. Multidrug-resistance, i.e. resistance to 3 or more of the 12 antimicrobial classes in the *E. coli* test panel, was observed in 12% of all isolates ($n = 20$). Eighteen of the 20 isolates were resistant to ampicillin, sulfonamide, and trimethoprim, some isolates also in combinations with quinolones, tetracycline and/or chloramphenicol.

Compared to 2017, minor fluctuations in the occurrence of resistance to any antimicrobial were observed (Figure 7.2). However, over the last 10-year period, there has been a slow but statistically significant increase in resistance to chloramphenicol, nalidixic acid, ciprofloxacin, gentamicin, sulfonamide and trimethoprim.

The noticeable fluctuations observed in 2013-2015 have been associated to changes in antimicrobial usage patterns due to disease outbreaks in several flocks in 2015 [DANMAP 2015]. Thus, multidrug-resistant *E. coli* were significantly lower in 2014 compared to 2015 and 2016 (Figure 7.1). Afterwards, decreased antimicrobial use seemed to be accompanied by a decreasing trend in occurrence of multidrug-resistant isolates. However, the association between antimicrobial use and resistance is not straightforward, and in particular the occurrence of nalidixic acid and ciprofloxacin (quinolones) resistance at levels around 10% for at least the last 10 years has no clear explanation since there is virtually no fluoroquinolone use in the Danish poultry industry. Vertical transmission of *E. coli* resistant to fluoroquinolones and also other antimicrobials has been hypothesised [Bortolaia et al. 2010. *Vet Microbiol.* 142(3-4):379]. No statistically significant five-year trend was observed for the fully susceptible isolates.

7.2.2 Indicator *E. coli* from cattle

From 198 representative cattle caeca collected at Danish slaughterhouses, 187 *E. coli* isolates were obtained and 99 of these were tested for antimicrobial resistance (Table 7.1). The vast majority of isolates (89%) was susceptible to all tested antimicrobials. Very low (1%) occurrence of trimethoprim resistance and low (5-7%) occurrence of ampicillin, chloramphenicol and sulfonamide resistance were observed. Moderate occurrence of tetracycline resistance (11%) and no occurrence of resistance to the remaining antimicrobials tested were detected (Table 7.1).

Eight resistance profiles were detected in the 11 resistant isolates (web annex Table A7.2). Tetracycline resistance was detected in all resistant isolates either alone (one isolate) or in combination with resistance to additional compounds.

Compared to 2017, there were minor fluctuations in occurrence of resistances observed (Figure 7.2). When analysing the trends over a 10-year period a slow but statistically significant increase in ampicillin and chloramphenicol resistance was observed, whereas over the last five-year period a slow but significant decrease in occurrence of sulfonamide resistance occurred. There were no significant five-year trends in the occurrence of multidrug-resistant isolates and fully susceptible isolates (Figure 7.1).

7.2.3 Indicator *E. coli* from pigs

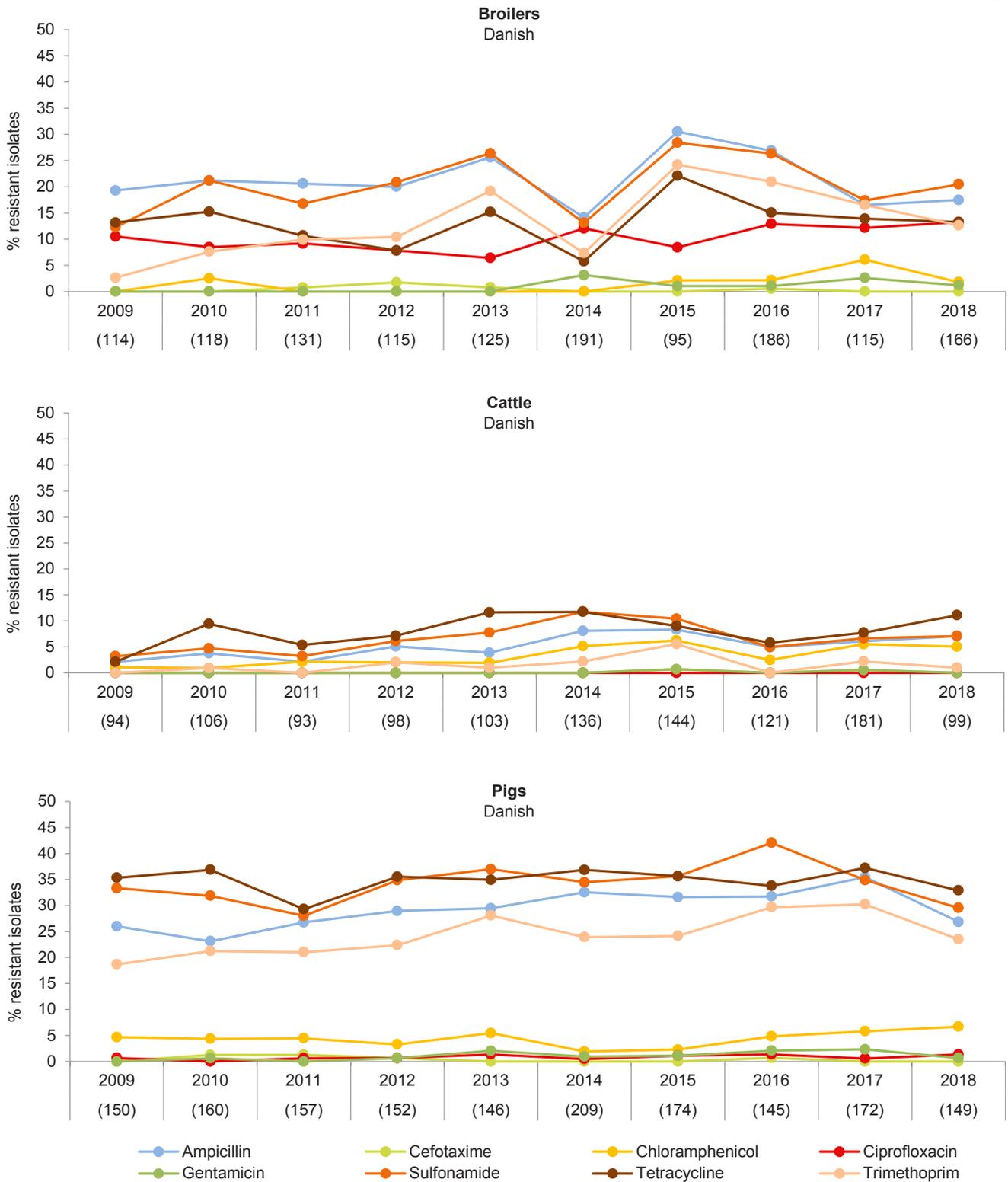
From 154 representative pig caeca collected at Danish slaughterhouses, 150 *E. coli* isolates were obtained and 149 of these were tested for antimicrobial resistance (Table 7.1). Approximately half (53%) of the isolates from pigs were susceptible to all antimicrobials tested. High (23-33%) occurrence of resistance to ampicillin, sulfonamide, tetracycline and trimethoprim and low (4-7%) occurrence of resistance to azithromycin and chloramphenicol were observed. Occurrence of resistance to the remaining antimicrobials tested was very low ($\leq 1\%$, gentamicin, nalidixic acid and ciprofloxacin) or not detected (Table 7.1).

Noteworthy, the occurrence of azithromycin resistance increased from 1% in 2014-2017 to 4% in 2018, it is unknown whether increase in macrolide use since 2016 has induced this, but will be monitored carefully in the coming years.

Resistance to ampicillin, azithromycin, chloramphenicol, tetracycline and trimethoprim was significantly higher in indicator *E. coli* from pigs than in those from broilers. Furthermore, resistance to ampicillin, azithromycin, sulfonamide, tetracycline and trimethoprim was significantly higher in indicator *E. coli* from pigs than in those from cattle.

Figure 7.2 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark

DANMAP 2018



Note: Number of isolates included each year is presented in the parenthesis

A total of 22 resistance profiles were detected among the 70 resistant isolates (see web annex Table A7.2). One third of the resistant isolates displayed resistance to one antimicrobial only. Thus, 20%, 6%, 4%, 3% and 1% of resistant isolates showed resistance to tetracycline, sulfamethoxazole, trimethoprim, ampicillin and chloramphenicol, respectively.

The remaining resistant isolates displayed varying resistance profiles, where 11 isolates exhibited resistance to antimicrobials of two classes (combinations of resistance to ampicillin, sulfonamide, tetracycline and trimethoprim). The remaining isolates (23% of all isolates) were classified as multidrug-resistant ($n = 35$) where co-resistance to ampicillin, sulfonamide, tetracycline and trimethoprim was common (ASuTTm resistance profile, $n = 21$). Each of the remaining resistance profiles was exhibited by few isolates indicating a wide diversity, and resistance to azithromycin ($n = 6$) were only observed in isolates resistant to three, four or five additional antimicrobial classes.

Compared to 2017, occurrence of resistance to several antimicrobials including ampicillin, gentamicin, sulfonamide, tetracycline and trimethoprim decreased (Figure 7.2). In addition, a minor increase in occurrence of chloramphenicol and ciprofloxacin resistance was observed. However, these changes in resistance levels were not statistically significant.

When analysing the trends over a 10-year period a slow but significant increase in ampicillin, gentamicin and trimethoprim resistance was observed whereas there was no statistically significant five-year trend in the occurrence of resistance to any antimicrobial. However, in the last five years, there has been a statistically significant increase in the percentage of fully susceptible isolates from 43% to 53% (Figure 7.1).

7.2.4 Perspectives

Antimicrobial resistance monitoring in commensal *E. coli* is considered a useful indicator of the selective pressure exerted by antimicrobial use on the intestinal microbiota of food-producing animals.

In 2018, there were relatively minor fluctuations in occurrence of antimicrobial resistance in indicator *E. coli* from broilers, cattle and pigs compared to previous years. However, the occurrence of multidrug-resistance was the lowest and the second-lowest detected in isolates from pigs and broilers, respectively, since 2014. In addition, the occurrence of fully

susceptible *E. coli* increased significantly in pigs since 2014. This likely reflects the efforts undertaken in Denmark to control antimicrobial use in food-producing animals. Genomic characterisation would be relevant to understand the population structure of susceptible and resistant *E. coli* populations from food-producing animals, which may provide the basis to design interventions to boost the fully susceptible *E. coli* population.

The antimicrobial resistance phenotypes detected in animal-origin indicator *E. coli* mostly relevant to human health were ciprofloxacin resistance in *E. coli* from broilers and azithromycin resistance in *E. coli* from pigs, as in 2017. A slow but increasing trend in resistance to ciprofloxacin has occurred in the *E. coli* isolates from broilers over the last ten years (13% in 2018). Although the molecular bases of ciprofloxacin resistance have not been investigated, the phenotype indicated chromosomal mutations (in 88 of 91 ciprofloxacin resistant broiler isolates from 2014 to 2018), consequently linking the main risk to human health to the disease-causing potential of these strains. Resistance to azithromycin in isolates from pigs increased from 1% in 2017 to 4% in 2018. The potential human risk derived by infections with these strains and/or transfer of azithromycin resistance to pathogenic strains remains low - but will be monitored closely the following years.

Resistance to other antimicrobials relevant for human medicine such as colistin, cefotaxime, ceftazidime, meropenem and tigecycline was not detected, which indicates that the prevalence of these resistance phenotypes was not more than 2% in *E. coli* from pigs and cattle and below 3% in *E. coli* from broilers (see section 9.7). However, cefotaxime- and ceftazidime-resistant *E. coli* were detected, when using selective enrichment, which is more sensitive.

From a European perspective based on the last published data from 2016 and 2017, indicator *E. coli* from Danish broilers and calves < 1 year show noticeably low occurrence of resistance to any antimicrobial compared to the indicator *E. coli* from other countries apart from the Nordic countries [EFSA/ECDC 2018. EFSA journal 16(2):5182; EFSA/ECDC 2019. EFSA journal 12(2):5598]. Denmark is among the countries reporting the lowest occurrence of chloramphenicol and, more importantly, ciprofloxacin resistance in indicator *E. coli* from pigs, whereas the reported occurrence of ampicillin, azithromycin, sulfonamide, trimethoprim and tetracycline resistance was comparable to the average reported in the EU Member States.

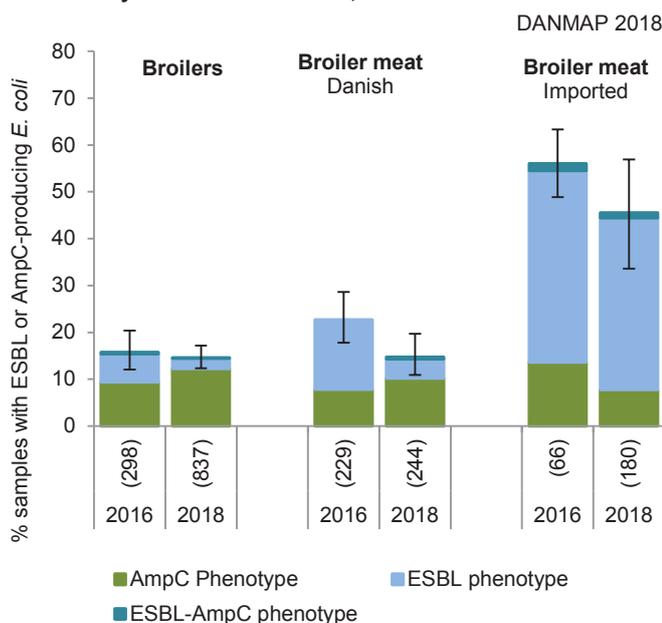
7.3 ESBL/AmpC- and carbapenemase-producing *E. coli*

DANMAP 2018 includes ESBL/AmpC- and carbapenemase-producing *E. coli* from caeca of domestic broilers at slaughter and from Danish and imported broiler meat at retail in concordance with the EU regulation on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. Samples were collected randomly and cultured directly in a selective enrichment for detection of cefotaxime-resistant *E. coli* and carbapenemase-producing *E. coli* (including oxacillinase producing OXA-48-like enzymes). Subsequently, obtained *E. coli* isolates were phenotypically antimicrobial susceptibility tested by MIC determination against the panel of antimicrobials recommended by EFSA. In parallel, for most isolates whole genome sequencing (WGS) and in silico bioinformatics were applied to detect the ESBL/AmpC/CPE-encoding genes. MIC distributions and occurrence of resistance among ESBL/AmpC-producing *E. coli* isolates are presented in the web annex (Table A7.3 and A7.4).

7.3.1 ESBL/AmpC- and carbapenemase-producing *E. coli* from Danish broilers and broiler meat

A total of 837 samples from broilers and 244 samples from domestically produced broiler meat resulted in 124 (15%) and 36 (15%) ESBL/AmpC-producing *E. coli* isolates, respectively (Figure 7.3). In 2018, the number of investigated caeca samples of broilers at slaughter increased two-fold compared to 2016 (N = 298).

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from broilers and broiler meat recovered by selective enrichment, Denmark



Note: Number of samples tested per year is presented in the parenthesis. Confidence intervals for total proportion of samples positive for phenotypic ESBL and/or AmpC producing *E. coli* - calculated as 95% binomial proportions presenting Wilson intervals. Classification of CPE, ESB and AmpC phenotypes according to the scheme provided by EFSA (see section 9.7)

Table 7.2. Resistance (%) and beta-lactam resistance phenotype distributions (%) in ESBL/AmpC-producing *Escherichia coli* from broilers and broiler meat recovered by selective enrichment, Denmark
DANMAP 2018

Antimicrobial agent	Broilers		
	Danish %	Danish %	Import %
Ampicillin	100	100	100
Azithromycin	0	0	4
Cefepime	81	83	93
Cefotaxime	100	100	100
Cefotaxime/clavulansyre	85	69	17
Cefoxitin	85	72	20
Ceftazidime	100	100	95
Ceftazidime/clavulansyre	82	69	17
Chloramphenicol	<1	6	23
Ciprofloxacin	23	22	72
Colistin	0	0	4
Ertapenem	0	11	1
Gentamicin	0	0	11
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	23	22	70
Sulfonamide	16	22	70
Temocillin	0	0	0
Tetracycline	18	17	56
Tigecycline	0	0	0
Trimethoprim	9	14	50
CPE phenotypes	0	0	0
AmpC phenotypes	83	69	17
ESBL phenotypes	15	28	80
ESBL-AmpC phenotypes	1	3	2
Other phenotypes	2	0	0
Number of isolates	124	36	82

Note: Classification of CPE, ESBL and AmpC phenotypes according to the scheme provided by EFSA (see section 9.7)

For broilers, the prevalence of samples positive for ESBL/AmpC-producing *E. coli* was similar to what was observed in 2016 (15% vs. 16%), whereas the prevalence in broiler meat was lower in 2018 compared to 2016 (15% vs. 23%, respectively), though not statistically significant. The reduction was mainly due to a statistically significant reduction in ESBL phenotypes (4% in 2018 vs. 15% in 2016). The prevalence of ESBL phenotypes among broilers also declined significantly between 2016 and 2018 from 6% to 2% (Figure 7.3).

No CPE isolates were recovered, suggesting that we can be 95% certain that CPE isolates are only present in 0.4% or less of domestic broilers at slaughter (see section 9.7). Low level phenotypic resistance to ertapenem was, however, observed in four isolates from broiler meat suggesting these to be

Table 7.3 Number of ESBL and AmpC enzymes detected in *E. coli* isolates from broilers and broiler meat recovered by selective enrichment, Denmark DANMAP 2018

Enzymes	Broilers		Broiler meat			
	Danish		Danish		Import	
	2016	2018	2016	2018	2016	2018
CMY-2	12	52	16	23	8	10
CMY-98	0	0	0	1	0	0
CTX-M-1	7	11	22	9	22	23
CTX-M-14	0	0	0	1	0	1
CTX-M-14b	0	0	0	0	1	2
CTX-M-32	0	0	0	0	0	1
SHV-12	1	2	4	1	3	19
SHV-2	0	0	0	0	0	1
TEM-52B	3	0	7	0	2	11
TEM-52C	1	0	0	0	0	5
Chromosomal AmpC	10	15	3	1	1	2
Unknown	0	0	0	0	0	1
Not available	14	44	0	0	0	6
Number (%) positive samples	48 (16%)	124 (15%)	52 (23%)	36 (15%)	37 (56%)	82 (46%)
Number of tested samples	298	837	229	244	66	180

Note: ESBL/AmpC enzymes and MLSTs are determined by WGS. For 2018 data, all MLST and ESBL/AmpC enzyme combinations are listed in web annex table A7.6

CPEs. WGS typing, however, revealed neither known carbapenemase nor oxacillinase encoding genes present in these *E. coli* isolates. In contrast, all four isolates harboured the CMY-2 encoding gene (Table 7.3) causing the phenotypic expression of the weak carbapenemase activity caused by porin-deficiency as described by Manneri et al. 2018 [FEMS Microbiol. Lett. 282:238].

Overall, the 124 ESBL/AmpC-producing *E. coli* isolates from broilers exhibited 100%, 100%, 85%, and 81% resistance to cefotaxime, ceftazidime (3rd generation cephalosporins), cefoxitin (2nd generation cephalosporin), and cefepime (4th generation cephalosporin), respectively (Table 7.2). Similar levels of resistance to cephalosporins were observed among the 36 ESBL/AmpC-producing *E. coli* isolates from domestically produced broiler meat that conferred resistance to cefotaxime (100%), ceftazidime (100%), cefoxitin (72%), and cefepime (83%), respectively (AMR profiles listed in web annex Table A7.5).

A total of 80 of the 124 ESBL/AmpC-producing *E. coli* isolates from broilers were whole genome sequenced (WGS). The MLST and ESBL/AmpC Enzymes combinations are listed in web annex Table A7.6. Among the AmpC phenotypes, 52 harboured the CMY-2 encoding gene. Of those, 43 (83%) belonged to the same clonal lineage of multi-locus sequence type (MLST) ST2040, indicating a clonal expansion from a point source. ST2040 harbouring an IncX plasmid replicon has been described as associated with the production of poultry in Finland [Oikarainen et al. 2019. Vet. Microbiology 231:100]. CMY-2 encoded ST2040 *E. coli* isolates appear

to be newly introduced in Danish broilers from an unknown origin. Only one *E. coli* isolate of this genotype was observed in 2016. Furthermore, singletons or a few isolates of CMY-2 encoded *E. coli* isolates were also attributed to other MLSTs such as ST48, ST580, ST752, ST1101, ST295 and ST1286; where the two latter types also harbouring CMY-2 were observed in 2016. Similarly to previous years, a subset of 15 (29%) AmpC-producing *E. coli* isolates conferred resistance due to the presence of an upregulated AmpC promoter (Table 7.3).

In broilers, 11 ESBL-producing *E. coli* isolates were also detected. They all harboured the CTX-M-1 encoding gene and belonged to a variety of different MLSTs including ST155, ST212, ST1818, ST88, ST1640 and ST752, of which the latter two types also harbouring CTX-M-1 were observed in 2016 (see web annex Table A7.6).

All of the 36 ESBL/AmpC-producing *E. coli* isolates from domestically produced broiler meat were typed by WGS. The vast majority, 23 (64%) AmpC phenotypes harboured the CMY-2 encoding gene, of which 16 belonged to ST2040 as observed in the Danish broilers. Similarly, the most common ESBL gene from the broiler meat was also CTX-M-1 and was found in nine isolates, attributed to ST162, ST1640, ST2309, ST3232, ST1640, ST752, and ST1818. The later three MLSTs harbouring CTX-M-1 were also observed among isolates originating from Danish broilers.

The ESBL/AmpC-producing *E. coli* isolates from Danish broilers and broiler meat exhibited varying levels of resistance to

other antimicrobials. No resistance to tigecycline, temocillin, gentamicin, azithromycin and colistin was detected. (Table 7.2). Resistance to quinolones was moderate in both sources, and lower than in 2016 in broilers (23% vs. 40%) but not in broiler meat (22% v. 15%). In 2018, a 100% concordance between resistance to ciprofloxacin and nalidixic acid was observed for both sources, suggesting chromosomal mutations in the topoisomerases genes *gyrA* and *parC*.

Different combinations of multidrug-resistance were observed among the ESBL/AmpC-producing *E. coli* isolates (web annex Table A7.5). The multidrug-resistance was primarily including ampicillin, chloramphenicol, tetracycline, sulphonamides, and trimethoprim in addition to the cephalosporins. Thus, co-resistance, likely attributable to the presence of class 1 integrons, was observed: i) to tetracycline in 18% and 17% of the isolates, ii) to sulfonamides in 16% and 22% of the isolates and iii) to trimethoprim in 9% and 14% of the isolates from Danish broilers and broiler meat, respectively (Table 7.2).

7.4.2 ESBL/AmpC- and carbapenemase-producing *E. coli* from imported broiler meat

A total of 180 samples from imported broiler meat resulted in 82 (46%) ESBL/AmpC-producing *E. coli* isolates (Table 7.2), which was significantly higher than the 36 (15%) in the domestically produced broiler meat. No CPE isolates were recovered in any of the imported broiler meat samples.

The prevalence of ESBL/AmpC-producing *E. coli* positive samples was similar to 2016 (46% vs. 56%), and no significant change in prevalence from 2016 to 2018 for neither ESBL nor AmpC phenotypes was observed (Figure 7.3). In contrast to the isolates from Danish broiler meat, the ESBL phenotype (80%) was more common than the AmpC phenotype (17%) in imported broiler meat. Two isolates exhibited both ESBL and AmpC phenotype.

The ESBL/AmpC-producing *E. coli* isolates exhibited 100%, 95%, 20%, and 93% resistance to cefotaxime, ceftazidime, ceftiofur, and cefepime, respectively (Table 7.2).

All of the 82 ESBL/AmpC-producing *E. coli* isolates from imported broiler meat were available for WGS. Among the 80% displaying an ESBL phenotype, the CTX-M-1-encoding gene was the most common found in 23 isolates belonging to 18 different MLSTs (see web annex Table A7.6). Moreover, 19 and 11 ESBL-producing *E. coli* isolates harboured the SHV-12- and TEM-52-encoding genes, respectively. Among the 19 *E. coli* isolates encoding SHV-12, the majority belonged to two MLSTs, ST1011 (n = 6) and ST117 (n = 6). For the *E. coli* isolates encoding TEM-52B, ST115 (n = 5) was dominant. The CMY-2-encoding gene was the most common among the 17% of *E. coli* isolates exhibiting the AmpC phenotype. All the AmpC-producing *E. coli* isolates were singleton MLSTs except for two isolates belonging to ST2040, which was the most prevalent MLST in Danish broilers and broiler meat.

The 82 ESBL/AmpC-producing *E. coli* isolates from imported broiler meat generally exhibited higher levels of resistance and co-resistance compared with those from Danish broiler meat. See AMR profiles listed in web annex Table A7.5. No resistance to tigecycline and temocillin was observed and only low levels of resistance to gentamicin (11%), azithromycin (4%) and colistin (4%) were detected (Table 7.3).

Resistance to quinolones was very high with 72% (n = 59) and 70% (n = 57) resistance to ciprofloxacin and nalidixic acid, respectively. This suggests that chromosomal mutations in the topoisomerases genes *gyrA* and *parC* have occurred and is similar to observations in isolates from Danish sources.

7.4.5 Perspectives

The number of samples collected for the monitoring of ESBL/AmpC-producing *E. coli* isolates from Danish broilers and imported broiler meat increased in 2018 compared to previous years, strengthening the monitoring system and the results hereof. In 2018, we observed a similar scenario as in previous years with a consistent lower level of ESBL/AmpC-producing *E. coli* in Danish broilers and broiler meat compared to imported broiler meat.

The most common ESBL/AmpC enzymes identified across the broiler sources were again the AmpC enzyme, CMY-2, and the ESBL enzyme: CTX-M-1. As in previous years we observed that all the ESBL/AmpC enzymes identified, were associated with large number of MLSTs. Interestingly, the majority of the AmpC enzyme, CMY-2, from Danish broilers and broiler meat were ST2040, a relative new introduction of an unknown origin in 2018. The pattern of MLSTs seems to fluctuate across years and in 2016 different MLSTs were most prevalent such as ST429 harbouring CMY-2.

Each year, the ESBL/AmpC enzymes and the MLSTs are compared by whole genome sequencing and phylogenetic SNP analysis to isolates from human bloodstream infections to elucidate potential zoonotic transmission. The vast majority of the food and veterinary ESBL/AmpC-producing *E. coli* isolates are rarely congruent to those of human bloodstream infections. This is supported by similar observations in other European countries. Only in very few cases, have commensal non-pathogenic MLSTs with similar ESBL/AmpC enzymes been identified in both sectors. A higher number of similar cases might be identified expanding the monitoring to also include ESBL/AmpC-producing *E. coli* isolates from human diarrheal and urine tract infections.

Rene S. Hendriksen, Valeria Bortolaia, and Helle Korsgaard
For further information: Helle Korsgaard, hkor@food.dtu.dk

Textbox 7.1

Antimicrobial resistance in prawns and pangasius fillets imported from Asia

Background: Global aquaculture production is expanding as the worldwide demand for protein increases [1]. Asia and other low and middle-income countries (LMIC) produce more than 90% of the global aquaculture both for domestic use and for export. In Denmark and other Northern European countries, pangasius fillets (*Pangasianodon hypophthalmus*) and especially, the Vannemei Prawns (*Litopenaeus vannamei*) are popular imports from Asia. Low to middle income countries generally struggle with sanitation, infrastructure and separation of clean and used water [2]. LMIC with a lower level of governance and less regulation including use of antibiotics are more likely to develop more antimicrobial resistance [3].

Despite strict requirements to products imported into the EU, it may be difficult to avoid microbial contamination in products from third countries and all bacteria can carry resistance genes independent of their ability to cause disease. This also includes resistance to antimicrobials critically important to humans (CI) and resistance types not present in the importing country. WHO note that among the CI antimicrobial classes; quinolones, 3rd and higher generation cephalosporins, macrolides, glycopeptides and polymyxins (Table 1) are drugs classified as highest priority (CIHP) [4].

To assess the risk of importing fish and seafood products contaminated with bacteria resistant to critically important antibiotics, we carried out a survey of antimicrobial resistance in indicator bacteria found in Vannemei prawns and pangasius fillets in Danish supermarkets.

Data and data sources: A total of 67 *Escherichia coli*, 204 *Enterococcus faecalis* and 65 *Enterococcus faecium* strains were isolated from 97 pangasius fillet and 203 Vannemei prawn samples collected in Danish supermarkets [5]. The samples were selected representatively during the period of May 2017 to May 2018. All *E. coli* and a total of 140 *E. faecalis* and 65 *E. faecium* isolates were MIC tested. Furthermore, all samples were screened for ESBL-, AmpC-, carbapenem- and OXA-48-producing *E. coli* using selective enrichment. Isolation, MIC testing and whole genome sequencing was performed as described in section 9.4-9.7 with a few moderations.

Results and discussion: Among the 67 *Escherichia coli* isolates, 60% were resistant to at least one antimicrobial in the test panel. Interestingly, the levels of resistance to the antimicrobials, such as ampicillin (9%), tetracycline (22%), sulphonamide (9%) and chloramphenicol (6%) were lower than in isolates originating from Danish pigs and broilers and comparable to isolates from Danish cattle (Figure 7.1). In contrast, resistance to CIHP antimicrobials were observed in 54% (n = 36) of the *E. coli* isolates from imported fish and prawns, and of these, all were resistant to the flouroquinolones (ciprofloxacin). One of the 36 isolates

Table 1 Classification of antimicrobials used to assess antimicrobial resistance in bacteria from prawns and fish from Asia

DANMAP 2018

Critically Important and of highest priority:

3rd, 4th and 5th gen. cephalosporins (cefepime, cefotaxime, ceftazidime); glycopeptides (teicoplanin, vancomycin, azithromycin); macrolides (azithromycin, erythromycin); polymyxins (colistin); quinolones and fluoroquinolones (ciprofloxacin, nalidixic acid)

Critically Important:

Aminoglycosides (gentamicin); aminopenicillins (ampicillin, temocillin); carbapenems and other penems (ertapenem, imipenem, meropenem); glycolcyclines (tigecycline); Lipopeptides (daptomycin) and oxazolidinones (Linezolid)

Highly important:

Amphenicols (chloramphenicol); 1st and 2nd gen. cephalosporins and cephamycins (cefoxitin); streptogramins (quinupristin, dalfoprisitn); sulfonamides, inhibitors and combinations (sulphamethoxazole) and tetracyclines (trimethoprim, tetracycline)

Note: Based on the 6th revision of the WHO report on critically important antimicrobials for human medicine, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 2019

was resistant to 3rd. generation cephalosporins (cefepime and ceftazidime) and one to polymyxins (colistin). None of the isolates was resistant to macrolides (azithromycin), carbapenems (meropenem) or glycolcyclines (tigecycline).

Only 7 of the 36 ciprofloxacin resistant isolates were also resistant to nalidixic acid, suggesting that the ciprofloxacin resistance may be due to plasmid-mediated resistance rather than the chromosomal mutation driven resistance (Figure 1). A total of 49% of the *E. coli* isolates from the imported fish and prawns carried the phenotypic profile suggesting plasmid-mediated resistance. Ciprofloxacin resistance levels are quite low in Denmark and plasmid-mediated quinolone resistance is considered very rare in Danish foods and animals [6]. A study examined the DANMAP indicator *E. coli* isolates from 2014-18 from Danish broilers, cattle, pigs and meat. Here only 122 of the 2,863 Danish *E. coli* isolates were resistant to ciprofloxacin (4.3%) and only 6 of these (0.2%) isolates were susceptible to nalidixic acid.

Four ESBL-producing *E. coli* isolates from prawns were detected on the selective media. The WGS analysis showed that two strains harboured *bla*_{CTX-M-15} and *qnrS1*, as the only identified resistance genes. Both of these isolates exhibited phenotypic resistance to ciprofloxacin, but not nalidixic acid. The other two strains carried *bla*_{CTX-M-55} and were multidrug-resistant, of which one also had the *qnrS1*. In the fourth isolate, 15 resistance genes were detected in addition to the ESBL-gene (Table 2), encoding for all the investigated classes of the CIHP antimicrobials as well as plasmid-mediated quinolone and colistin resistance.

No carbapenemase- or OXA-48 producing *E.coli* was detected by the selective enrichment screening.

Among the 104 *Enterococcus faecalis* isolates, 34% were resistant to at least one of the eleven compounds in the test panel. *E. faecalis* is intrinsically (i.e. naturally) resistant to streptogramin A and B (quinupristin-dalfopristin), and interpretation of the MIC testing for this drug was therefore not evaluated. Tetracycline was the most common resistance found in 32% of the isolates, and 4% were resistant to chloramphenicol. One isolate was resistant to gentamycin (CI) and 7% of the isolates were resistant to erythromycin (CIHP). All multidrug-resistant strains (n = 3) exhibited resistance to chloramphenicol, tetracycline, and erythromycin.

Almost all of the 65 *Enterococcus faecium* isolates had MIC values higher than the microbiological cut-off of MIC < 1 µg/ml for streptogramins (97%), however only four isolates exceeded the clinical breakpoint of MIC > 4 µg/ml. Most isolates were also resistant to tetracycline (64%). Resistance to erythromycin (CIHP) was detected in 22% of the isolates. Resistance to chloramphenicol and ciprofloxacin were each detected in one strain only. Multidrug-resistance was found in 20% of the strains.

Table 2 Phenotypic and genotypic traits of the four ESBL-producing *E. coli* isolates from Asian prawns recovered by selective enrichment

DANMAP 2018

Isolate number	Phenotypic resistance profile	Genotypic resistance profile
1	AMP CIP FEP FOT TAZ	<i>bla</i> _{CTX-M-15} , <i>qnrS1</i>
2	AMP CIP FEP FOT TAZ	<i>bla</i> _{CTX-M-15} , <i>qnrS1</i>
3	AMP CHL CIP FEP FOT TAZ TET TMP	<i>bla</i> _{CTX-M-55} , <i>aadA5</i> , <i>bla</i> _{TEM-1B} , <i>dfrA17</i> , <i>floR</i> , <i>qnrS1</i> , <i>tet(A)</i>
4	AMP AZI CHL CIP COL FEP FOT FOX GEN NAL SMX TAZ TET TMP	<i>bla</i> _{CTX-M-55} , <i>aac(3)-lid</i> , <i>aadA22</i> , <i>aadA5</i> , <i>aph(3')-Ia</i> , <i>aph(6)-Id</i> , <i>dfrA17</i> , <i>mcr-1</i> , <i>mph(A)</i> , <i>Inu(F)</i> , <i>FloR</i> , <i>GyrA S83L</i> , <i>qnrS1</i> , <i>Sul2</i> , <i>sul3</i> , <i>tet(A)</i>

Note: AMP=Ampicillin; AZI=Azithromycin; CIP=Ciprofloxacin; CHL=Chloramphenicol; COL=Colistin; FEP=Cefepime; FOT=Cefotaxime; FOX=Cefoxitin; GEN=Gentamicin; NAL=Nalidixic acid; SMX=Sulphamethoxazole; TAZ=Ceftazidime; TET=Tetracycline; TMP=Trimethoprim

continued ... Textbox 7.1

No resistance to the last-line CI drugs; linezolid or vancomycin was detected in any of the enterococci isolates by the methods applied. It is possible that more sensitive methods such as WGS or culture on selective media may have detected resistance to some of the last-line drugs.

Conclusion: Most of the observed phenotypic resistances have previously been observed in Danish food or production animals. However, we cannot exclude that these products may pose a risk to consumers by introducing AMR genes that are very rare in domestic food sources. No carbapenem, linezolid or daptomycin resistance was found, all of which are antimicrobials prioritised for treatment of human infections with multidrug-resistant enterococci and staphylococci. Plasmid-mediated quinolone resistance is quite rare in Danish foods, but was quite abundant in the imported prawns and fish products. One isolate carried resistance genes to ESBL, macrolide and plasmid-mediated colistin and flouoroquinolone resistance alongside genes coding resistance to several of the critically important and highly important antimicrobial groups.

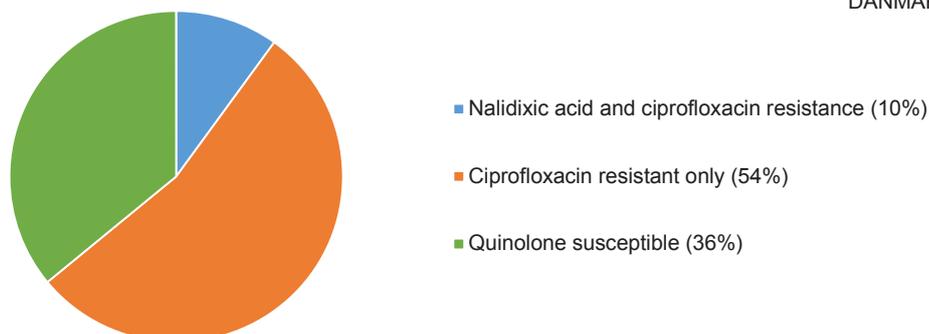
Johanne Ellis-Iversen, Helle Korsgaard
For further information: hkor@food.dtu.dk

References

- [1] Globefish, 2018. Information and Analysis on World Fish Trade, FAO. Retrieved April 19, 2019, from <http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1156018/>
- [2] Thornber et al, 2019. Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture* 1-21, <https://doi.org/10.1111/raq.12367>
- [3] FAO, 2016. The FAO action plan on Antimicrobial Resistance 2016-2020. Retrieved from <http://www.fao.org/fsnforum/resources/fsn-resources/fao-action-plan-antimicrobial-resistance-2016-2020>
- [4] WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 2019. Critically Important Antimicrobials for Human Medicine, 6th Revision 2018. Retrieved from <https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/>
- [5] Anonymous, 2018. Annual Report on Zoonoses in Denmark 2018, National Food Institute, Technical University of Denmark
- [6] Veldman K et al, 2011. International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *J Antimicrob Chemother.*66(6):1278-86

Figure 1 Quinolone resistance distribution in 67 *E. coli* isolated from prawns and fish from Asia

DANMAP 2018



Textbox 7.2

ESBL- and pAmpC-producing *Escherichia coli* - comparison of isolates of animal origin and isolates obtained from human bloodstream infections

Background: ESBL- and pAmpC-producing bacteria are widespread in both humans and animals worldwide. Several studies have suggested that the presence of *E. coli* with identical Multilocus Sequence Types (STs) and ESBL-/pAmpC-genes in animals, meat products and human infections could be caused by zoonotic transmission. In this study, we investigated possible zoonotic links between isolates originating from different origins based on MIC determination and whole genome sequencing combined with bioinformatic analysis.

Materials and methods: ESBL- and pAmpC-producing *E. coli* isolates from production animals and meat obtained in 2017 and 2018 (Table 7.3 and DANMAP 2017) were compared with *E. coli* 2018-isolates from human bloodstream infections (BSI) having similar phenotypic characteristics (Textbox 8.2). Possible clonal relationships between isolates sharing the same combination of ST and ESBL-/pAmpC-genes, were identified by whole-genome-based single-nucleotide polymorphism (SNP) analysis. Genomes were further characterized and phylogenetic analysis initiated if < 100 SNPs were observed between isolates of both domains. Horizontal gene transfer by plasmids encoding ESBL/pAmpC enzymes was not investigated.

Results: During 2017-2018, a total of 209 isolates from production animals and meat products were carrying ESBL/pAmpC-encoding genes, including: 80 isolates from broilers, 112 isolates from broiler meat (Danish and imported), 10 isolates from beef and seven isolates from pork. From human BSI, a total of 352 ESBL/pAmpC positive isolates were collected in 2018.

When comparing the *E. coli* isolates from human BSI and *E. coli* isolates of animal origin, the same combinations of STs and ESBL/pAmpC were detected 11 times; ST10, ST23, ST69, ST88, ST162, ST362, ST74, and ST1434 in combination with CTX-M-1, ST10 in combination with CTX-M-32, ST69 in combination with CMY-2, and ST69 in combination with SHV-12. For each of the 11 combinations, a SNP-based comparison was performed. Only for ST69 with CTX-M-1 less than 100 SNPs between isolates of human and animal origin were observed.

The ST69 CTX-M-1-producing *E. coli* encompassed one isolate from imported beef obtained in 2017 and four isolates from patients with BSI in 2018. Twenty-one, 126, 154, and 227 SNPs were observed between the isolate from beef and the four isolates from human infections indicating a close clonal relatedness.

Discussion and conclusion: ST69 *E. coli* is a frequent cause of urinary tract infections and BSI in humans but also found in poultry, pork/pigs and beef/cattle [Amee *et al.* 2012, CID 55: 712-719]. The number of SNP (>10) observed between the isolate from beef and the human BSI do not indicate an outbreak or a direct transmission, but the difference of 21 SNPs indicates high clonal relationship and a possible zoonotic link of *E. coli* ST69 with CTX-M-1. A more comprehensive analysis of the zoonotic transmission in Denmark could in the future also include isolates from other human sources than BSI.

*Louise Roer, Anette M. Hammerum, Frank Hansen, Helle Korsgaard,
Valeria Bortolaia, Pimlapas Leekitcharoenphon, Henrik Hasman and Rene S. Hendriksen*
For further information for the human isolates: Anette M. Hammerum (ama@ssi.dk)
For further information for the meat isolates: René S. Hendriksen (rshe@food.dtu.dk)

Textbox 7.3

Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries

Background: Traditional AMR monitoring, as performed in DANMAP and similar national programmes, is limited to a handful of bacterial species with pathogenic or indicator potential. The majority of AMR genes are however located in species that are not being monitored. Since many bacterial species have unknown requirements and have therefore never been cultured, it would be impractical or even impossible to meet their growth conditions. In order to elucidate the livestock-associated AMR gene reservoir, also called the resistome, we use shotgun metagenomics.

Materials and method: As part of the European Union-funded EFFORT project (www.effort-against-amr.eu) we collected 25 animal faecal samples from each of 181 pig and 178 poultry herds located in nine European countries and pooled each set for a herd-level sample. From each pool, DNA was extracted, sheared and paired-end sequenced on an Illumina HiSeq instrument. On average, more than 50 million pieces of DNA were sequenced per sample and were analysed *in silico* against the ResFinder database. For details, please see the publication [Munk et al. 2018, Nat. Microbio. 3: 898].

The numbers of assigned sequences were adjusted to both the sample-specific number of bacterial sequences and gene-specific lengths, such that the “Fragments Per Kilobase reference gene per Million bacterial fragments” (FPKM) was obtained.

Results: The total AMR load varied drastically between samples, both as a function of host animal species and country of origin (Figure 1). For both pig and poultry, the Dutch and Danish farms had lower combined AMR loads than other countries. Over half the Danish poultry farms were below 500 FPKM AMR, whereas the Italian pig farms had the highest loads, frequently in excess of 10,000 FPKM.

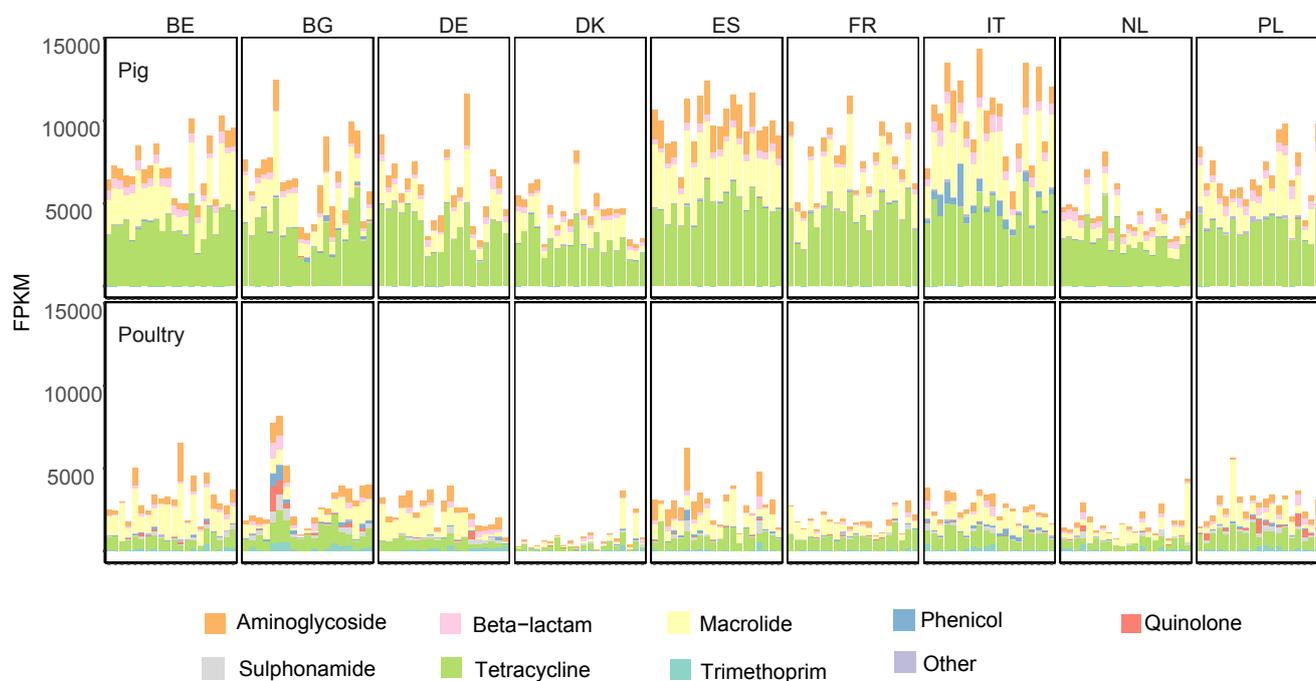
In addition to the abundance, we calculated AMR gene diversity and richness. Across all samples, more than 400 different AMR genes were detected. Interestingly, high AMR gene richness in one livestock species was associated with high richness in the other species in the same country. Despite the lower combined load, poultry had more unique AMR genes. Danish pig herds had lower expected gene richness (66 unique genes) compared to any other herds.

Several important AMR genes, including linezolid-resistance gene *optrA* and colistin-resistance gene *mcr-1*, were detected and found to differ significantly in abundance between countries. National veterinary drug use level was significantly associated with the combined AMR load measured by metagenomics.

Conclusion: The detailed metagenomic snapshot of European livestock showed a staggering diversity of AMR that is not normally observed in traditional monitoring. This baseline study is being followed by additional monitoring in more livestock species including turkey, fish and veal calves. The shotgun sequence data are being used to answer completely new questions and highlights the significant value of metagenomic monitoring going forward.

For further information Patrick Munk, pmun@food.dtu.dk

Figure 1 Distribution of antimicrobial resistance genes across livestock species and countries. Each stacked bar represents a single herd in which AMR was quantified using a metagenomic approach



Note: The overall height of the bar represents the combined load, whereas the individual colors denote the antimicrobial drug classes corresponding to the individual 400+ AMR genes that were quantified. Top and bottom panels are pig and poultry respectively. Source: EFFORT projec published in Munk et al. 2018, Nat. Microbio. 3: 898

BE=Belgium; BG=Bulgaria; DE=Germany; DK=Denmark; ES=Spain; FR=France; IT=Italy; NL=Netherlands; PL=Poland.
FPKM=Fragments Per Kilobase reference gene per Million bacterial fragments





8

RESISTANCE IN HUMAN
CLINICAL BACTERIA

8. Resistance in human clinical bacteria



Highlights: In DANMAP 2018, the Danish Microbiology Database (MiBa) has been used as primary data source for monitoring prevalence and resistance in several human clinical bacteria (Textbox 8.1).

Increasing trends in the number of invasive cases continued for the majority of the surveilled species in 2018 (section 8.1).

Blood infections with *Staphylococcus aureus* (subsection 8.3.8) have increased gradually from 1400 cases in 2010 to nearly 2300 cases in 2018. In 2018 1.6% of these were methicillin resistant (MRSA). The prevalence of **LA-MRSA CC398**-positive pig farms and the number of LA-MRSA CC398 infections in the general population seem to have reached a maximum (Textbox 8.2).

Resistance rates to 3rd generation cephalosporins have increased slowly during the past 10 and five years in urinary *Escherichia coli* (subsection 8.2.1). In invasive *E. coli* no significant increase in 3rd generation cephalosporin resistance was observed. A significant increase in the prevalence of the **ESBL** enzyme, CTX-M-15 (subsection 8.3.1), was found in 3rd generation cephalosporin resistant *E. coli* from bloodstream infections for 2018.

Mecillinam resistance in urinary cases of *Klebsiella pneumoniae* (subsection 8.2.2) remained in 2018 at a high level of 16-17%. Thereby the resistance rates to the standard per oral treatment against urinary tract infections with pivmecillinam are high. This places a pressure on the usage of ciprofloxacin, for which the resistance rate also increased from 2017 (5.4-7.6%) to 2018 (6.4-8.9%).

Increasing resistance towards ciprofloxacin was also observed for *Neisseria gonorrhoeae* (subsection 8.3.9) - reaching a level of 40% in 2018 (28% in 2017 and 18% in 2016). No isolates were ceftriaxone resistant.

For invasive *Enterococcus faecium* cases (subsection 8.2.5), continued escalation of vancomycin-resistance was observed (12 % compared to 7.1% in 2017). Detection and spread of **VVE** (vancomycin-variable enterococci), as part of this steep increase, is described in subsection 8.3.3.

In 2018, an overall 44% increase of submitted **carbapenemase-producing organisms** (CPO) (subsection 3.2.1) was observed compared to 2017 (177 CPO from 160 patients compared to 123 CPO from 115 patients). Several outbreaks with carbapenemase-producing enterobacteriaceae (CPE) were observed during 2018. In September 5th 2018 the Danish Health Authority made CPO notifiable.

8.1 Introduction

In Denmark all hospitals and general practitioners are serviced by 10 departments of clinical microbiology (DCMs) located at hospitals in the five regions of Denmark. The national surveillance of resistance in human clinical bacteria is based on either data from routine diagnostics performed at the 10 departments of clinical microbiology (DCMs) in Denmark or on

resistance and typing results from isolates received at the reference laboratories at SSI for further characterisation. Isolates are received either based on a mutual agreement of voluntary submission of specific species and/or types of resistances or as part of a mandatory surveillance program of diseases made notifiable by the Danish Health Authority (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of national resistance surveillance in isolates from humans, 2018

DANMAP 2018

Routine diagnostics from all 10 DCMs in Denmark. Data are directly identified and extracted from MiBa	
Species	Sampling
<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	First isolate per patient per year from blood or cerebrospinal fluid, from urine in hospitalised patients and from urine from primary health care
<i>Pseudomonas aeruginosa</i> <i>Acinetobacter species</i> <i>Enterococcus faecalis</i> <i>Enterococcus faecium</i>	First isolate per patient per year from blood or cerebrospinal fluid
Voluntary submission of isolates to the reference laboratories at SSI	
Species or type	Sampling
<i>Staphylococcus aureus</i> Beta-haemolytic streptococci	One isolate per patient per episode from blood or cerebrospinal fluid
<i>Neisseria gonorrhoeae</i>	One isolate per patient per episode from all sample sites
Third-generation cephalosporin resistant <i>Escherichia coli</i>	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months irrespective of sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode irrespective of sample site
Mandatory submission of isolates to the reference laboratories at SSI	
Species or type	Sampling
Carbapenemase-producing organisms	First isolate per patient within 12 months irrespective of sample site (clinical and screening samples)
Methicillin-resistant <i>Staphylococcus aureus</i>	First isolate from all new cases of MRSA positive patients irrespective of sample site (clinical and screening samples)
<i>Streptococcus pneumoniae</i>	One isolate per patient per episode from blood or cerebrospinal fluid
<i>Haemophilus influenzae</i> serotype b, Hib	All invasive isolates

Regarding submissions of isolates to the reference laboratories normally more isolates are received, but in the statistics the patient only counts once

8.1.1 Surveillance based on MiBa data

The surveillance of resistance in invasive isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* is based on data from routine diagnostics from the DCMs in Denmark. Surveillance has been performed since 1995 - in the very beginning based on reporting from two DCMs, but quickly joined and supported by most DCMs in Denmark. From 2009 to 2014, DANMAP received data from all but one DCM resulting in a coverage of approx. 95% of the population. Since 2015, all DCMs participate in the program resulting in a 100% population coverage. In 2018, all these data were extracted directly from the Danish Microbiology Database (MiBa) (<https://miba.ssi.dk/Service/English.aspx>). A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1. Due to the quality of data in MiBa, this register-based surveil-

lance gives the best achievable monitoring of prevalence of resistance in the surveilled species. Materials and methods are described in chapter 9.

8.1.2 Surveillance based on data from the reference laboratories

In addition to the MiBa-based monitoring of resistance a surveillance program exists, based on submission of specified strains to the reference laboratories at SSI. Isolate-based surveillance gives the opportunity to further characterise strains and resistance mechanisms and type the isolates; since 2015-2016, this has been mainly performed by the use of whole genome sequencing (WGS). Voluntary submission of specified strains has existed since 1957; beginning with the submission of all strains of *Staphylococcus aureus* from bloodstream infections. The submission of invasive beta-haemolytic streptococci is also voluntary, while invasive *Streptococcus pneumoniae*

and *Haemophilus influenzae* serotype b (Hib) are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable but the submission of the isolated strains of MRSA is mandatory, while the submission of isolated strains of *N. gonorrhoeae* is voluntary. In addition, the DCMs voluntarily submit isolates of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) from all clinical sites, based on a mutual agreement to survey the development and spread of these often multiresistant bacteria at Danish hospitals. The Danish Health Authority made carbapenemase-producing organisms (CPO) notifiable, and submission of all clinical and screening isolates irrespective of sample site has been mandatory as of 5th September 2018. Before that, CPO was submitted on a voluntary basis.

8.1.3 Surveillance of invasive cases

A key function in the monitoring of antimicrobial resistance for DANMAP is to surveil the number of resistant bacteria in invasive cases (blood and cerebrospinal fluid). This is harmonised with the monitoring performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Figure 8.1 presents total numbers of invasive cases in Denmark from 2009 to 2018 for the bacterial species included in the surveillance programmes for both DANMAP and EARS-Net. Excluded from the figure is *Acinetobacter* species - these have only been registered since 2012 in DANMAP and the number of cases are low (55 to 70 cases annually). For all registered species, the

following case definitions applies: The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates within the year of observation from the same patient are removed.

Since 2010, the total number of registered invasive cases increased by 44% (from 8,021 to 11,589 cases). The largest increase observed was for *S. aureus* (68%). The only species with an overall decreasing number of cases was *S. pneumoniae* (-16%). From 2017 to 2018 the number of invasive *S. pneumoniae* increased for the first time in the decade.

Figure 8.2a shows the number of invasive cases per 100,000 inhabitants in Denmark per year for 2010 to 2018. During this period, the Danish population increased by 4.5% (from 5,534,738 to 5,781,190 inhabitants). Figure 8.2b shows the number of blood cultures taken per 100,000 inhabitants per year for the same period. In addition, the number of unique patients being blood cultured per 100,000 inhabitants per year is shown. This demonstrates that the number of unique patients with at least one blood culture taken per year has increased dramatically from approximately 2,060 patients per 100,000 inhabitants in 2010 to approximately 2,940 patients per 100,000 inhabitants in 2018 (an increase of 43%). The total number of blood cultures taken (as registered with a unique sample ID in MiBa) per 100,000 inhabitants has increased even more (52%). Thus, on average more patients have more blood cultures taken each year.

Figure 8.1 Number of submitted invasive isolates for each species under surveillance, Denmark

DANMAP 2018

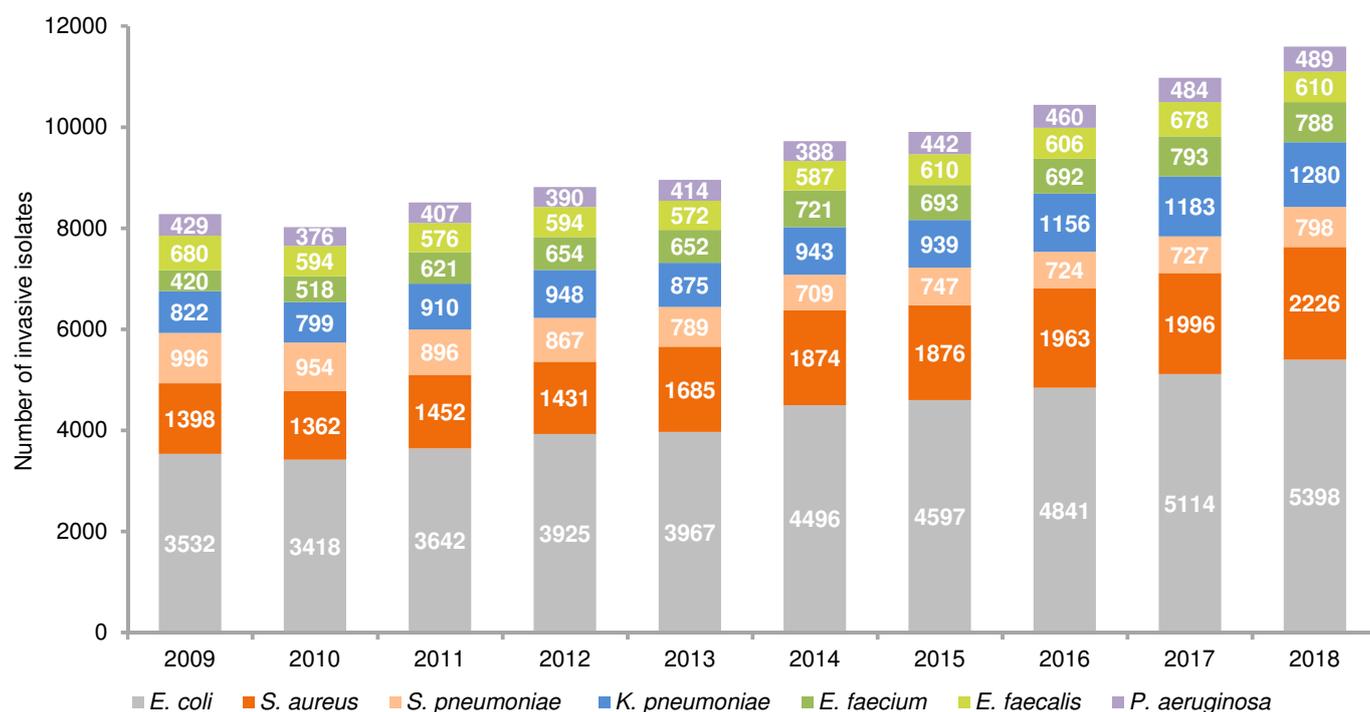
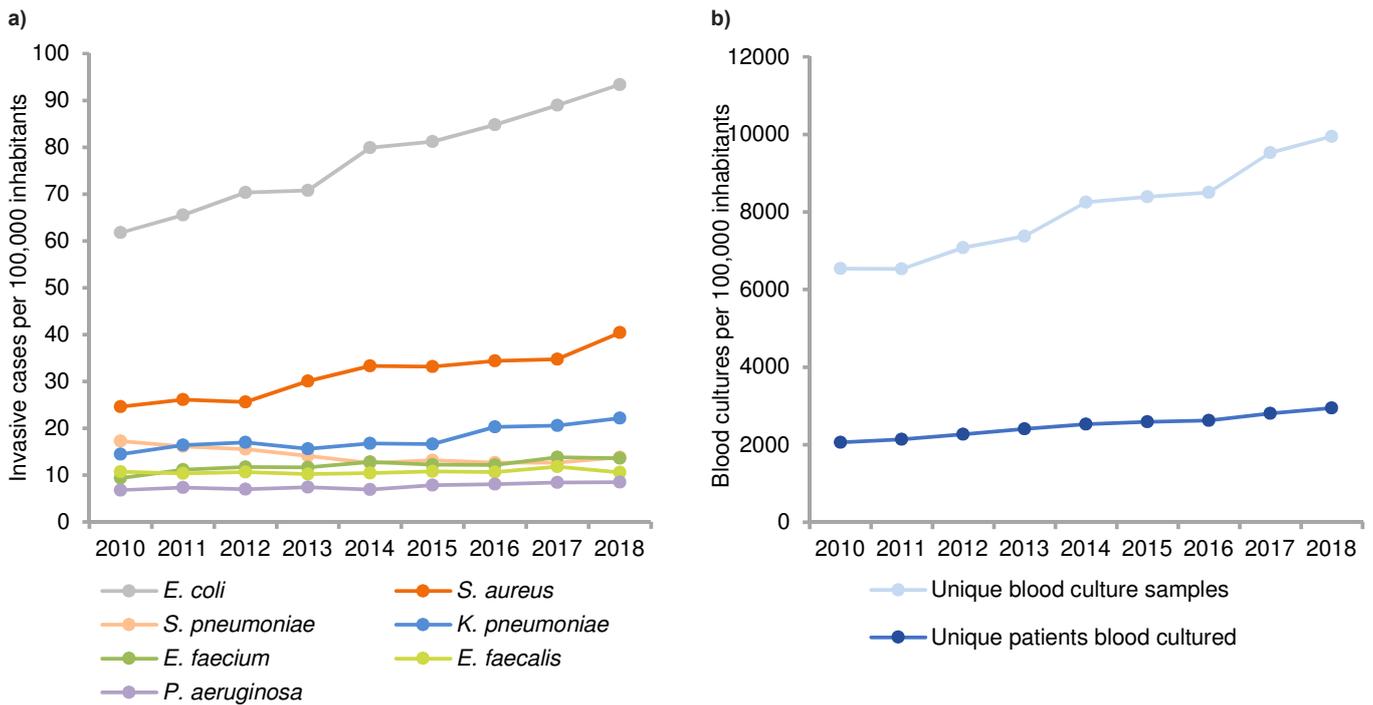


Figure 8.2 Invasive cases and blood cultures taken per 100,000 inhabitants, Denmark

DANMAP 2018



Changes in hospital workflow, improved culturing methods and demographic changes with a growing population of elderly and chronically ill or immunocompromised patients may explain some of the observed changes. The increasing number of invasive infections is of concern to a health care system that is under pressure. It demands fast and effective antibiotic treatment, while simultaneously increasing the risk for the development and selection of resistant bacteria due to a higher consumption of antimicrobials. These resistant bacteria can then be spread in hospital environments with

fragile patient populations underlining the need for a health care system with firmly established infection prevention and control. The importance of proper diagnostics combined with a rational use of antibiotics, reserving the most broad-spectrum antibiotic classes to the patients with multiresistant infections is underlined as well.

The next sections in this chapter present the individual results for the species and/or resistance types under surveillance.

Textbox 8.1

MiBa, the Danish Microbiology Database - now prepared for use in national AMR-surveillance

MiBa is a milestone in the development of a national digital surveillance system for infectious diseases in Denmark and an example of how surveillance systems can be an integrated part of national healthcare infrastructure.

MiBa is a nationwide, automatically updated database containing all test results from all departments of clinical microbiology (DCMs) in Denmark..

The objectives of MiBa are:

- To provide access for healthcare professionals to microbiological test results from all of Denmark for patients in their care
- To provide the foundation for a flexible, timely and complete national surveillance of all laboratory confirmed infectious diseases and microorganisms
- To serve as a shared resource for research
- To ensure automatic transfer of data to other databases monitoring e.g. AMR data and hospital acquired infections
- To aid informed decision making on the treatment of individual patients as well as development of local and national antibiotic guidelines

MiBa is integrated into regional electronic health record systems, providing overview and access at a national level to microbiological test reports for relevant health care personnel. This is important e.g. when patients are moved between hospitals and regions or between different sectors within the health care system. The patients themselves and doctors outside hospital settings, for instance general practitioners, have access via the national health portal www.sundhed.dk.

MiBa is also a primary data source for national surveillance of laboratory confirmed infectious diseases and microorganisms. The development of a fully automated MiBa-based surveillance system is a complex, ongoing process, which includes standardisation of data-coding, clarification of concepts and a close collaboration between stakeholders, in particular all Danish DCMs.

When MiBa was launched in 2010, only information relevant for patient treatment was included in the test reports. The copy of the report transferred to MiBa from the local laboratory information system (LIS) was identical to the report sent to the clinician for diagnostic information and treatment purposes. Denmark has a longstanding tradition for selected reporting of susceptibility results supporting local antibiotic treatment guidelines. Hence, not complete laboratory data, but only the antibiogram of relevance to the clinician's choice of antimicrobial treatment was transferred to MiBa.

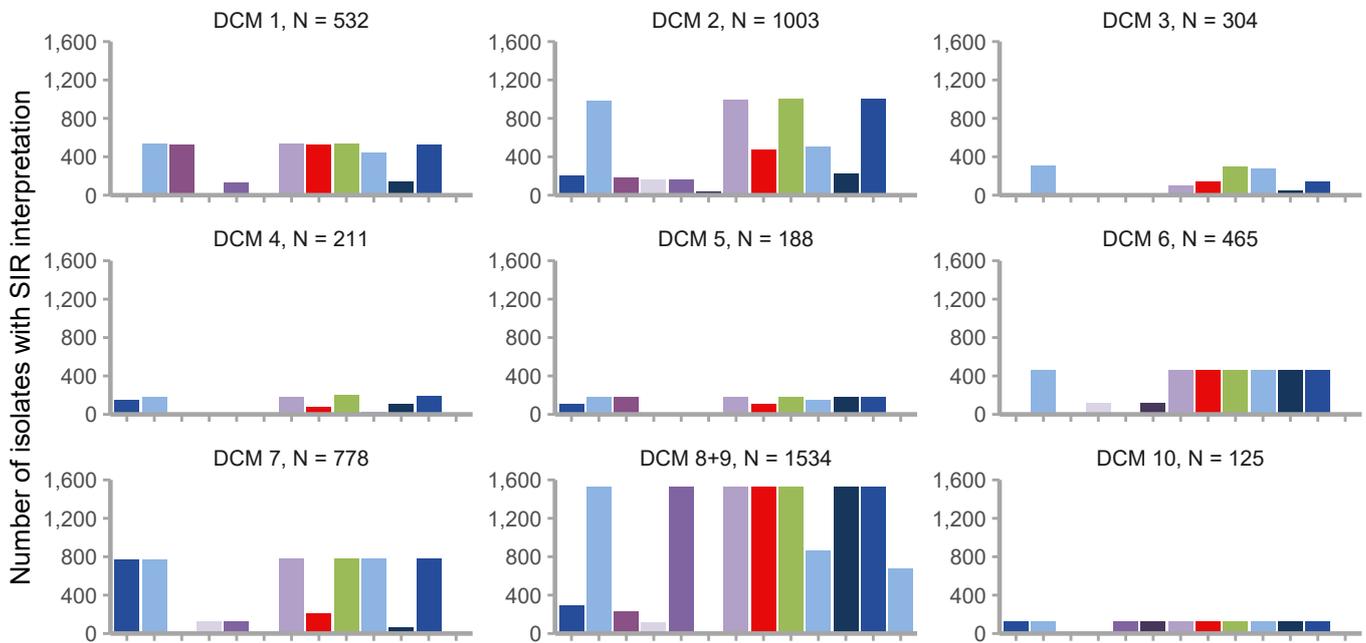
In 2017, a new and expanded standard data-transfer protocol was developed and implemented, allowing more complete laboratory data and specifically data relevant for surveillance purposes to be included in the copy transferred from the local LIS to MiBa. From 2018 and onwards, practically all tests for AMR performed in the departments of clinical microbiology have been included in the copy of the report transferred to MiBa, enabling a comprehensive national surveillance of AMR. These "extended" AMR-data are not visible in the version of the report accessed by the clinician. This new and expanded data model for microbiology in Denmark allows MiBa to be used for DANMAP and to form the future basis for national AMR surveillance.

Figure 1 shows examples of antibiograms from human invasive *E. coli* analysed at the Danish DCMs available from MiBa before and after the implementation of the new protocol. It demonstrates clearly, how AMR-data are now transferred systematically and almost completely to MiBa.

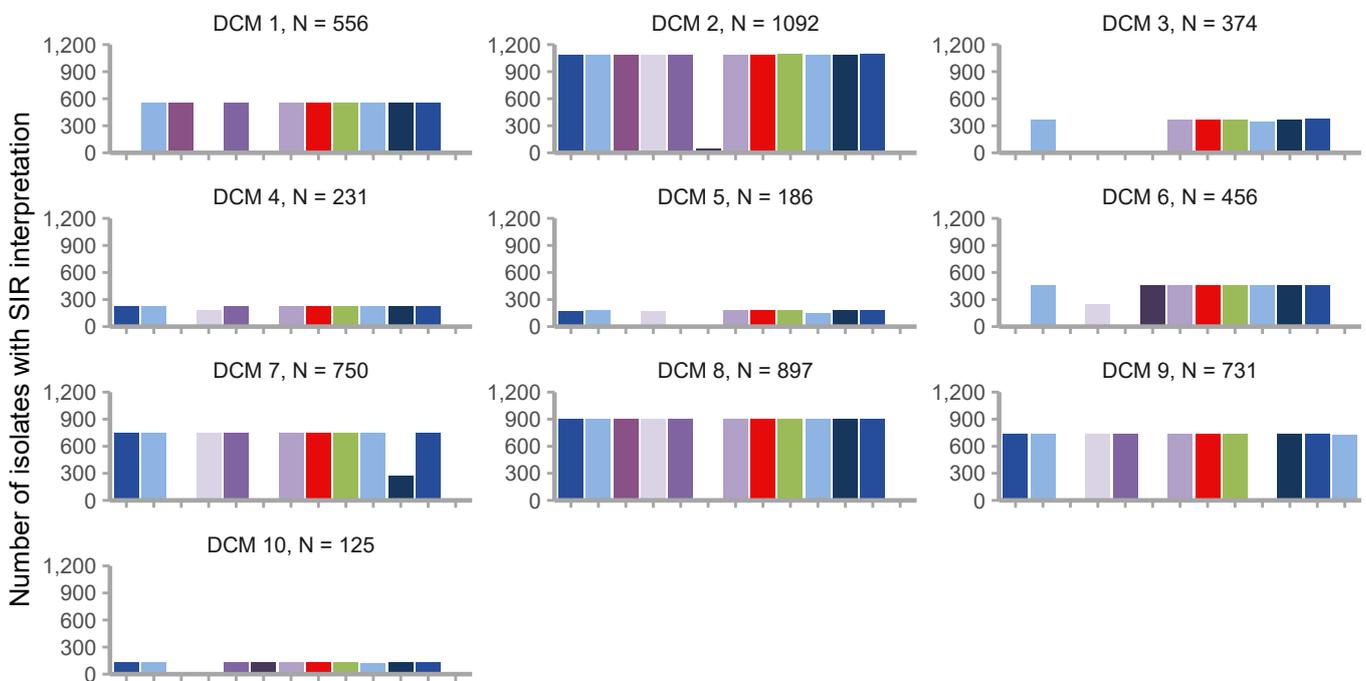
Figure 1 Number of invasive *E. coli* (from unique patients) identified in MiBa for, a) 2017 and b) 2018, from the 10 DCMs in Denmark, and the available parts of their antibiograms

DANMAP 2018

a) 2017



b) 2018



continued ... Textbox 8.1

Data for DANMAP used to be reported manually and non-standardized from the DCMs to SSI - a time consuming, cumbersome task demanding active reporting from the labs and data cleaning at SSI. In DANMAP 2018, MiBa was used as the primary data source for monitoring reported resistance in clinical bacteria in humans (calculations and Figures in section 8.2).

To ensure a smooth and safe shift from the old to the new surveillance system, a comprehensive validation of the data, focusing on completeness and correctness, was performed:

1. Was it possible to extract complete national data from MiBa to fulfil the purposes of the surveillance as usual?
2. Were there any mismatches between MiBa data and data delivered from the individual DCM - and can mismatches be explained?

Selected results are presented in Figure 2 (A full dataset of the comparisons is available online).

In general, MiBa data were identical or nearly identical to the DCM data. When differences turned up, we were able to identify the individual cases and explain the cause of the difference.

Most differences could be explained by:

- The data provided by the individual DCM from their own laboratory information system differed a bit in the criteria for in-/exclusion. Examples of this would be: Inclusion of other specimen than blood, e.g. peritoneal fluid collected in a blood culture flask or inclusion of a specimen, identified by DNA detection or microscopy but never cultured
- Differences in the way of sorting and selecting, when only data from the first isolate per patient per sample type per year were supposed to be included
- Missing data from the dataset provided by the individual DCM
- Some S-I-R interpretations were not registered in MiBa, even though they were present in the data provided by the DCMs

In the process of clarifying mismatches we obtained new insight in details of the data, which is important when MiBa-data on AMR are processed automatically. The fact that the selection criteria can be standardised across all DCMs when performed centrally will further increase data quality. Such standardisations and the principles for interpretation of data will be decided in close collaboration with the DCMs in near future.

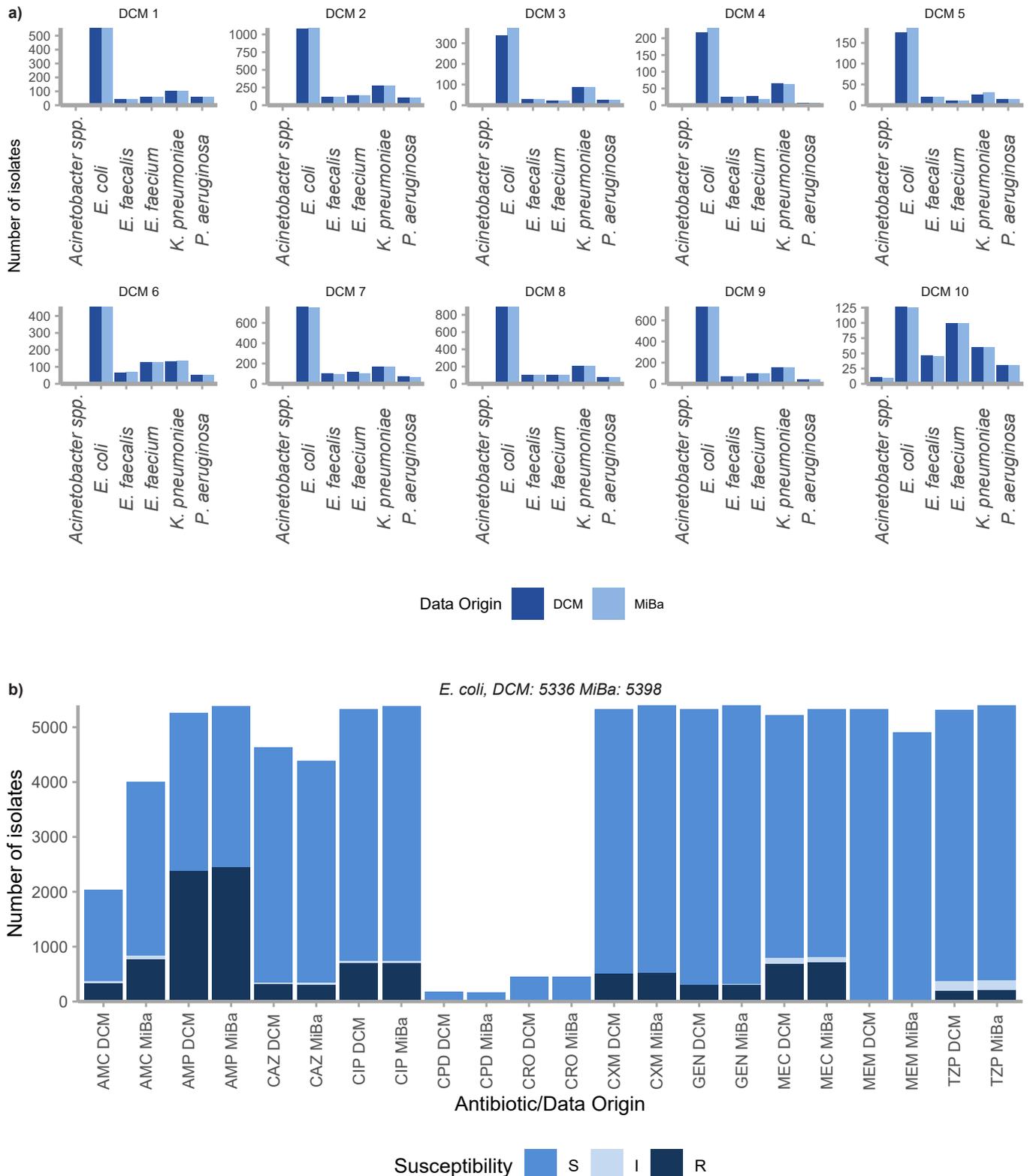
This new MiBa based reporting will provide timely, harmonised and cleaned AMR data, ready for analysis, with a tremendous reduction in workload. This will lead the way to online publishing of AMR data in (close to) real time.

In conclusion, MiBa data (for surveillance purposes) were as comprehensive as the combined data provided individually from each DCM. Basing DANMAP and national AMR surveillance on MiBa reduces workload considerably, increases data quality and allows real time analysis of AMR data. The next step is to implement standardised search queries and refined algorithms for automatic data processing. The next goal is to develop a full automatic online visualisation tool for national AMR surveillance data in close collaboration with the DCMs.

*Sissel Skovgaard, Anna E. Henius, Stefan S. Olsen, Jonas Kähler and Marianne Voldstedlund
For further information: Marianne Voldstedlund, mav@ssi.dk and Sissel Skovgaard, sisk@ssi.dk*

Figure 2 Comparing MiBa data on invasive isolates with data reported from the DCMs (2018)

DANMAP 2018

Figure 2a) Comparisons of total numbers of invasive isolates subdivided on species-level and on individual DCM. 2b) Comparisons of S-I-R interpretations of invasive *E. coli* isolates for all 10 DCMs added together

Glossary: AMC = Amoxicillin/Clavulanate, AMP = Ampicillin, CAZ = Ceftazidime, CIP = Ciprofloxacin, CPD = Cefpodoxime, CRO = Ceftriaxone, CXM = Cefuroxime, GEN = Gentamicin, MEC = Mecillinam, MEM = Meropenem, TZP = Piperacillin/Tazobactam

8.2 Surveillance based on MiBa data

8.2.1 *Escherichia coli*

Escherichia coli (*E. coli*) is by far the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark accounting for 55%, 45% and 21%, respectively, of all registered positive cultures in MiBa for 2018. It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferred resistance mechanisms from these to *E. coli* are thus frequently seen as is the development of resistance through mutations. Since *E. coli* is the most frequent cause of urinary tract infections and bacteraemia, it is also one of the bigger drivers of antibiotic use.

Invasive cases from hospitals

For 2018, altogether 5,398 unique patients with invasive *E. coli* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, ciprofloxacin, piperacillin/tazobactam, gentamicin, cefuroxime and mecillinam. In addition, nine DCMs routinely registered antimicrobial susceptibility to 3rd generation cephalosporins and meropenem and seven routinely registered antimicrobial susceptibility to amoxicillin/clavulanic acid. Tested 3rd generation cephalosporins were either ceftazidime, ceftriaxone or cefpodoxime, while the tested carbapenem was meropenem for all DCMs in 2018. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Zone diameters have also been registered since 2015 and will be commented on in specific cases.

Resistance rates for all tested antimicrobials, presented as a national mean for each antibiotic class, are summarized in Table 8.2. In Figure 8.3 rates of resistance are shown for the past decade - here data are presented as a national mean, when at least six DCMs have registered routine testing. Time trends and significance levels of these, based on the resistance rates five and ten years back, respectively, are presented in Figure 8.3c. Test results for mecillinam resistance in invasive *E. coli* are excluded from Figure 8.3, since the S-I-R interpretation rules for the individual DCM differ and/or vary over time, making comparison of the results difficult and time trends unreliable.

A continuous increase in the number of invasive *E. coli* cases was observed throughout the years, from 3,426 cases in 2010 to 5,398 cases in 2018. This corresponds to 61.8 cases and 93.4 cases per 100,000 inhabitants respectively and an increase of 51%. Simultaneously, the total number of blood cultures taken also increased steeply with 52% per 100,000 inhabitants (subsection 8.1.3).

In 2018, the proportion of ciprofloxacin resistant strains (13.0%) was comparable to 2017 (12.8%) after a marked increase from 2016 to 2017. This increase mainly reflected a change in the interpretation of S-I-R more than a true epidemiologic change, since new EUCAST breakpoints for ciprofloxacin were implemented in most of the Danish DCMs as of January 2017.

For cefuroxime resistance in invasive *E. coli* significant increasing trends were observed for the past decade as well as for the past five years. For 3rd generation cephalosporins in invasive *E. coli* there is an increasing trend in resistance rates from 2017 to 2018, but the five year trend analysis shows no significance.

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, 2018

DANMAP 2018

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	45	42	37
Mecillinam	13	7.4	5.1
Piperacillin/tazobactam	3.8	3.5	2.9*
Amoxicillin/clavulanic acid	19	12*	6.3*
Sulfonamide		31*	28
Trimethoprim		25	23
Nitrofuratoin		1.2*	0.8*
Gentamicin	5.7	4.7	3.8*
Ciprofloxacin	13	11	8.1
Cefuroxime	9.8	7.2	5.6*
3 rd generation cephalosporins	7.3	6.4	4.9
Carbapenem	0.0	0.0	0.0*
Max. number of isolates tested for resistance to the presented antibiotics	5397	47828	80703

The presented resistance rates are means of the resistance rates determined by the individual DCMs. Included are results from all DCMs where > 75% of the isolates in each antibiotic/sample group were susceptibility tested. The * marks where less than 6 (out of totally 10 DCMs) tested a sufficient percentage of their samples. For carbapenems in urines from primary health care this were only two DCMs

Resistance to piperacillin/tazobactam and gentamicin both showed significant decreasing trends for the past five years. For more details see Figure 8.3.

The number of carbapenem resistant invasive *E. coli* isolates remained very low with two carbapenem resistant and four

intermediate susceptible strains in 2018. The level of multi-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained at around 2% (Table 8.3). For colistin none of the invasive *E. coli* were registered resistant. However, colistin resistance is not tested for routinely.

Figure 8.3 *Escherichia coli*. Resistance (%) in invasive isolates from humans, Denmark

DANMAP 2018

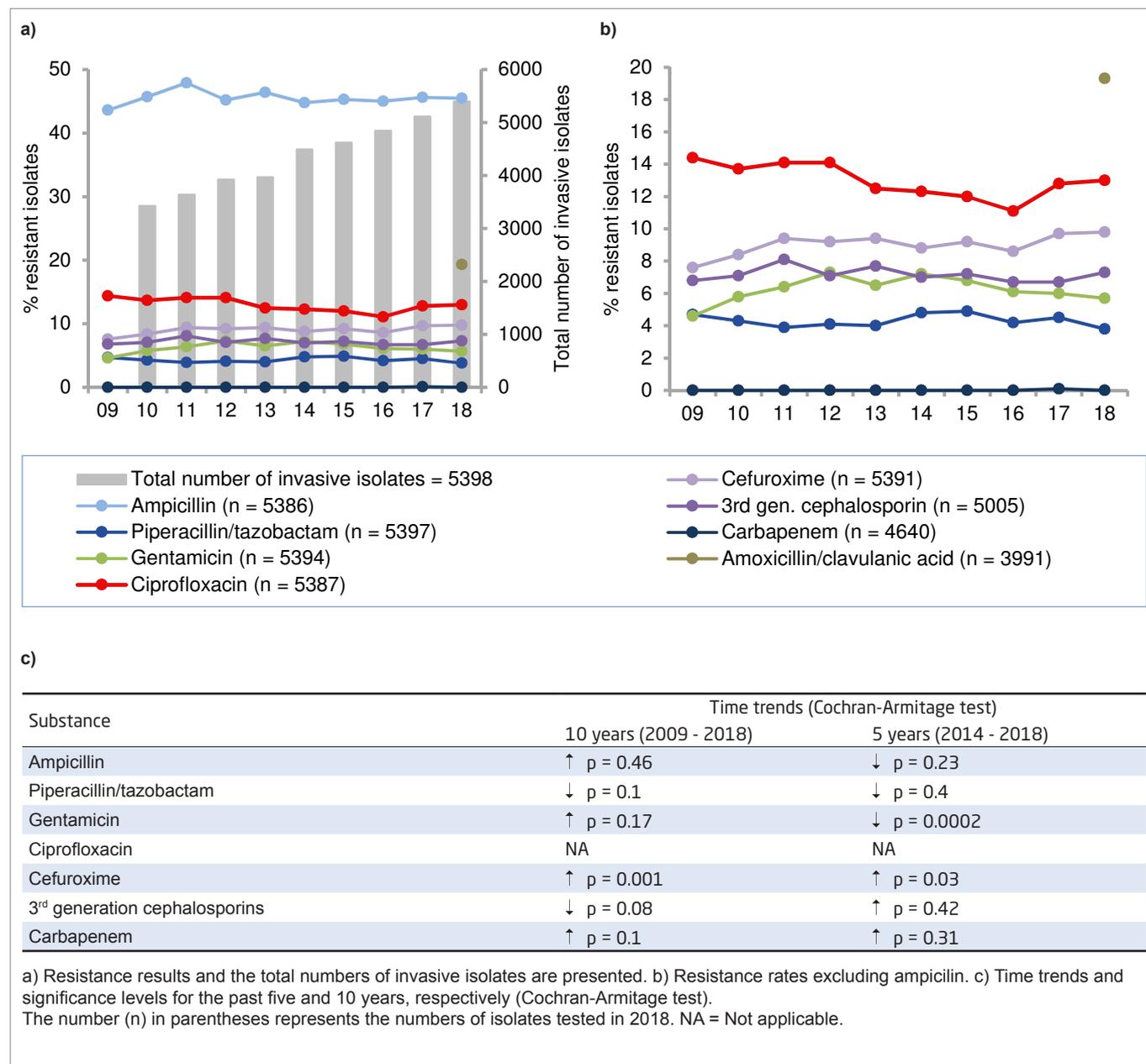


Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark

DANMAP 2018

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)
Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	90 (4039)	88 (4071)	98 (4763)	95 (4883)	93 (4997)
Total number of invasive isolates	4495	4614	4841	5114	5398

Urinary cases from hospitals

For 2018, altogether 47,914 unique patients with *E. coli* isolates, cultured in urine samples from hospital patients from all DCMs in Denmark, were identified in MiBa. 3,946 unique patients with *E. coli* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary urines. However for 1,273 of these samples the patients were already represented in either hospital or primary care or both, with other *E. coli* urine cultures in 2018.

All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, mecillinam, piperacillin/tazobactam and ciprofloxacin. In addition nine DCMs routinely registered antimicrobial susceptibilities to gentamicin and eight DCMs to cefuroxime and trimethoprim. Seven DCMs routinely registered antimicrobial susceptibilities to 3rd generation cephalosporins, six DCMs to carbapenem and five DCMs to amoxicillin/clavulanic acid and sulfonamide. Four DCMs routinely registered antimicrobial susceptibilities to nitrofurantoin.

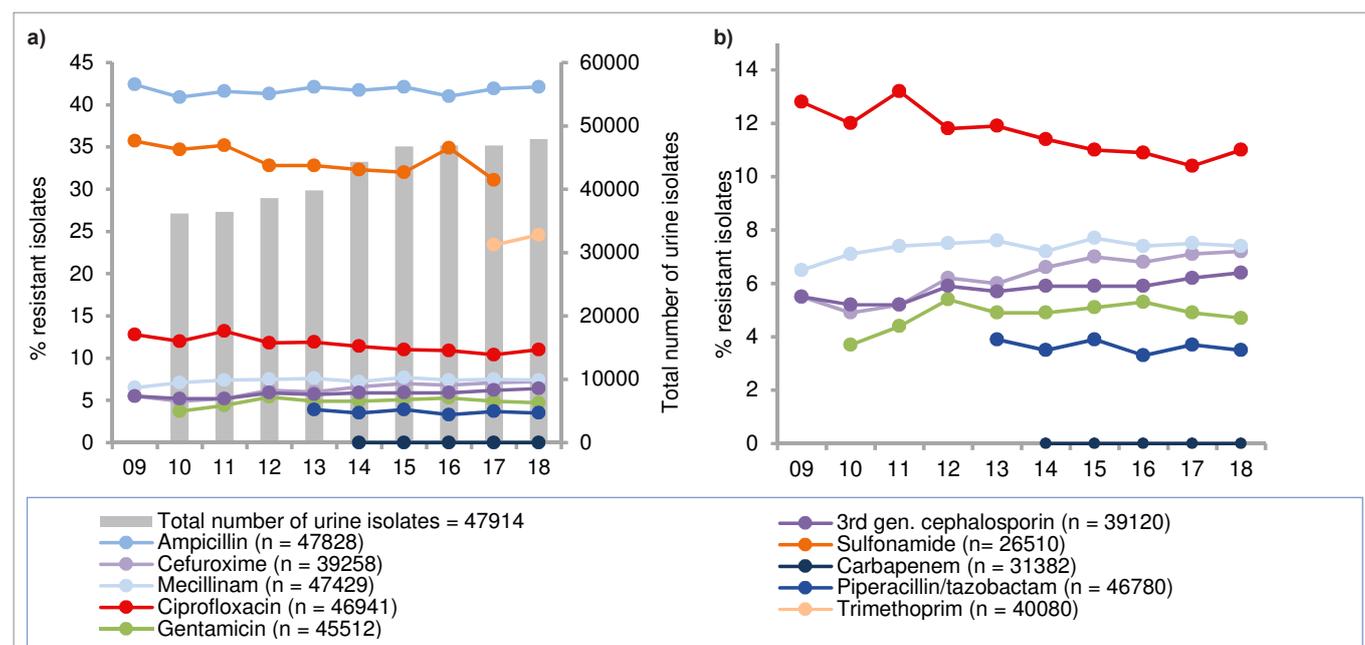
In Table 8.2. resistance results for all tested antimicrobials are summarised together with the results from the invasive isolates as a national mean for each antimicrobial. In Figure 8.4, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing for a given drug. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.4c.

Time trend analysis revealed that mecillinam, cefuroxime and 3rd generation cephalosporins all had significantly increasing resistance rates when looking back 10 years. Looking back five years increases were no longer significant for mecillinam, while cefuroxime and 3rd generation cephalosporin resistance rates were still increasing. For more details see Figure 8.4.

In 2018, 24 carbapenem resistant and 20 intermediate susceptible *E. coli* urine isolates from hospital patients were registered.

Figure 8.4 *Escherichia coli*. Resistance (%) in urine isolates from humans in hospitals, Denmark

DANMAP 2018



c)

Substance	Time trends (Cochran-Armitage test)	
	10 years (2009 - 2018)	5 years (2014 - 2018)
Ampicillin	↑ p = 0.32	↑ p = 0.16
Mecillinam	↑ p = 4.578e-10	↑ p = 0.22
Piperacillin-tazobactam		↓ p = 0.32
Sulfonamide	↓ p < 2.2e-16	↓ p = 3.633e-05
Gentamicin		↓ p = 0.05
Ciprofloxacin	NA	NA
Cefuroxime	↑ p < 2.2e-16	↑ p = 0.0005
3rd generation cephalosporins	↑ p = 1.052e-14	↑ p = 0.0009

a) Resistance rates and total numbers of urine isolates are presented, b) Resistance rates excluding ampicillin, sulfonamide and trimethoprim,

c) Time trends and significance levels for the past five and 10 years respectively

The number (n) in parentheses represents the numbers of isolates tested in 2018. NA = Not applicable

Urinary cases from primary health care

For 2018, altogether 80,851 unique patients with *E. coli* isolates, cultured in urine samples from primary health care, from nine DCMs in Denmark, were identified in MiBa. The general practitioners in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom are serviced by one private laboratory.

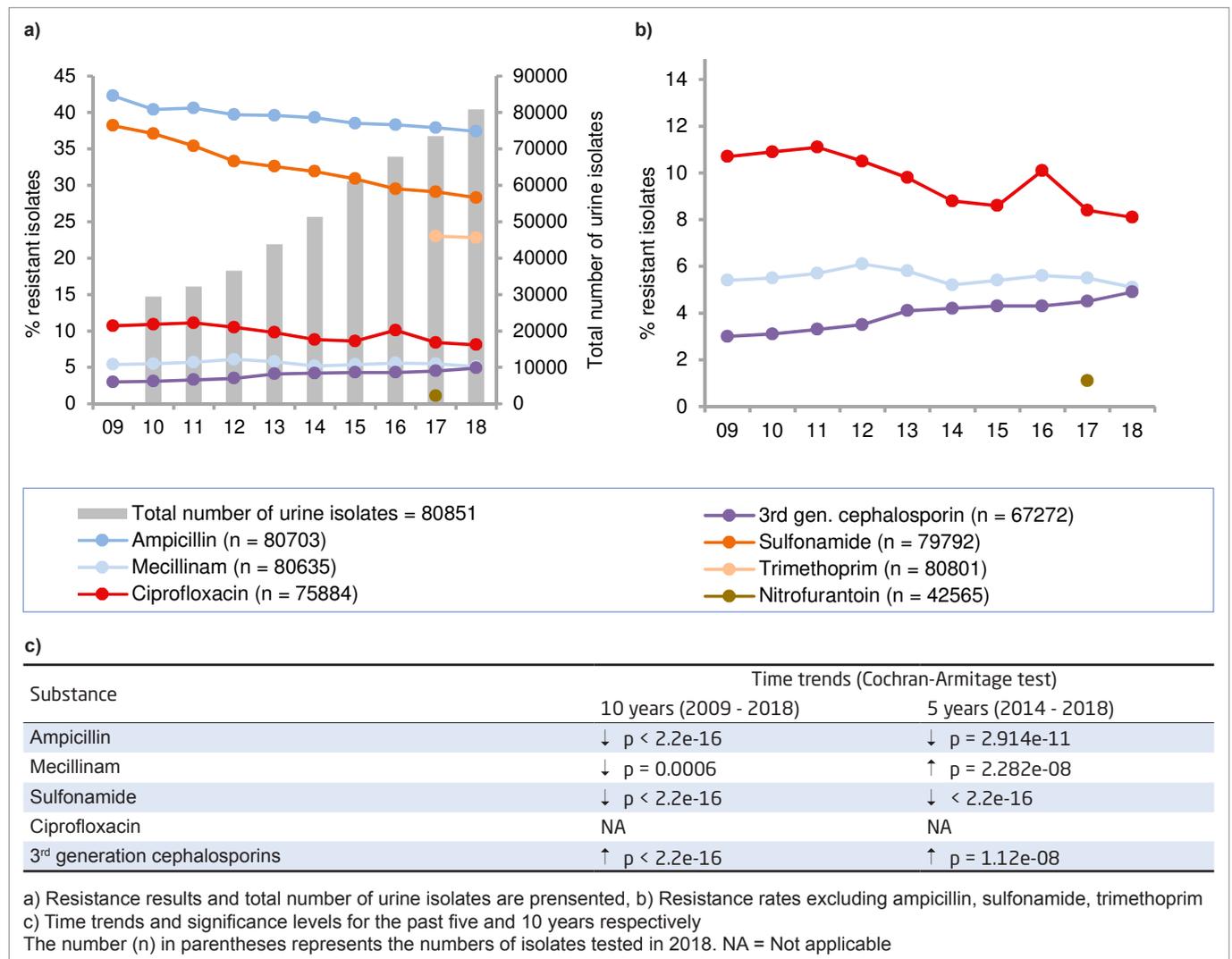
All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ampicillin, mecillinam, sulfonamide and trimethoprim. In addition eight DCMs routinely registered antimicrobial susceptibilities to ciprofloxacin, seven DCMs to 3rd generation cephalosporins and five DCMs to nitrofurantoin. Four DCMs routinely registered antimicrobial susceptibilities to amoxicillin/clavulanic acid, gentamicin and cefuroxime, three DCMs to piperacillin/tazobactam and two DCMs to carbapenem.

As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.2. In Figure 8.5, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.5c.

As for the number of invasive isolates, a steep increase (175%) in registered *E. coli* isolates cultured from urine samples from primary health care has been observed since 2010. Data extractions from MiBa for the same period show a steep increase in the total number of submitted urines for culturing as well. In 2010 between 164,000 and 206,000 urine samples were submitted to the DCMs from the primary sector, compared to 455,500 urine samples in 2018, representing an increase between 120% and 178%. The imprecision in the numbers extracted from MiBa is caused by, in some cases, difficulties in categorisation of hospital and primary care urines.

Figure 8.5 *Escherichia coli*. Resistance (%) in urine isolates from humans in primary health care, Denmark

DANMAP 2018



Time trend analysis revealed that resistance to 3rd generation cephalosporins have increased significantly both for the past decade and for the past five years. Mecillinam resistance rates have increased for the past five years. Sulfonamide and ampicillin resistance rates have decreased both for the past decade and for the past five years. Ciprofloxacin resistance rates must be interpreted with caution because of changed EUCAST break points, but the trend seems decreasing. For more details see Figure 8.5.

In 2018, eight carbapenem resistant and two intermediate susceptible *E. coli* isolates from primary health care urinary cases were registered. As noted, registering of carbapenem susceptibility results in urine samples from PHC is only routinely done at two of nine DCMs. However, since carbapenem resistant isolates are often multiresistant, most DCMs recognise them and perform additional testing.

Conclusion

A substantial increase in the total number of invasive and of primary health care urinary *E. coli* cases were observed since 2010. Within the same time period, a corresponding increase in the total numbers of blood cultures taken and urinary samples registered from the primary sector occurred. The number of urinary *E. coli* cases from hospitals showed less increase as did the total number of urine samples registered from hospitals. It could be that at least part of the increase in the number of *E. coli* cases was due to an increased number of cultures taken.

A trend for all three categories of *E. coli* isolates were the increasing cephalosporin resistance rates for both the past decade and the past five years. An exception was the resistance to 3rd generation cephalosporins in invasive *E. coli*, which showed no increases in resistance rates for the past ten and five years, but an increase was observed from 2017 to 2018. See also subsection 8.3.1 for genetic characterisation of ESBLs from bloodstream infections. An additional analysis of zone diameters for cefuroxime in invasive *E. coli*, registered since 2015, revealed the same upward trend, and thereby the increase was not caused by changes in interpretations. In Europe, an increase in resistance to 3rd generation cephalosporins in invasive *E. coli* was observed (EU/EEA population-weighted mean 14.9%). The majority of these (almost 90%) were extended-spectrum beta-lactamase (ESBL)-positive [EARS-Net annual report, 2017]. When the number of ESBL-positive *E. coli* isolates increases, this can lead to increased use of broader spectrum antimicrobials and thereby a vicious circle selecting for even more resistance can start. Whenever possible, a deescalation to narrow spectrum antimicrobials therefore is necessary.

Although still at a relatively low level, an increase in total numbers of carbapenem resistant isolates was observed in *E. coli* urine isolates from hospitals in 2018. The risk of further increasing levels of carbapenem resistance in the future is worrisome.

It is positive to note that the resistance rates for piperacillin/tazobactam remained low and that time trends were stable. Also for gentamicin, the resistance rates were rather low and time trends even decreasing for the past five years. In urine isolates, mecillinam resistance rates were still relatively low and resistance to nitrofurantoin was rare in *E. coli*. There have been no reports of pan-resistant *E. coli* in Denmark yet.

Sissel Skovgaard, Stefan S. Olsen and Anna E. Henius
For further information: *Sissel Skovgaard, sisk@ssi.dk*

8.2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) is capable of colonising the gastrointestinal and respiratory tract in humans, especially in hospitalised patients. It may cause infections such as urinary tract infections, severe pneumonia and blood stream infections - the latter especially in patients with indwelling devices - and may give rise to nosocomial outbreaks. *K. pneumoniae* rather easily acquires and is able to transfer plasmid-borne resistance traits. *K. pneumoniae* is the fourth most common species in blood cultures, constituting 5% of positive blood cultures registered in MiBa in 2018. It is the third most common species in urine from hospitals, constituting 8% of positive urine cultures in 2018 and the second most common species in urine from primary health care, constituting 6% of positive urine cultures in 2018.

Invasive cases from hospitals

In 2018, altogether 1,280 unique patients with invasive *K. pneumoniae* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, piperacillin/tazobactam, gentamicin and cefuroxime in MiBa. In addition, nine DCMs routinely registered antimicrobial susceptibilities to mecillinam, 3rd generation cephalosporins and meropenem and seven routinely registered antimicrobial susceptibilities to amoxicillin/clavulanic acid. Tested 3rd generation cephalosporins were either ceftazidime, ceftriaxon or cefpodoxime, while the tested carbapenem was meropenem for all DCMs in 2018. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. Direct zone diameters have also been registered since 2015 and will be commented on in specific cases.

Resistance results for 2018 for all tested antimicrobials, presented as a national mean for each antimicrobial class, are summarised in Table 8.4. In Figure 8.6 rates of resistance are shown for the past decade - here data are presented as a national mean, when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.6b. Test results for mecillinam resistance in invasive *K. pneumoniae* are excluded from Figure 8.6, since the S-I-R interpretation rules for the individual DCM

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark

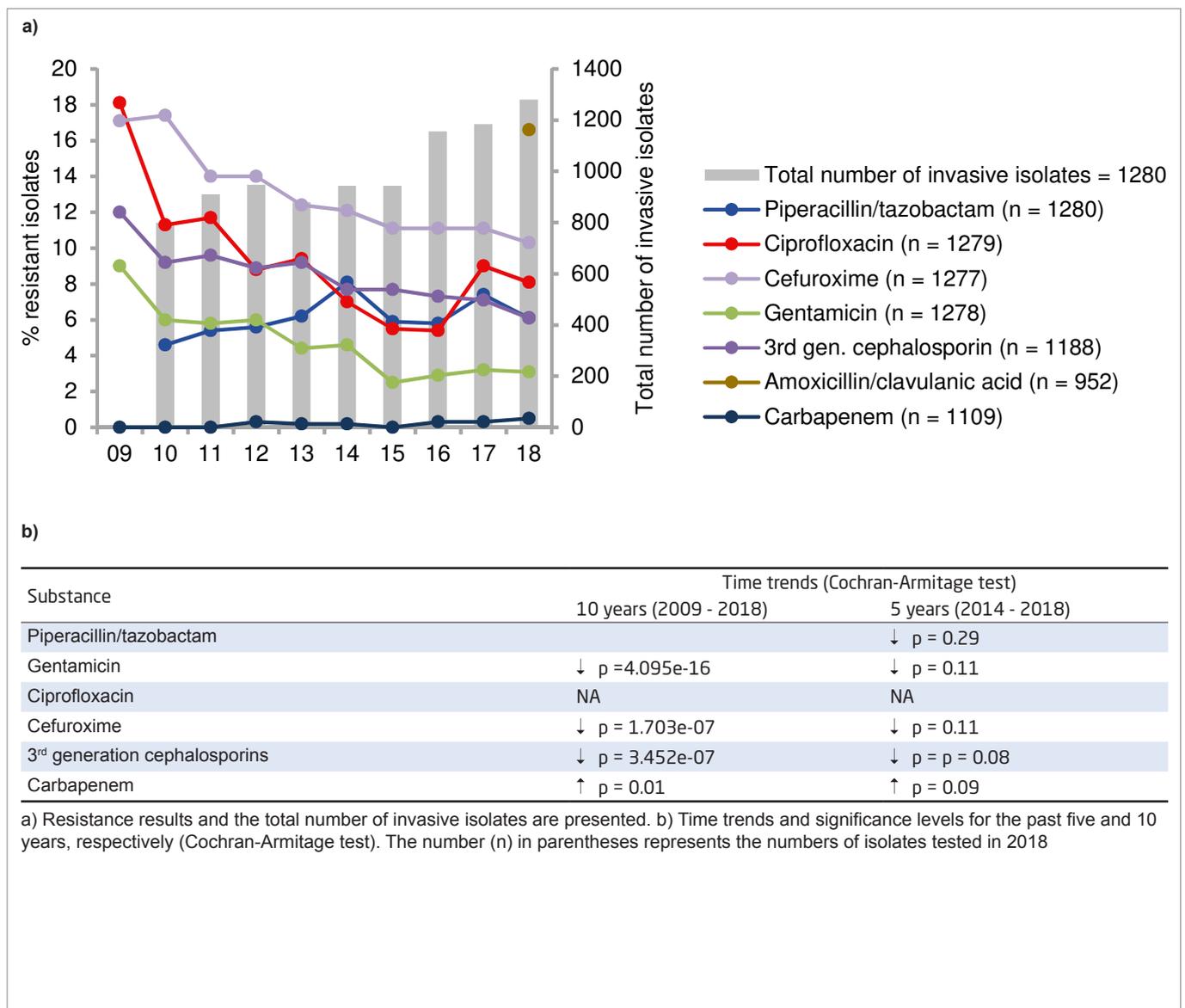
DANMAP 2018

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	18	17	16
Piperacillin/tazobactam	6.1	8.7	7.4*
Amoxicillin/clavulanic acid	17	17	12*
Sulfonamide		22*	25
Trimethoprim		26	26
Nitrofurantoin		14*	15*
Gentamicin	3.1	3.2	2.2*
Ciprofloxacin	8.1	8.9	6.4
Cefuroxime	10	9.5	5.3*
3 rd generation cephalosporins	6.1	6.8	5.3
Carbapenem	0.5	0.2	0.0*
Max. number of isolates tested for resistance to the presented antibiotics	1280	8030	9222

The presented resistance rates are means of the resistance rates determined by the individual DCM. Included are results from all DCMs where > 75% of the isolates in each antibiotic/sample group were susceptibility tested. The * marks where less than 6 (out of totally 10 DCMs) tested a sufficient percentage of their samples. For carbapenems in urines from primary health care this were only two DCMs.

Figure 8.6 *Klebsiella pneumoniae*. Resistance (%) in invasive isolates from humans, Denmark

DANMAP 2018



differ and/or vary over time, making comparison of the results difficult and time trends unreliable.

As for invasive *E. coli* cases, continuous increases in the number of invasive *K. pneumoniae* cases was observed throughout the years, from 799 cases in 2010 to 1,280 cases in 2018. This corresponds to 14.4 cases and 22.1 cases per 100,000 inhabitants respectively, and an increase of 53%. Simultaneously, the total number of blood cultures taken also increased steeply with 52% per 100,000 inhabitants (commented on in subsection 8.1.3).

Graphs and time trend analysis revealed that resistance rates have decreased markedly over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with lesser or insignificant decreases for the past five years (Figure 8.6). The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a change in interpretation of S-I-R more than a true epidemiologic change because of a new EUCAST breakpoints for ciprofloxacin implemented in most Danish DCMs as for January 2017. The downward trend for ciprofloxacin resistance in invasive *K. pneumoniae* seems to have continued when comparing 2018 to 2017 where the same breakpoint have been used.

For carbapenem resistance in invasive *K. pneumoniae* a very small but significant increase was observed for the past 10 years. Total numbers of meropenem resistant invasive isolates are low, but slowly increasing with seven resistant and one intermediary resistant invasive *K. pneumoniae* isolates in 2018 compared to three and none in 2017. The level of multiresistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* remained at around 2% (Table 8.5). For colistin none of the invasive *K. pneumoniae* were registered resistant. However, colistin resistance was not tested for routinely.

Urine cases from hospitals

For 2018, altogether 8,047 unique patients with *K. pneumoniae* isolates, cultured in urine samples from hospitalised patients from all DCMs in Denmark, were identified in MiBa. 564 unique patients with *K. pneumoniae* isolates, cultured in urine samples were excluded because it was not possible to categorise them as hospital or primary care urines. However

198 of those patients were already represented in either hospital or primary care or both, with other *K. pneumoniae* urine cultures in 2018.

All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to mecillinam, piperacillin/tazobactam and ciprofloxacin in MiBa. In addition nine DCMs routinely registered antimicrobial susceptibilities to gentamicin and trimethoprim, eight DCMs to cefuroxime, seven DCMs to 3rd generation cephalosporins, six DCMs to carbapenem and amoxicillin/clavulanic acid, five DCMs to sulfonamide and three DCMs to nitrofurantoin.

Resistance results for all tested antimicrobials are summarised together with the results from the invasive isolates as a national mean for each antimicrobial in Table 8.4. In Figure 8.7, rates of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years, respectively, are presented in Figure 8.7c.

As for the number of invasive cases, an increase (40%) in urinary *K. pneumoniae* cases from hospitals has been observed since 2010. Data extractions from MiBa for the same period show a smaller increase of only 5.9% to 23% in the total number of submitted hospital urine cultures - the imprecision is due to uncertainty regarding the correct categorisation of some urines as hospital or primary care samples.

In 2018, a further increase in the resistance to mecillinam was observed in urine isolates from hospitals. The very steep increase observed in 2017 is thereby, at least, confirmed in 2018. In 2018 also a rather steep increase in resistance to piperacillin/tazobactam and ciprofloxacin was observed. For ciprofloxacin the increase in resistance must be interpreted with caution because of the change in EUCAST breakpoints, but from 2017 to 2018 it should be the same breakpoints used. The only downward trend in resistance for the past five years was for gentamicin. For more details see Figure 8.7.

In 2018, 19 carbapenem resistant and six intermediate susceptible *K. pneumoniae* isolates from hospital urinary cases were registered.

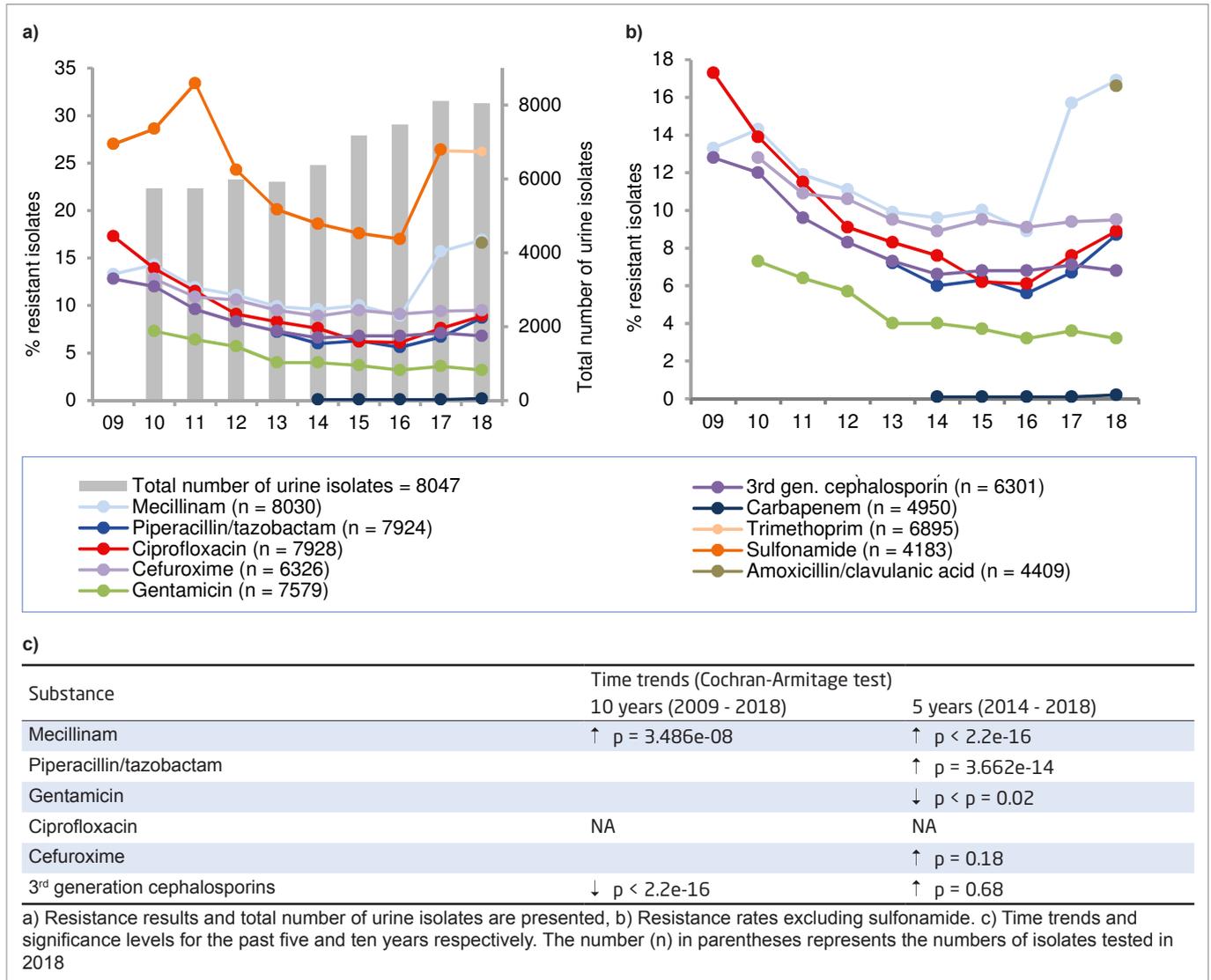
Table 8.5 *K. pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in invasive isolates from humans, Denmark

DANMAP 2018

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)
Resistance	3.0 (26)	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	91 (859)	89 (840)	98 (1131)	95 (1122)	93 (1188)
Total number of invasive isolates	943	943	1156	1183	1280

Figure 8.7 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in hospitals, Denmark

DANMAP 2018



Urinary cases from primary health care

For 2018, altogether 9,227 unique patients with *K. pneumoniae* isolates, cultured in urine samples from primary health care, from nine DCMs in Denmark, were identified in MiBa. The general practitioners in Denmark are all serviced by those nine DCMs except for a few GPs in the Capital Region of Denmark whom are serviced by one private laboratory.

All nine DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to mecillinam, sulfonamide and trimethoprim in MiBa. In addition eight DCMs routinely registered antimicrobial susceptibilities to ciprofloxacin, seven DCMs to 3rd generation cephalosporins, four DCMs to amoxicillin/clavulanic acid, gentamicin and cefuroxime, three DCMs to piperacillin/tazobactam and nitrofurantoin and two DCMs to carbapenem.

As for the results from invasive isolates and isolates from hospital urines resistance results for all tested antimicrobials are shown as national means in Table 8.4. In Figure 8.8, rates

of resistance are presented as a national mean when at least six DCMs have registered routine testing. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.8c.

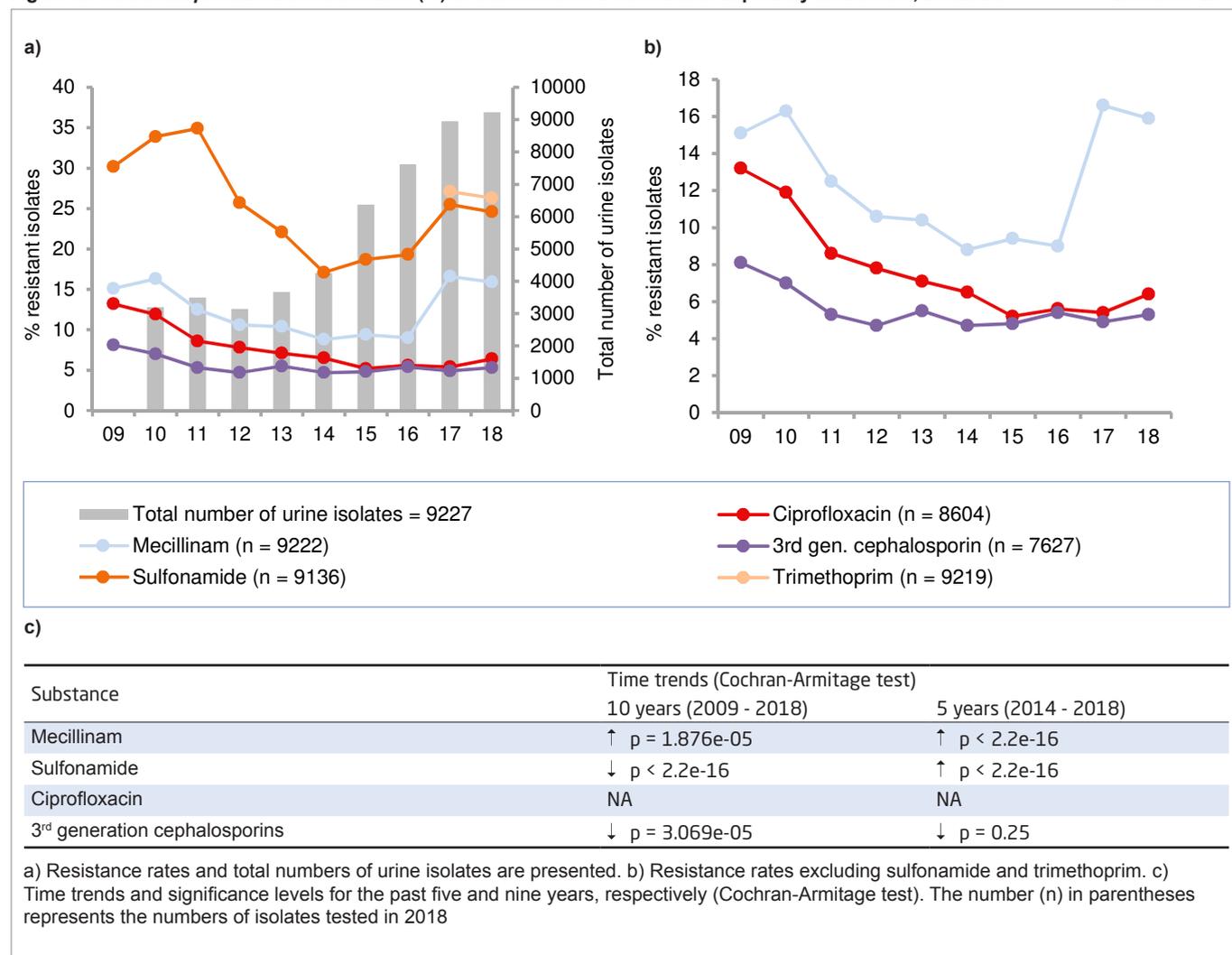
Also the number of urinary *K. pneumoniae* cases from primary health care, saw a very steep increase (189%) since 2010. As mentioned in subsection 8.2.1 *E. coli* in urine from primary health care, the increase in the total number of urine samples submitted to the DCMs from the primary sector, was between 120% and 178%.

In 2018, no further increase in the resistance to mecillinam was observed in urine isolates from primary health care compared to 2017, but still a very steep increase when comparing 2018 to 2016. For more details see Figure 8.8.

Four carbapenem resistant and two intermediate susceptible isolates were registered in 2018.

Figure 8.8 *Klebsiella pneumoniae*. Resistance (%) in urine isolates from humans in primary health care, Denmark

DANMAP 2018



Conclusion

As for *E. coli*, the continuing increase in total numbers of *K. pneumoniae* cases is worrisome and needs to be further investigated. Especially worrisome is the relatively high resistance rates in *K. pneumoniae* to antimicrobials with per oral formulations. Rates of resistance to mecillinam, amoxicillin/clavulanic acid, sulfonamide, trimethoprim and nitrofurantoin between 12% and 26% can lead to more usage of ciprofloxacin. Campaigns, with focus on the unnecessary treatment of asymptomatic bacteriuria in Denmark, can hopefully contribute to counteract the problem. In *E. coli*, which counts for half of all urinary tract infections, the susceptibility rates to antimicrobials with per oral formulations are more feasible.

The significant increase in resistance in *K. pneumoniae* to mecillinam is planned to be further investigated in the future.

The small but increasing levels of carbapenem resistance in *K. pneumoniae* also worries. Often these isolates carry several resistance mechanisms. Despite the worrying tendencies, the proportion of *K. pneumoniae* with combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is not increasing, which is encouraging. In the southern and south-eastern part of Europe high levels of carbapenem resistance as well as combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin is truly problematic [EARS-Net annual report, 2017].

There have been no reports of pan-resistant *K. pneumoniae* in Denmark yet.

Sissel Skovgaard, Stefan S. Olsen and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen causing relatively rare but significant disease in humans. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and can cause bloodstream infections as well. It is a relatively frequent colonizer of medical devices (e.g. indwelling catheters). *P. aeruginosa* infection is a serious problem in immunocompromised patients with e.g. cancer and in patients with cystic fibrosis. The case fatality rate in these patients is high. *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial agents. The antimicrobial classes which can be used for treatment include: some fluoroquinolones, some aminoglycosides, some beta-lactams (piperacillin/tazobactam, ceftazidime and carbapenems) and colistin.

Invasive cases from hospitals

In 2018, altogether 489 unique patients with invasive *P. aeruginosa* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. All 10 DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, piperacillin/tazobactam and gentamicin. Nine DCMs routinely registered antimicrobial susceptibilities to ceftazidime and meropenem in MiBa. Resistance testing was mainly performed by disc diffusion or E-test. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

Data are presented in Figure 8.9.

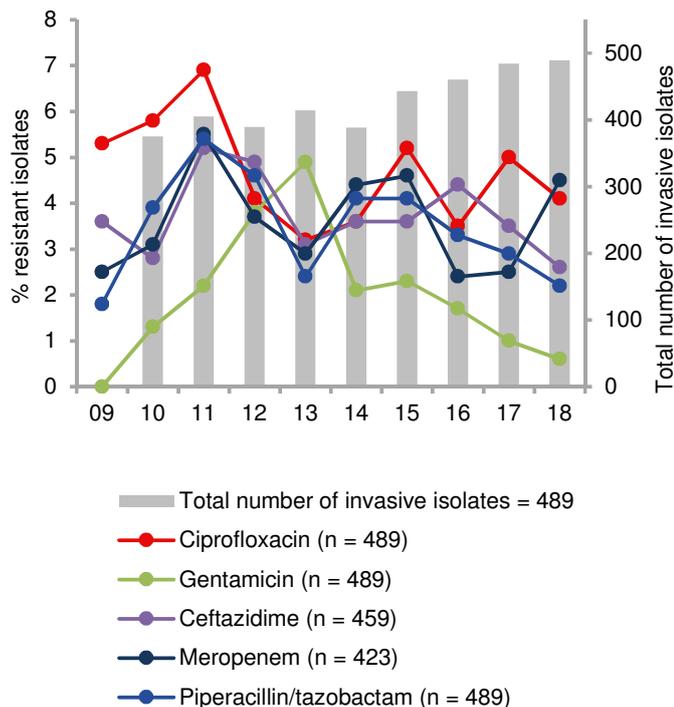
One invasive isolate of *P. aeruginosa* identified in MiBa for 2018 was registered colistin resistant with an MIC of 3 mg/L.

Conclusion

Regarding resistance in invasive *P. aeruginosa* the situation in Denmark is quite stable and with relatively low prevalence. EARS-Net 2017 reported a small decreasing trend in resistance in the EU/EEA population-weighted mean for all antimicrobial groups under surveillance during the period 2014-2017. EU/EEA population-weighted mean in 2017 were for ceftazidime 14.7%, fluoroquinolones 20.3%, aminoglycosides 13.2%, piperacillin/tazobactam 18.3% and carbapenems 17.4%. Large inter-country variations are reported between south-east Europe and north Europe [EARS-Net annual report, 2017]. Denmark was below 5% resistance proportions for all antibiotics under surveillance in invasive *P. aeruginosa* isolates in 2018.

Sissel Skovgaard, Stefan S. Olsen and Anna E. Henius
For further information: *Sissel Skovgaard, sisk@ssi.dk*

Figure 8.9 *Pseudomonas aeruginosa*. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018



The number (n) in parentheses represents the numbers of isolates tested in 2018

8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature, in soil, water and/or animals and humans. In humans *Acinetobacter* can colonize the skin and wounds but may also be the cause of hospital-acquired infections like central line-associated bloodstream infections, nosocomial pneumonia, urinary tract infections and wound infections. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* species possess an inherent resistance to a broad range of antimicrobials because of a low membrane permeability and constitutive expression of efflux systems. The antimicrobial classes which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. For especially *A. baumannii*, multiresistant clones are widespread in the hospital environment in many south- and east European countries, where they cause problems with outbreaks in fragile patient subpopulations at e.g. intensive care units. Of worldwide concern are severely war-wounded soldiers colonised or infected with multiresistant *A. baumannii*.

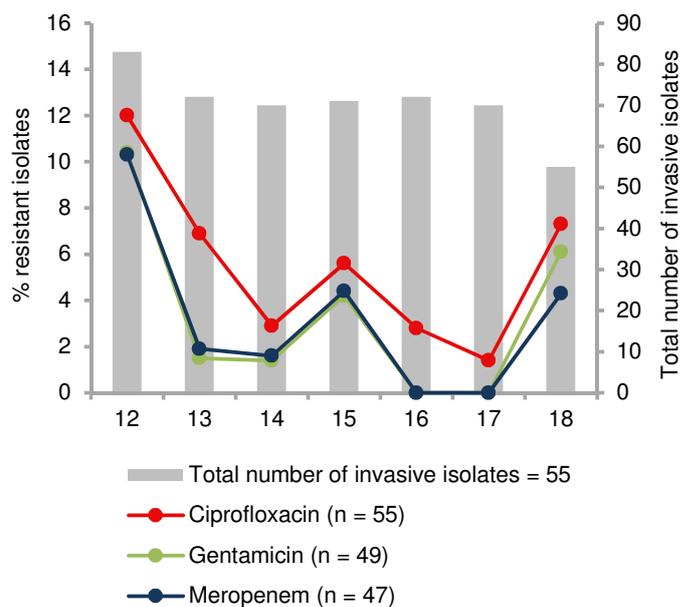
Invasive cases from hospitals

In 2018, 55 unique patients with *Acinetobacter* species invasive isolates from eight departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. For two DCMs, there were not identified any invasive *Acinetobacter* species in MiBa in 2018. All eight DCMs routinely (>75% of isolates) registered antimicrobial susceptibility interpretations to ciprofloxacin, and seven to meropenem and gentamicin. Resistance testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, and are based on the S-I-R system.

Data are presented in Table 8.6 and in Figure 8.10.

Compared to the previous six years, less invasive *Acinetobacter* species were registered in 2018. Two isolates (both *A. baumannii*) had combined resistance to ciprofloxacin, gentamicin and meropenem. None of the invasive *Acinetobacter* species were reported colistin resistant. However, colistin resistance is not tested for routinely.

Figure 8.10 *Acinetobacter* spp. Resistance (%) in invasive isolates from humans, Denmark DANMAP 2018



The numbers (n) in parentheses represents the number of isolates tested in 2018

Conclusion

In general, low total numbers of invasive *Acinetobacter* species is registered in Denmark, as well as low total numbers of resistant invasive *Acinetobacter* species. In EARS-Net, markedly differences in resistance profiles across Europe have been reported with more than 50% of the isolates being resistant to at least one of the three surveilled antimicrobials (fluoroquinolones, aminoglycosides and carbapenems). Particularly the Baltic and southern and south-eastern countries of Europe reported on problems with high resistance and the most common was combined resistance to fluoroquinolones, aminoglycosides and carbapenems. The northern countries reported between 0% and 3.4% combined resistance in 2017 [EARS-Net annual report, 2017].

Sissel Skovgaard, Stefan S. Olsen and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

Table 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates

DANMAP 2018

	2012		2013		2014		2015		2016		2017		2018	
	res.	n												
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72	1	70	4	55
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70	3	49
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67	2	47
Total number of invasive isolates	84		72		72		71		72		70		55	

res. = number of resistant isolates. n = number of tested isolates

8.2.5 Enterococci

Enterococci constitute a part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus enterococcus have been described, but the majority of the human infections are caused by *E. faecalis* and *E. faecium*. Most common clinical infections include urinary tract infections, bacteraemia and bacterial endocarditis. Enterococci are inherently resistant to many groups of antimicrobials and thereby get a selective advantage in e.g. hospitalised patients under antibiotic treatment where they can lead to colonisation or infection. The source of hospital infection is often associated with the use of medical supplies, such as catheters, as well as other instruments and medical devices. Use of antimicrobials in these patients increases the risk for an enterococcal infection.

Therapy of enterococcal infections may be challenging. For *E. faecium*, where the vast majority are ampicillin resistant, severe infections are treated with vancomycin. Antimicrobials, such as linezolid and daptomycin are options for treatment of the multiresistant, vancomycin-resistant enterococcus (VRE). Combinational therapy based on a synergistic effect of beta-lactam antimicrobials (penicillin/ampicillin) with an aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis.

Invasive cases from hospitals

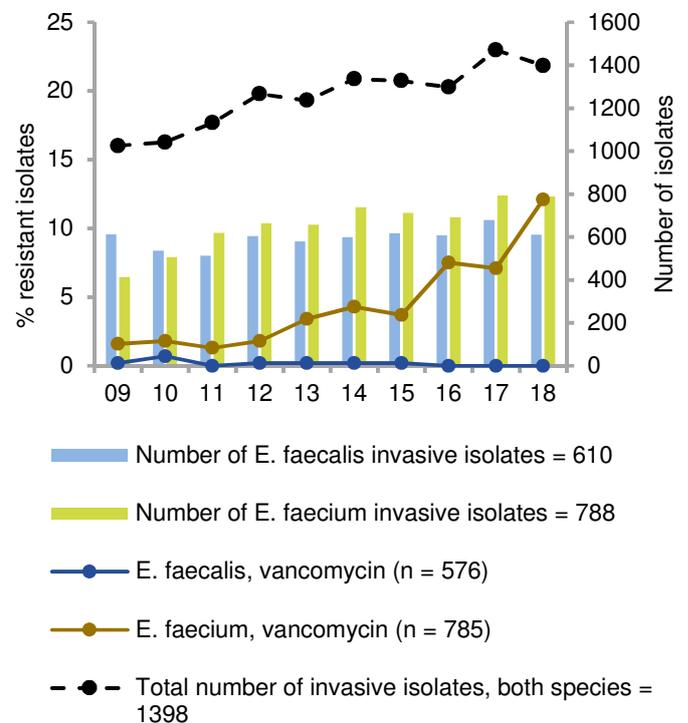
For 2018, altogether 610 unique patients with invasive *E. faecalis* isolates and 788 unique patients with invasive *E. faecium* isolates from all departments of clinical microbiology (DCMs) in Denmark, were identified in MiBa. For *E. faecalis*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations to ampicillin. In addition, nine DCMs routinely reported antimicrobial susceptibilities to vancomycin, five DCMs to linezolid, four DCMs to gentamicin (high-level resistance to gentamicin), two DCMs to teicoplanin and one DCM to tigecycline. For *E. faecium*, all 10 DCMs routinely (>75% of isolates) reported antimicrobial susceptibility interpretations to ampicillin and vancomycin in MiBa. In addition, six DCMs routinely reported antimicrobial susceptibilities to linezolid, four DCMs to gentamicin (high-level resistance to gentamicin), two DCMs to teicoplanin and one DCM to tigecycline. Resistance

testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the DCMs, based on the S-I-R system. One exception is high-level gentamicin resistance from one DCM, where MIC and/or zone diameters in MiBa were used because interpretations were not reported in MiBa.

Resistance to all tested antibiotics are presented as a national mean of the combined DCMs reporting in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the ratio of resistance to vancomycin in both, for the past decade, are shown.

Figure 8.11 Enterococci. Number of isolates and rates of resistance to vancomycin (%) in invasive isolates from humans, Denmark

DANMAP 2018



The number (n) in parentheses represents the numbers of isolates tested in 2018. In 2009 the presented data covers 75% of the Danish population. From 2010 to 2014 data covers 95% of the Danish population and from 2015 the total Danish population is covered

Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2018

DANMAP 2018

	<i>E. faecalis</i>	<i>E. faecium</i>	Number of tested isolates (number of DCMs)	
	%	%	<i>E. faecalis</i>	<i>E. faecium</i>
Ampicillin	0.3	94	608 (10)	787 (10)
Vancomycin	0.0	12	576 (9)	785 (10)
Linezolid	1.2	0.5	427 (5)	602 (6)
High-level gentamicin	11	40	326 (4)	396 (4)
Teicoplanin	0.0	16	213 (2)	246 (2)
Tigecycline	0.0	0.0	105 (1)	103 (1)

All proportions of resistance are presented for each antibiotic as a mean of the resistance rates reported by the DCMs. Included are all DCMs that reports routine testing (> 75% of the isolates). The number in parentheses tells the number of included DCMs

From 2017 to 2018, the total number of invasive cases decreased from 678 to 610 for *E. faecalis* and from 793 to 788 for *E. faecium*.

A continuing high prevalence of ampicillin resistance in invasive *E. faecium* have been observed with rates between 92% and 95% since 2010. In 2002 the resistance rate was 65%.

In 2018, yet a steep increase in the proportion of invasive vancomycin resistant *E. faecium* (12% compared to 7.1% in 2017) was observed. A part of this was due to the spread and detection of the "VVE clone", ST1421-CT1134 vanA *E. faecium*, which even though variable in vancomycin resistance, has the ability to revert to vancomycin resistance, and therefore is interpreted as vancomycin resistant in the clinical setting. For more, go to subsection 8.3.3, VVE and VRE. In this subsection there is no distinction between VRE and VVE. None vancomycin resistant invasive *E. faecalis* were reported in 2018. In total numbers 97 unique patients had invasive VRE/VVE as the first invasive enterococcus isolate in 2018. This number was 56 patients in 2017 and 51 in 2016. Thirty-four out of 90 of those patients (38%) in 2018, died within 30 days of diagnosis. Many of the VRE patients were chronically ill patients and the high mortality might be due to this.

The proportion of high-level gentamicin resistance (MIC > 128 mg/L and/or zone diameters < 8 mm) was reported in MiBa from four DCMs (>75% of isolates tested), Table 8.7. In the previous years, only one DCM reported on the proportion of high-level gentamicin resistance to DANMAP. Based on these rather sparse data, a decreasing trend in high-level gentamicin resistance in invasive *E. faecalis* has been observed over the decade, from 35% in the first years to 20% in 2016, 7.1% in 2017 and 11% in 2018. In *E. faecium* the level has been oscillating between 55% and 75% in the same time period, but decreasing to 43% in 2017 and 40% in 2018.

In 2018, five isolates of *E. faecalis* and three isolates of *E. faecium* from unique patients were reported linezolid resistant from the five and six DCMs routinely reporting interpretations to linezolid (Table 8.7). In 2017 the numbers were six *E. faecalis* (three reporting DCMs) and eight *E. faecium* (six reporting DCMs). Of all linezolid resistant invasive isolates identified in MiBa in 2018, two *E. faecium* were also VVE but still susceptible to tigecycline and daptomycin, the rest were susceptible to vancomycin.

Conclusion

An increase of invasive enterococci, mainly caused by an increase in invasive *E. faecium*, has been observed during the past 17 years (Figure 8.1.1. DANMAP 2015). The increase was combined with firstly, an increase in the proportion of ampicillin resistant *E. faecium* (65% in 2002 and more than 90% since 2010) and since 2013, an increase in vancomycin resistant *E. faecium*. In 2018, yet a steep increase in the percentage of vancomycin resistant invasive *E. faecium* was observed but

the total number of invasive enterococci did not increase any further compared to 2017. The proportion of invasive vancomycin resistant *E. faecium* is high in Denmark, especially when compared to the other Nordic countries, where Norway has the next highest percentage (4.5%). But also southern European countries like France and Spain have lower percentages of vancomycin resistant invasive *E. faecium* than Denmark [EARS-Net annual report, 2017]. The increase is worrisome and reflects the sharp increase in Denmark since 2013 in all types of clinical VRE (subsection 8.3.3). A high mortality was observed in patients with invasive VRE/VVE. There is an ongoing focus, on dealing with the VRE/VVE problem in Denmark.

Sissel Skovgaard, Stefan S. Olsen and Anna E. Henius
For further information: Sissel Skovgaard, sisk@ssi.dk

8.3 Surveillance based on data from the reference laboratories

8.3.1 Characterisation of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections, 2018, Denmark

Background

Resistance to 3rd generation cephalosporins in *Escherichia coli* often occur through production of extended-spectrum beta-lactamases (ESBL), carbapenemases, plasmid-mediated AmpC (pAmpC) or through mutations within the promoter/attenuator region of chromosomal AmpC (cAmpC).

Since 2014, the Danish departments of clinical microbiology have, on a voluntary basis, submitted 3rd generation cephalosporin resistant *E. coli* (3GC-R Ec) from bloodstream infections for characterisation at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut. The 3GC-R Ec were defined by the EUCAST criteria to cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

The 3GC-R Ec collected in Denmark through 2018, were characterized according to Multilocus Sequence Types (MLSTs), and the encoding ESBL-, pAmpC- and carbapenemase genes. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promoter mutations presumed to up-regulate cAmpC.

Results

In 2018, whole genome sequencing data were obtained from 369 *E. coli* isolates from unique patients. Genes encoding ESBL and/or pAmpC were detected in 352 (95%) of the isolates while 17 isolates were cAmpC hyper producers only; these 17 isolates were not further investigated.

Demographic data was available for all 352 *E. coli* isolates in 2018; 185 (53%) of the patients were men compared to 209 (62%) in 2017, and 167 (47%) were women compared to 128 (38%) in 2017. The average age at diagnosis was 69 years,

ranging from below one to 96 years. Fifty patients (14%; 28 men and 22 women) of the 352 patients died within 30 days of diagnosis (average age at death was 78 years; ranging from 53 to 96 years).

The regional distribution of the 352 isolates with ESBL- and pAmpC encoding genes was compared to data from previous years (Table 8.8 and Figure 8.12).

From 2014 to 2018, the reported cases of ESBL/pAmpC *E. coli* in bloodstream infections have changed from 245 to 352 per year, a 44% increase. In the same time period the total number of unique patients with invasive *E. coli* (irrespective of the resistance profile) only increased by 20%.

In the Capital Region, the number of reported cases increased significantly from 112 cases in 2017 to 154 cases in 2018 (p

= 0.005), whereas the reported number of cases in the Zealand Region decreased significantly from 38 cases in 2017 to 23 cases in 2018 (p = 0.029). For the remaining three regions, the reported number of cases were stable in 2018 compared to 2017.

In 2018, 24 different ESBL-, pAmpC- and carbapenemase-enzymes were detected among the 352 isolates (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, with a significant increase from 164 cases in 2017 to 200 cases in 2018 (p = 0.032), whereas the presence of CTX-M-14 and CTX-M-55 decreased significantly from 48 and 13 cases in 2017 to 31 and four cases in 2018, respectively (p = 0.025 and p = 0.021). In five cases, a carbapenemase-enzyme was detected along with an ESBL-enzyme.

Table 8.8 Distribution of ESBL/pAmpC producing *E. coli* from bloodstream infections, Denmark

DANMAP 2018

Region	2014		2015		2016		2017		2018	
	Numbers	%								
The Capital Region of Denmark	110	45	116	42	111	36	112	33	154	44
The Zealand Region	27	11	14	5	36	12	38	11	23	7
Region of Southern Denmark	43	18	45	16	67	21	76	23	75	21
Central Denmark Region	43	18	59	21	66	21	80	24	74	21
North Denmark Region	22	9	41	15	32	10	31	9	26	7
Total Numbers	245		275		312		337		352	

Figure 8.12 Regionwide distribution of ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark

DANMAP 2018

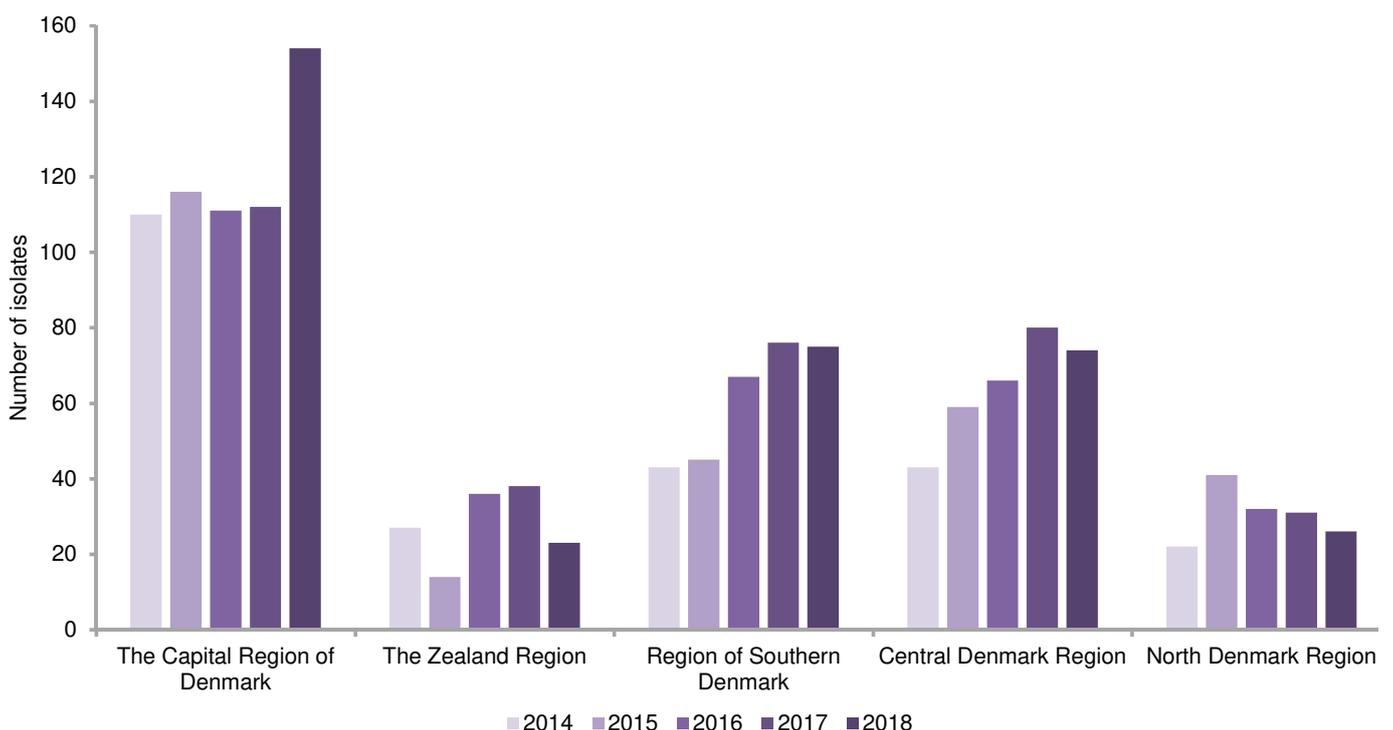


Table 8.9 Most common ESBL enzymes, pAmpC enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark

DANMAP 2018

Enzyme	2014		2015		2016		2017		2018	
	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
CTX-M-1	10	4%	7	3%	8	3%	17	5%	25	7%
CTX-M-101	12	5%	15	5%	14	4%	9	3%	4	1%
CTX-M-14	38	16%	33	12%	40	13%	48	14%	31	9%
CTX-M-14b	5	2%	5	2%	9	3%	3	1%	10	3%
CTX-M-15	121	49%	139	51%	157	50%	164	49%	200	57%
CTX-M-27	25	10%	33	12%	44	14%	52	15%	53	15%
CTX-M-3	4	2%	4	1%	7	2%	8	2%	5	1%
CTX-M-55	8	3%	14	5%	6	2%	13	4%	4	1%
CMY-2	10	4%	6	2%	10	3%	7	2%	6	2%
DHA-1	0	-	3	1%	5	2%	6	2%	10	3%
SHV-12	2	1%	5	2%	5	2%	3	1%	4	1%
Other CMY variants	4	2%	10	4%	3	1%	3	1%	3	1%
Other ESBL enzymes	12	5%	8	3%	17	5%	10	3%	10	3%
Carbapenemase enzymes	3	1%	3	1%	1	< 1%	1	< 1%	5	1%

In some isolates more than one enzyme was detected

Table 8.10 Distribution of MLSTs in ESBL/pAmpC-producing *E. coli* from bloodstream infections, Denmark

DANMAP 2018

MLST	2014		2015		2016		2017		2018	
	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
ST131	124	51%	135	49%	177	57%	175	52%	189	54%
ST38	18	7%	23	8%	21	7%	23	7%	22	6%
ST405	13	5%	12	4%	7	5%	9	3%	9	3%
ST410	4	2%	11	4%	6	4%	6	2%	3	1%
ST69	10	4%	10	4%	16	3%	20	6%	27	8%
ST648	7	3%	10	4%	5	2%	8	2%	6	2%
ST12	5	2%	9	3%	14	2%	6	2%	5	1%
ST88	2	1%	1	< 1%	0	-	5	1%	2	1%
ST1193	2	1%	5	2%	10	2%	7	2%	8	2%
ST10	0	-	6	2%	2	2%	4	1%	7	2%
ST73	3	1%	2	1%	4	2%	2	1%	6	2%
Other STs ¹	57	24%	51	19%	50	16%	72	21%	70	20%

¹ less than 5 isolates per ST in 2018

In 2018, the 352 *E. coli* isolates belonged to 62 different MLSTs, with the most common sequence type (ST) being ST131 (54%), followed by ST69 (8%) and ST38 (6%) (Table 8.10). No significant changes were observed in the MLSTs in 2018, compared to 2017.

Among the 189 *E. coli* isolates belonging to ST131, CTX-M-15 (67%) was most common, followed by CTX-M-27 (20%), and CTX-M-14 (7%). A significant increase ($p = 0.021$) of CTX-M-15 in ST131 was observed from 2017 (97; 55%) to 2018 (127; 67%), which explains the increase of CTX-M-15 in the collection of the 352 *E. coli* isolates. The five carbapenemase producers belonged to ST38 (2), ST405 (1), ST648 (1) and ST744 (1).

Conclusion

In 2018, the number of isolates carrying CTX-M-15 increased significantly in ST131, where 67% of the isolates were carrying the CTX-M-15 enzyme. In 2018, five isolates were also carbapenemase producers (OXA-48 group and NDM), and a minor part (3%) of the isolates carried CMY-variants. The distribution of sequence types for the 352 isolates did not change according to previous years; the worldwide disseminated ST131 clone was still strongly represented in 2018 (54%).

Louise Roer, Frank Hansen, Henrik Hasman and Anette M. Hammerum
For further information: Anette M. Hammerum, ama@ssi.dk

8.3.2 Carbapenemase producing bacteria in Denmark, 2018

Background

Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem-resistant bacteria are often none or suboptimal. Resistance can be caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integron-encoded metallo- β -lactamase (VIM), New Delhi metallo- β -lactamase (NDM) and Imipenemase (IMP).

In recent years, Danish departments of clinical microbiology (DCMs) have on a voluntary basis submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut. The Danish Health Authority made CPO notifiable as of 5th September 2018 [<https://www.sst.dk/da/udgivelser/2018/~media/52D5C295BCEA48E6BC596C0083367FF3.ashx>]. The present textbox describes carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

Carbapenemase-producing organisms

During 2018, 177 carbapenemase-producing organisms (CPO) were detected from 160 patients compared with 123 CPO from 115 patients in 2017 leading to a 44% overall increase of submitted CPO isolates compared to 2017. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Eighteen of the CPO (15 Enterobacterales and three *Acinetobacter* spp.) were from bloodstream infections compared with five of the CPO in 2017.

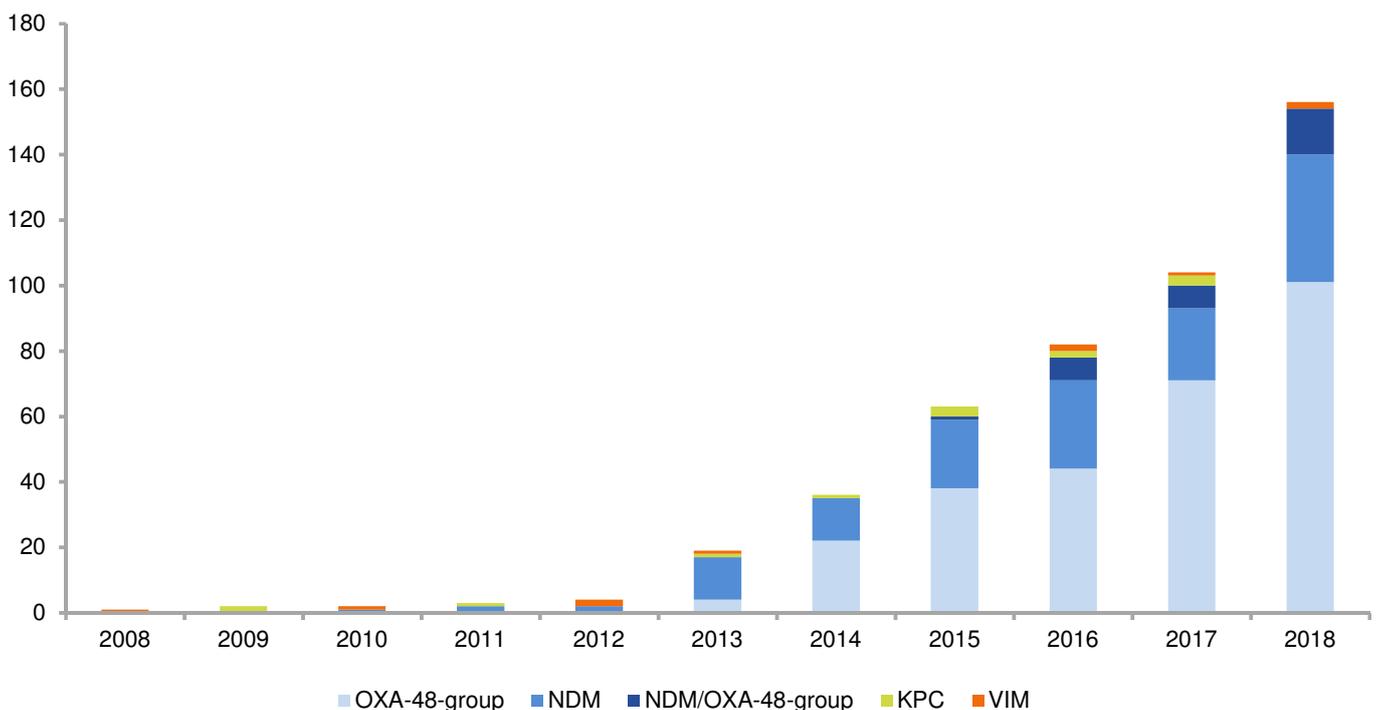
Enterobacterales

In 2018, 156 CPE isolates were detected from 141 patients compared to 104 CPE from 96 patients in 2017 (Figure 8.13) leading to a 50% increase of submitted CPE isolates compared to 2017. In 2018, 35 of the patients had reported travelling abroad prior to detection of the CPE; three of the patients had no reported history of recent travel and for the remaining 103 patients, travel information was unavailable. As many of the patients with unavailable travel information were involved in the detected outbreaks, it seems that the CPE were obtained in Denmark, at least for these patients. Better reporting will be obtained in the future, since CPE has become notifiable.

Fourteen of the 156 CPE isolates produced both NDM and OXA-48 group enzymes, 101 produced OXA-48-like enzymes and 39 were NDM-producing (Figure 8.13). Furthermore, two VIM-producing isolates were detected.

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), 2008-2018, Denmark

DANMAP 2018



Larger outbreaks with CPE during 2018

The ST18 NDM-1-producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2018 [Hammerum et al. 2016, J. Antimicrob. Agents, 71:3117-3124]. During 2018, an ST18 NDM-1-producing *C. freundii* was also detected from a patient in the Central Denmark Region, with epidemiologic link to the North Denmark Region. Furthermore, the NDM-1-encoding plasmid (pT1) was detected in an ST8 *C. freundii*, indicating possible plasmid transfer from an ST18 NDM-1-producing *C. freundii*. Until the end of 2018, 27 patients had been involved in this outbreak in the period 2012-2018. During 2018, eight new patients were part of this outbreak. None of these patients had a prior history of travel noted in their hospital records. The origin of the NDM-1-producing *C. freundii* was unknown.

Another large outbreak was detected in Zealand with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer et al. 2018, mSphere;3(4)]. The first patient with ST410 NDM-5/OXA-181 *E. coli* was hospitalised in the Capital Region in 2015 after hospitalisation in Egypt [Overballe-Petersen et al. 2018, Genome Announc.6(5): e01542-17]. The other patients with ST410 NDM-5/OXA-181 *E. coli* were hospitalised in the Region Zealand in 2016-2018. By the end of 2018, 28 patients had been involved in this outbreak in the period 2015-2018 and 18 of these were detected during 2018.

In 2013, OXA-436, a novel carbapenemase belonging to the OXA-48 enzyme group, was detected in Denmark [Samuelsen et al. 2017, Antimicrob. Agents and Chemother 62(1): e01260-17]. From 2013 to the end of 2018, 14 patients had had OXA-436-producing CPE isolates, both plasmid and clonal spread have been seen. Spread of ST90 OXA-436-producing *E. cloacae* between three patients occurred in the Region of Southern Denmark in 2018.

During 2018, spread of ST231 OXA-232-producing *K. pneumoniae* were detected between five patients in the Central Denmark Region. Furthermore, spread of ST101 OXA-48-producing *K. pneumoniae* was detected between eight patients in the Capital Region during 2018.

Besides these larger outbreaks, possible spread of CPE between two patients were observed several times during 2018. It seems very likely that the increase in OXA-48-producing CPE was due to plasmid transfer, but this was not investigated further.

***Acinetobacter* spp.**

In 2018, 18 carbapenemase-producing *Acinetobacter* spp. isolates were detected compared to 15 isolates in 2017. Ten of the 18 patients with carbapenemase-producing *Acinetobacter* spp. had been travelling abroad prior to detection. In 2018, 16 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were detected: OXA-23 (12), NDM-1 (1), NDM-1/OXA-23 (1), OXA-239 (1) and OXA-72 (1). Furthermore,

one OXA-58 -producing *Acinetobacter bereziniae* and one OXA-499 -producing *Acinetobacter calcoaceticus* were detected.

***Pseudomonas* spp.**

In 2018, one VIM-2-producing *Pseudomonas aeruginosa*, one NDM-1-producing *P. aeruginosa* and one VIM-2-producing *Pseudomonas monteilii* were detected. All three patients had been travelling abroad prior to detection of the carbapenemase-producing *Pseudomonas* spp.

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continues to increase, a trend worrisome to patients and clinicians. Especially the spread of CPE among patients in Denmark is of concern, since Enterobacterales can be carried in the intestine for a long time (years) without any symptoms of infections, which makes outbreak control difficult.

Henrik Hasman, Frank Hansen, Lone Jannok Porsbo, Anne Kjerulf, Louise Roer, Hülya Kaya, Mette Bar Ilan, Brian Kristensen and Anette M. Hammerum
For further information: Anette M. Hammerum, ama@ssi.dk

8.3.3 Further increase of *vanA* *Enterococcus faecium* in Denmark**Background**

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins. Therefore, antibiotic treatment of enterococcal infections may be challenging. In addition, most hospital-acquired *E. faecium* are resistant to ampicillin, thus limiting the treatment possibilities. Vancomycin is an important drug for the treatment of severe *E. faecium* infections, but an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed in Denmark and internationally. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents have side effects and development of resistance has been reported.

In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically vancomycin susceptible, has been described in different countries. These enterococci are referred to as vancomycin-variable enterococci (VVE). VVE have caused nosocomial outbreaks and development of revertant mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. This makes the detection of VVE highly clinically relevant in order to avoid treatment failure with vancomycin. VVE can only be detected by molecular methods and cannot be cultured on selective vancomycin-containing media. In 2015 and 2016, sporadic VVE with different genetic background were detected in the Capital Region of Denmark, in relation to concurrent VRE-outbreaks [B Holzknecht, personal communication]. In 2016, a new VVE clone belonging to ST1421-CT1134 was detected, which displays variable vancomycin

susceptibility due to a deletion in the *vanX* gene. [TA Hansen et al., J. Antimicrob. Agents, 2018, 73: 2936-2940].

Surveillance of VRE/VVE

Since 2005, Danish departments of clinical microbiology (DCMs) have voluntarily submitted VRE (first isolate per patient per 12 months) for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI). In 2017, VVE isolates were included in the VRE surveillance. However, VVE diagnostics differ substantially in the different regions. In 2017, testing of invasive *E. faecium* isolates was introduced in some DCMs in the Capital Region, which was during 2018 expanded to testing of all clinical *E. faecium* isolates for the presence of *vanA/vanB* genes. During 2018, VVE screening was also implemented in one DCM in the Region of Southern Denmark. Furthermore, invasive *E. faecium* isolates were tested by PCR for *vanA/vanB* genes in another DCM in the Region of Southern Denmark and in the DCM in Central Denmark Region.

To determine any underreporting in the submissions, the number of VRE/VVE submitted to SSI in 2016, 2017 and 2018 were compared to data from clinical VRE reported by the DCMs to MiBa (the Danish Microbiology Database). This comparison showed that the number of submitted VRE/VVE isolates were not complete, since VRE/VVE isolates were missing from 80, 81 and 78 patients in surveillance in 2016, 2017 and 2018, respectively (Figure 8.14). In 2018, 525 VRE/VVE isolates

were submitted to SSI. One patient had both a vancomycin resistant *E. faecium* and a vancomycin resistant *E. faecalis*. By adding the 81 and 78 VRE/VVE isolates extracted from MiBa, this added up to 603 VRE/VVE isolates from 599 patients in 2018 compared to 510 VRE/VVE isolates from 508 patients in 2017 (Figure 8.14).

From 2013, a sharp increase in clinical VRE isolates has been observed. The increase has mostly been seen for *vanA E. faecium* (Figure 8.14).

From 2015 through 2018, all clinical VRE/VVE isolates have been submitted to whole-genome sequencing (WGS). In 2018, 525 VRE/VVE were submitted to WGS. From the WGS data, multilocus sequence type (MLST), core genome MLST (cgMLST) and *van*-genes were extracted *in silico*.

Of the 525 VRE/VVE isolates, 480 were *vanA E. faecium*, 16 *vanB E. faecium*, 19 *vanA/vanB E. faecium*, five *vanA E. faecalis* and five *vanB E. faecalis* (Figure 8.14). cgMLST analysis was performed on the 10 *E. faecalis* isolates and 515 *E. faecium* isolates using SeqSphere+. The 10 *E. faecalis* isolates were subdivided into nine different complex types (CTs) by cgMLST, whereas the 515 *E. faecium* isolates were subdivided into 65 CTs. Two types dominated: ST203-CT859 *vanA E. faecium* and ST1421-CT1134 *vanA E. faecium* (Table 8.11). The number of *vanA E. faecium* isolates belonging to ST203-CT859 was high during 2015-2017, but the number decreased during 2018.

Figure 8.14 Numbers of *Enterococcus faecium* and *Enterococcus faecalis* isolates carrying *vanA* and *vanB* genes from clinical samples submitted to SSI 2009-2018 supplemented with data obtained from MiBa from 2016-2018, Denmark

DANMAP 2018

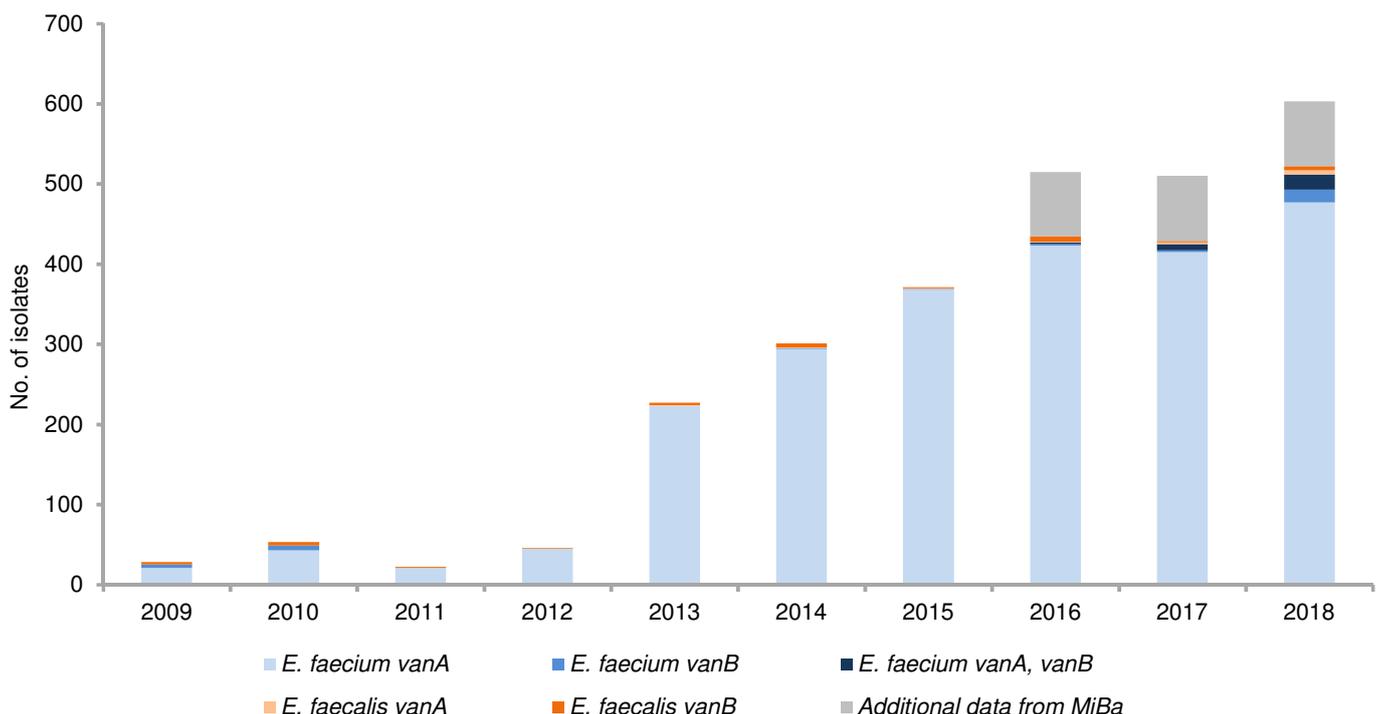


Table 8.11 Distribution of the most common types of *vanA* *Enterococcus faecium* according to MLST and cgMLST, Denmark DANMAP 2018

Types ^a	2015		2016		2017		2018	
	(n = 369)		(n = 427)		(n = 425)		(n = 515)	
ST80-CT14 <i>vanA</i>	81	22%	38	9%	15	4%	1	<1%
ST80-CT24 <i>vanA</i>	23	6%	19	5%	11	3%	2	<1%
ST80-CT860 <i>vanA</i>	7	2%	11	3%	N.D.	N.D.	N.D.	N.D.
ST80-CT866 <i>vanA</i>	14	4%	10	2%	7	2%	N.D.	N.D.
ST80-CT991 <i>vanA</i>	N.D.	N.D.	11	3%	9	2%	6	1%
ST80-CT1160 <i>vanA</i>	N.D.	N.D.	N.D.	N.D.	7	2%	10	2%
ST80-CT1064 <i>vanA/vanB</i>	N.D.	N.D.	2	<1%	8	2%	23	5%
ST80-CT1729 <i>vanA</i>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	22	4%
ST117-CT873 <i>vanA</i>	5	1%	12	3%	N.D.	N.D.	N.D.	N.D.
ST117-CT1180 <i>vanA</i>	N.D.	N.D.	N.D.	N.D.	9	2%	30	6%
ST203-CT859 (subtypes CT1051 and CT1507) <i>vanA</i>	188	51%	271	64%	265	63%	161	31%
ST1421-CT1134 <i>vanA</i>	N.D.	N.D.	2	<1%	13	3%	176	34%
other types	51	14%	51	12%	81	19%	84	16%

^aST, sequence type (MLST); CT, cluster type (cgMLST); N.D., not determined

In 2017, the VVE clone ST1421-CT1134 *vanA* *E. faecium* was only detected in clinical samples from the Capital Region and accounted for 3% of the *E. faecium* isolates. In 2018, 34% (n = 176) of the *vanA* *E. faecium* isolates belonged to ST1421-CT1134 (Table 8.11) and this clone was detected from all DCMs in the Capital Region, the DCM in Region Zealand and from one DCM in the Region of Southern Denmark. It seems very likely that VVE have been underreported, since not all DCMs in Denmark have implemented systematic molecular testing.

Conclusion

The still increasing number of VRE/VVE cases in 2018 in Denmark is worrying. VRE can be carried in the intestine for a long period without showing any symptoms. Moreover, VRE can persist in the hospital environment, which makes infection control difficult. Infection control should include proper cleaning, good hand hygiene, VRE/VVE screening and subsequent isolation of patients. The spread of the "VVE clone", ST1421-CT1134 *vanA* *E. faecium*, in Denmark is of concern, especially since VVE diagnostic is challenging and therefore, the clone is likely to be underdiagnosed. Close surveillance of VVE is important in the future.

Anette M. Hammerum, Barbara Holzknicht, Louise Roer, Hülya Kaya, Sissel Skovgaard, Stefan S. Olsen and Henrik Hasman
For further information: Anette M. Hammerum, ama@ssi.dk

8.3.4 Detection of the 23S rRNA mutations encoding linezolid resistance and the *optrA* in enterococci from Denmark

Background

Linezolid can be used for treatment of infections caused by vancomycin-resistant enterococci. Resistance to linezolid in

enterococci is often due to mutations in the V domain of the 23S rRNA gene. The G2576T mutation (*Escherichia coli* numbering) is the most common cause of the linezolid-resistant enterococci (LRE) phenotype. Another mutation in the 23S rRNA, G2505A, (*E. coli* numbering) has also been reported for LRE, but seems to be less frequent than the G2576T mutation. Furthermore, transferable resistance genes (*cfr*, *cfr(B)*, *optrA* and *poxtA*) encoding linezolid resistance, have been described in enterococci, whereas amino acid substitutions in the ribosomal proteins L3, L4 and/or L22, suspected to cause decreased susceptibility to linezolid in staphylococci, are rare in enterococci.

In 2018, a web tool for detection of the 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and *poxtA* genes from whole genome sequences from enterococci was developed [Hasman et al., J. Antimicrob. Chemother., 2019, 74:1473-1476].

Surveillance of linezolid-resistant enterococci (LRE)

Danish departments of clinical microbiology (DCMs) have, on voluntarily basis, submitted LRE for surveillance to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI).

During 2015-2018, eight linezolid-resistant *E. faecium* isolates and eight linezolid-resistant *E. faecalis* isolates were sent to SSI (only one isolate per patient were included). WGS data from the 16 LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and *poxtA* genes using the LRE-Finder (<https://cge.cbs.dtu.dk/services/LRE-Finder/>). Among the LRE isolates, LRE-Finder detected seven *E. faecium* with the G2576T mutation, one *E. faecium* with the G2505A mutation and eight *E. faecalis* isolates with *optrA* (Table 8.12).

Table 8.12 Characterisation of the 16 linezolid resistant enterococci (LRE) and the eight linezolid vancomycin resistant enterococci (LVRE), 2015-2018, Denmark

DANMAP 2018

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistant gene
LRE	1	<i>E. faecium</i>	G2505A	none
	7	<i>E. faecium</i>	G2576T	none
	8	<i>E. faecalis</i>	<i>optrA</i>	none
LVRE	6	<i>E. faecium</i>	G2576T	<i>vanA</i>

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also used for detection of LRE mutations and LRE genes in genomes from VRE isolates from the VRE Surveillance. In total, 1757 genomes from VRE isolates from 2015-2018 were investigated using the LRE-Finder. No linezolid vancomycin resistant *E. faecalis* were detected, whereas, six linezolid-vancomycin resistant *E. faecium* were detected. The linezolid resistant *E. faecium* isolates had the G2576T mutation and were positive for the *vanA* gene encoding vancomycin resistance (Table 8.12).

Conclusion

The findings of LRE and LVRE are of concern. Linezolid is used for treatment of VRE. Only a limited number of antimicrobial agents are available for treatment of infections with LVRE.

Anette M. Hammerum, Hülya Kaya, Frank Hansen and Henrik Hasman
For further information: Anette M. Hammerum, ama@ssi.dk

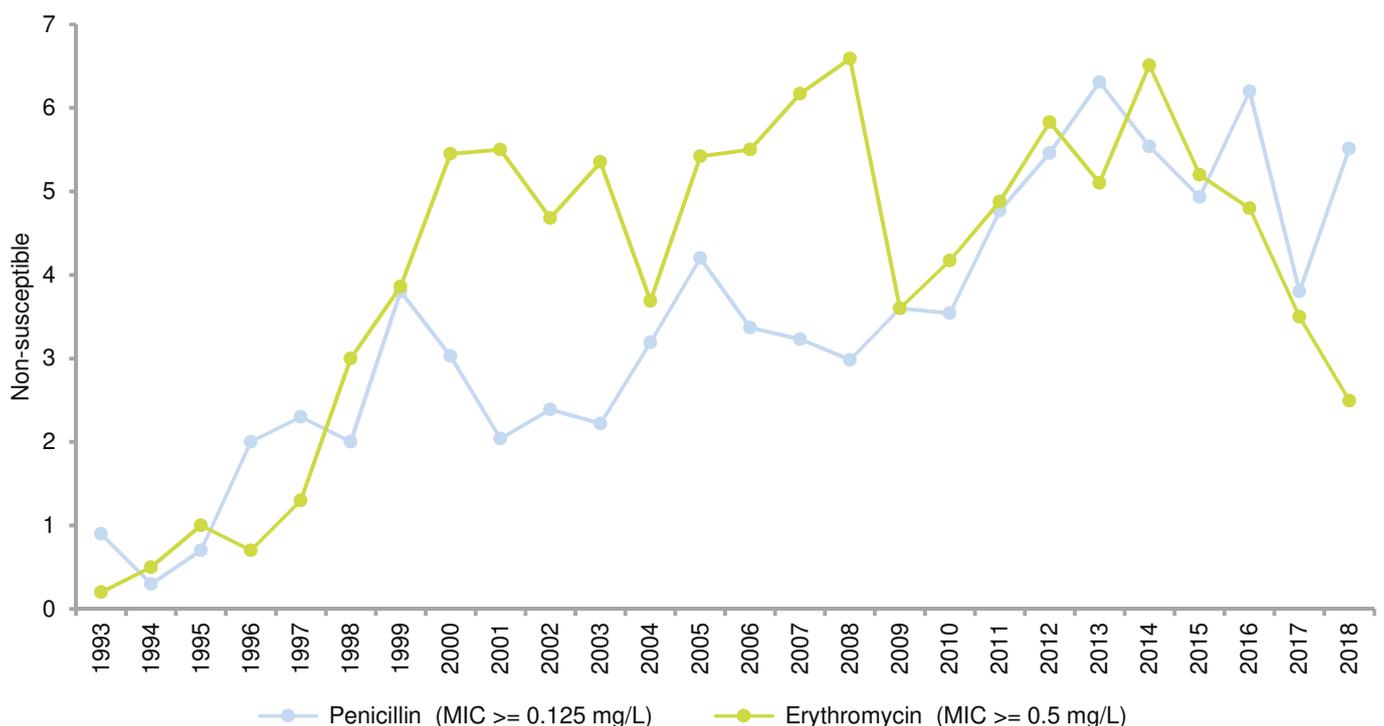
8.3.5 *Streptococcus pneumoniae*

The surveillance of pneumococci (*Streptococcus pneumoniae*) causing invasive disease in Denmark happens through mandatory submission of clinical isolates to Statens Serum Institut (SSI). At SSI, the isolates are serotyped and tested for antimicrobial susceptibility.

In Denmark, 798 cases of invasive pneumococcal disease were registered in 2018. Isolates were received from 768 of these cases. The isolates mainly originated from either blood (713 isolates from bacteraemias, nine of which the patient additionally had a positive identification of pneumococci in the cerebrospinal fluid) or from cerebrospinal fluid alone (51 isolates). Four isolates were moreover received from other, normally sterile sites (ascites, joint), but results from these are by tradition not included in this report. Two isolates could not be serotyped. The remaining 762 isolates from blood or cerebrospinal fluid belonged to 40 different serotypes, and 711 of those were fully susceptible to both penicillin and erythromycin (93.3%). For penicillin, 720 isolates were fully

Figure 8.15 Non-susceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark

DANMAP 2018



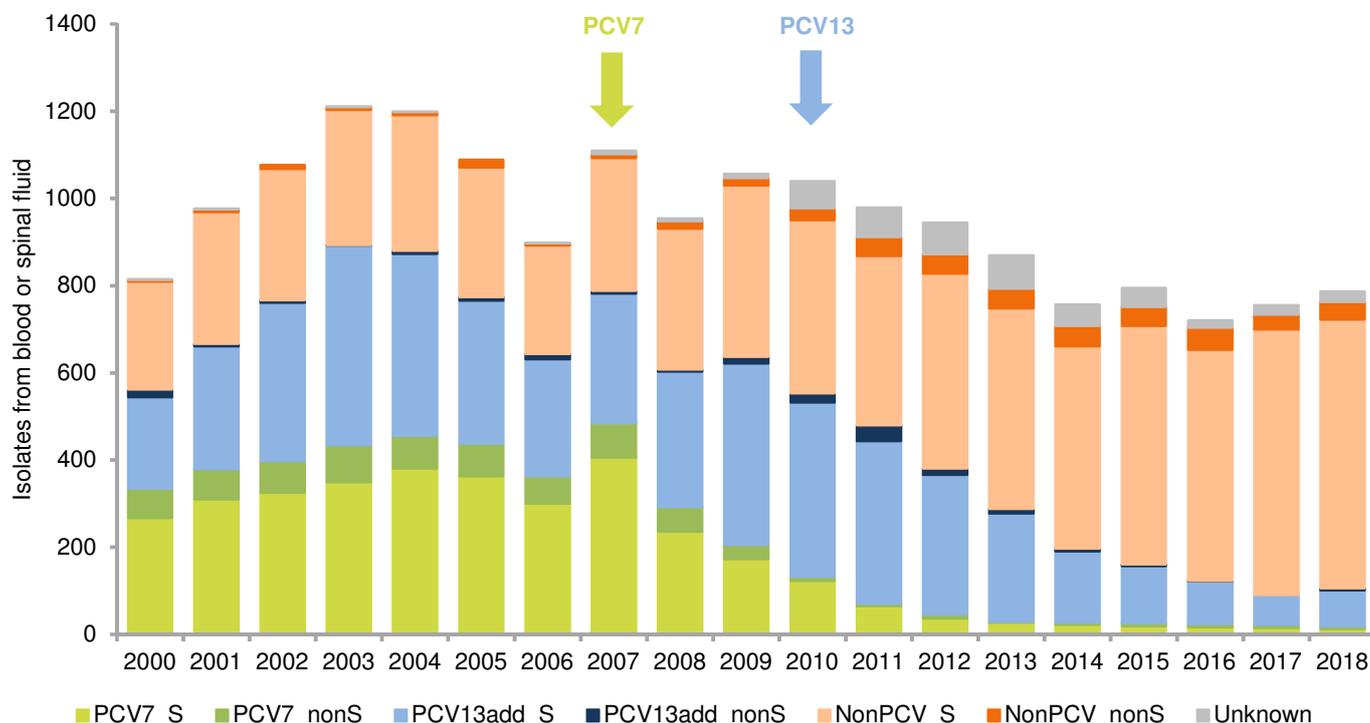
susceptible (94.5%), 41 isolates (5.4%) were intermediate susceptible and one (of serotype 14) was resistant (0.1%). For erythromycin, 743 isolates were fully susceptible (97.5%) and 19 isolates (2.5%) were resistant. For penicillin, the level of non-susceptibility in 2018 was higher than in 2017 (3.8%) but lower than in 2016 (6.2%). For erythromycin, the level of non-susceptibility in 2018 was the lowest since 1998, Figure 8.15.

Comparing these results to the data reported in 2017 from our neighbouring countries, the levels of penicillin non-

susceptibility reported to EARS-Net were: Sweden (6.1%), Norway (4.8%) and Germany (4.8%). The levels of erythromycin non-susceptibility were: Sweden (4.8%), Norway (5.9%) and Germany (7.1%). Thus, the results of non-susceptibility for invasive pneumococci from Denmark in 2018 were similar to the reported values from 2017 from neighbouring countries with respect to penicillin, but markedly lower with respect to erythromycin.

Figure 8.16 *Streptococcus pneumoniae* blood and spinal fluid isolates from humans. Vaccine serotypes and non-vaccine serotypes in combination with susceptibility to penicillin and erythromycin, Denmark

DANMAP 2018



PCV7_S : PCV7 serotypes, susceptible to both penicillin and erythromycin
 PCV7_nonS : PCV7 serotypes, non-susceptible to either penicillin or erythromycin
 PCV13add_S : PCV13 serotypes not in PCV7, susceptible to both penicillin and erythromycin
 PCV13add_nonS : PCV13 serotypes not in PCV7, non-susceptible to either penicillin or erythromycin
 NonPCV_S : serotypes not included in PCV7 or PCV13, susceptible to both penicillin and erythromycin
 NonPCV_nonS : serotypes not included in PCV7 or PCV13, non-susceptible to either penicillin or erythromycin
 Unknown : cases where either serotype or susceptibility to penicillin or erythromycin is unknown
 The two arrows indicate when PCV7 and PCV13 were introduced in the Danish childhood immunization programme.

Table 8.13 Number of invasive isolates and distribution of resistance in the most common sero-types of pneumococci, Denmark

DANMAP 2018

Serotype	N 2018	PenS, EryS	PenS, EryR	PenI, EryS	PenI, EryR	PenR, EryS	%PenSEryS	N 2017	%PenSEryS2017
8	194	194					100.0%	192	99.5%
3	70	68	2				97.1%	57	100.0%
22F	69	69					100.0%	58	100.0%
9N	62	61		1			98.4%	56	98.2%
12F	55	55					100.0%	69	98.6%
15A	25	19			6		76.0%	16	62.5%
20	24	24					100.0%	26	100.0%
11A	19	18		1			94.7%	19	89.5%
16F	19	16		3			84.2%	21	95.2%
24F	17	13	4				76.5%	19	78.9%
33F	17	15	2				88.2%	13	92.3%
10A	15	15					100.0%	9	100.0%
31	15	15					100.0%	8	87.5%
35B	15	15					100.0%	20	90.0%
23B	14	1		13			7.1%	11	27.3%
35F	14	14					100.0%	13	100.0%
23A	13	13					100.0%	18	100.0%
17F	12	6		6			50.0%	10	80.0%
19A	11	9		2			81.8%	5	100.0%
15B	9	9					100.0%	14	92.9%
19F	7	5	1		1		71.4%	13	69.2%
6C	5	2		2	1		40.0%	13	92.3%
Other	61	55		3	2	1	90.2%	54	90.7%

N = number of isolates, Pen = penicillin, Ery = erythromycin

Conclusion

There has been a trend of decreasing non-susceptibility to erythromycin since 2013, while the non-susceptibility levels to penicillin are more variable. Antimicrobial susceptibility is highly correlated to serotypes, and therefore fluctuations in susceptibility often reflects changes in the circulating serotypes. More information on the surveillance of invasive pneumococcal disease can be found on the SSI homepage (EPI-NEWS, No 40/41-2018, <https://en.ssi.dk/news/epi-news/2018/no-40-41---2018>).

Tine Dalby & Hans-Christian Slotved
For further information: Tine Dalby, tid@ssi.dk

8.3.6 Beta-haemolytic streptococci

Beta-haemolytic streptococci (BHS) can be divided into serological groups according to antigenic properties of the polysaccharide capsule. In human infections, groups A, B, C and G are the most frequent species. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group.

Streptococcus pyogenes (group A streptococci; GAS) cause pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections, e.g., bacteraemia, necrotizing myofasciitis, and rarely meningitis. In Denmark, the rate of asymptomatic throat carriage of GAS is approximately 2%. *Streptococcus agalactiae* (group B strepto-

cocci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immuno-compromised patients.

Streptococcus dysgalactiae subsp. *equisimilis* (group C streptococci; GCS, and group G streptococci; GGS) predominantly cause soft-tissue infections and sometimes bacteraemia.

This report presents data on antimicrobial resistance in non-duplicate isolates from normally sterile sites (e.g. blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) of BHS submitted in 2018 to the Neisseria and Streptococcus Reference laboratory. Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of BHS, and not all DCMs submit BHS of all groups.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS group A, B, C and G are therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. For all isolates of GAS the *emm* type (the M protein gene that dictates the M serotype) was determined by whole genome sequencing.

Figure 8.17 shows the resistance findings for the years 2014 through 2018. In 2018, the number of submitted isolates from

unique cases was 873, an increase of 25% compared to 2017. Corresponding changes for individual serogroups were: GAS;

+ 14%, GBS; + 37%, GCS; + 33%, and GGS; + 23%. All isolates were fully susceptible to penicillin.

Figure 8.17 Beta-haemolytic streptococci: Antimicrobial resistance testing results. Numbers of isolates and resistance in percent, Denmark

DANMAP 2018

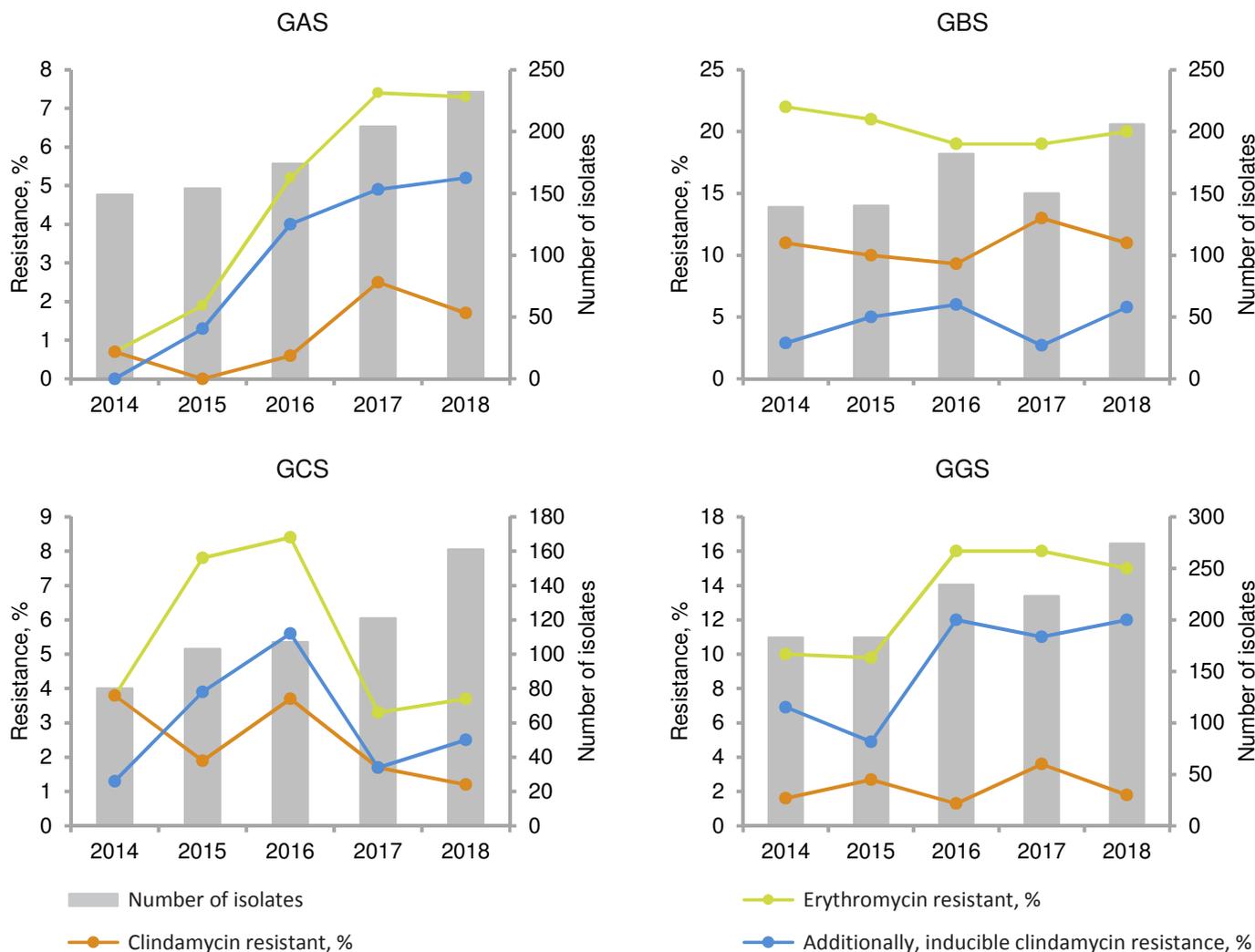


Table 8.14 Group A streptococci 2018: Distribution of *emm* types, clindamycin resistance and erythromycin resistance, Denmark

DANMAP 2018

<i>emm</i> type	CLI-S			Total
	CLI-R	ERY-R	ERY-S	
1.0	0	0	83	83
12.0	0	0	13	13
28.0	0	0	10	10
66.0	0	0	20	20
89.0	0	0	27	27
108.1	0	10	1	11
Subtotal	0	10	154	164
Other	3 (*)	4 (#)	61	68
Total	3 (*)	14 (#)	215	232

Note: Numbers of isolates are shown for individual *emm* types if ≥ 10 . Otherwise, if < 10 , the numbers are summarized in the "Subtotal" category.

(*) All were ERY-R and all were *emm* type 11.0

(#) One was *emm* type 11.0

CLI = clindamycin, ERY = erythromycin, R = resistant, S = sensitive

The erythromycin resistance rate remained virtually unchanged compared to 2017 for all four serogroups. The clindamycin resistance rate showed a slight decrease for all serogroups. The percentage of strains with inducible clindamycin resistance was virtually unchanged for GAS and GGS, but showed an increase for GBS and GCS. The percentage of fully susceptible isolates was unchanged for all four serogroups.

The GAS isolates belonged to 35 different *emm* types. The majority of the received isolates (164; 71%) belonged to six *emm* types, each of which were represented by at least ten isolates (Table 8.14). The remaining 68 isolates (29%) belonged to 29 different *emm* types. All three clindamycin resistant isolates and one clindamycin sensitive, erythromycin resistant isolate were *emm* type 11.0. There were no erythromycin sensitive isolates of *emm* type 11.0.

Conclusions

The number of submitted isolates in 2018 compared to 2017 increased for all four serogroups. The erythromycin resistance rate was unchanged for all four serogroups. The clindamycin resistance rate, including inducible resistance, decreased slightly for GAS and GGS and increased slightly for GBS and GCS.

Steen Hoffmann & Hans-Christian Slotved
For further information: Steen Hoffmann, hof@ssi.dk

8.3.7 *Haemophilus influenzae*

Haemophilus influenzae is part of the normal upper respiratory tract flora, where colonisation varies with age. *H. influenzae* can also be the cause of infections, with otitis media and bacterial sinusitis being the most common clinical manifestations.

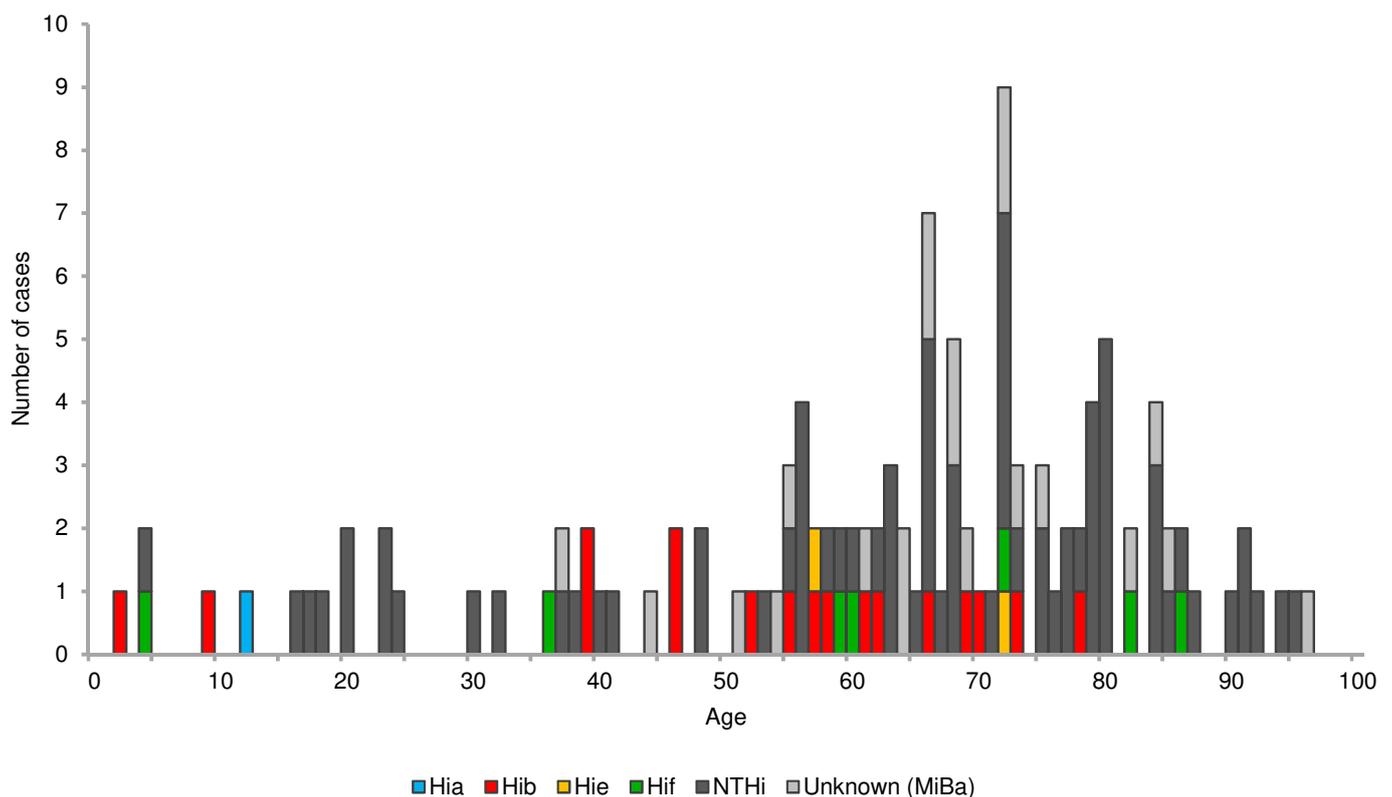
Invasive infections with *H. influenzae* happen relatively rarely and occur predominantly in the very young and the elderly but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. *H. influenzae* can be divided into six capsular serotypes (a, b, c, d, e and f, also known as Hia, Hib, Hic, Hid, Hie and Hif), as well as non-capsular (non-typeable, NTHi). Introduction of the polysaccharide type B vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. Before the vaccine was introduced, there were around 80 cases of Hib meningitis annually among infants in Denmark, and this is now down to 0-2 per year. NTHi for which no vaccine yet exists now dominates the invasive infections.

Invasive *Haemophilus influenzae*

Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, most departments of clinical microbiology are voluntarily submitting all isolates of invasive *H. influenzae* and not just Hib. The received isolates are then serotyped and bityped by the reference laboratory at SSI. Isolates are submitted for the

Figure 8.18 Different serotypes in invasive *H. influenzae* cases according to age, 2018, Denmark

DANMAP 2018



majority of cases, and the remaining cases can be identified through the Danish Microbiological Database (MiBa). Thus, all invasive infections with *H. influenzae* are registered in the surveillance database, and for the majority of cases serotypes are available. Whole genome sequencing is performed on the received isolates, and the data are analysed for the presence of the plasmid-borne beta-lactamase genes TEM-1 and ROB-1. Antimicrobial susceptibilities of the isolates are found through MiBa.

The present report includes all episodes of invasive *H. influenzae* identified in MiBa, where the date of sampling was in 2018. A total of 121 cases were identified, of which isolates from 100 (83%) were received at the reference laboratory. For five of the 121 cases, *H. influenzae* were isolated from cerebrospinal fluid, two were from pleural fluid, and 114 were from blood. The serotypes of the received isolates were one Hia (1%), 17 Hib (17%), two Hie (2%), seven Hif (7%) and 73 NTHi (73%). The age-distribution of the cases is presented in Figure 8.18.

Seventeen of the received isolates harboured the TEM-1 gene (five Hib and 12 NTHi) (17%), and all of these had corresponding resistance to penicillin as registered in MiBa. Susceptibility results, divided in to serotypes, for the antibiotics that were most frequently registered in MiBa, are presented in Table 8.15.

The results from antimicrobial susceptibility testings registered in MiBa showed that there was 26% resistance to penicillin, 1% to ciprofloxacin, 20% to ampicillin, 16% to cefuroxime and 10% to amoxicillin/clavunulate. Some variation across serotypes was observed.

In summary, the majority of isolates from invasive infections with *H. influenzae* are of the non-capsular type. This is similar to what is observed generally in Europe [R. Whittaker, Emerg Infect Dis, 2017, Mar;23(3):396-404]. Only 48% of the isolates were registered as fully susceptible to penicillin. However, 80% of the isolates were registered as susceptible to ampicillin.

Tine Dalby

For further information: Tine Dalby, tid@ssi.dk

Table 8.15 Distribution of antimicrobial susceptibilities in invasive *Haemophilus influenzae* according to serotypes, 2018, Denmark

DANMAP 2018

	Hia	Hib	Hie	Hif	NTHi	Unknown*	All
Penicillin_no result registered	-	-	-	-	1	-	1
Penicillin_I	-	3 (18%)	1 (50%)	3 (43%)	17 (24%)	7 (33%)	31 (26%)
Penicillin_R	-	5 (29%)	-	1 (14%)	21 (29%)	4 (19%)	31 (26%)
Penicillin_S	1 (100%)	9 (53%)	1 (50%)	3 (43%)	34 (47%)	10 (48%)	58 (48%)
Ciprofloxacin_no result registered	-	-	-	-	3	-	3
Ciprofloxacin_R	-	-	-	-	1 (1%)	-	1 (1%)
Ciprofloxacin_S	1 (100%)	17 (100%)	2 (100%)	7 (100%)	69 (99%)	21 (100%)	117 (99%)
Ampicillin_no result registered	-	-	-	-	3	1	4
Ampicillin_R	-	5 (29%)	-	-	15 (21%)	3 (15%)	23 (20%)
Ampicillin_S	1 (100%)	12 (71%)	2 (100%)	7 (100%)	55 (79%)	17 (85%)	94 (80%)
Cefuroxime_no result registered	-	1	-	1	13	3	18
Cefuroxime_I	-	-	-	-	-	1 (6%)	1 (1%)
Cefuroxime_R	-	4 (25%)	-	-	12 (20%)	-	16 (16%)
Cefuroxime_S	1 (100%)	12 (75%)	2 (100%)	6 (100%)	48 (80%)	17 (94%)	86 (83%)
Amoxi/Clav_no result registered	-	3	-	1	23	2	29
Amoxi/Clav_R	-	3 (21%)	-	-	6 (12%)	-	9 (10%)
Amoxi/Clav_S	1 (100%)	11 (79%)	2 (100%)	6 (100%)	44 (88%)	19 (100%)	83 (90%)

* The group "unknown" represent the 21 cases that were registered in MiBa, but where an isolate was not received at the reference laboratory at SSI, and therefore not serotyped.

8.3.8 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa. Approximately 50% of humans will be colonized with *S. aureus* in their nose or throat at any given time, some of these carrying the strain only intermittently, others for longer periods of time. *S. aureus* causes infections ranging from superficial skin infections e.g. impetigo and boils to deeper and more complicated infections. These may be health care associated such as post-operative wound infections and infections related to intravenous catheters and prosthetic devices

but they can also be caused by endogenous spread of bacteria causing septic arthritis, osteomyelitis, endocarditis and bacteraemia. Most of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteraemia cases was established in 1957. By comparison with the numbers of bacteraemia cases registered in the Danish Microbiology Database (MiBa) since 2010, the number of cases reported to SSI has been almost complete (94-97%).

Laboratory and clinical notification of all cases of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all referred isolates are initially tested using a multiplex PCR detecting: the *spa*, *mecA*, *hsd*, *scn* and *pvl* gene (lukF-PV). *Spa* is used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing, *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to the clonal complex (CC) 398, respectively. PVL has been closely linked to skin abscesses and the very rare condition of severe necrotizing pneumonia. PVL is rarely found in methicillin-susceptible *S. aureus* (MSSA) causing bacteraemia but has been associated with certain community acquired (CA) MRSA strains. Isolates positive for *mecA* and the CC398 specific *hsd* fragment but negative for *scn* (human adaptive factor) and *pvl* genes are considered typical livestock associated MRSA (LA-MRSA) and are not *spa* typed. All others, including human adapted CC398 isolates, are *spa* typed. In addition, all bacteraemia cases and *mecA* negative presumptive MRSA are tested for presence of the *mecC* gene.

A representative selection of bacteraemia isolates is tested for antimicrobial susceptibility against 17 antimicrobials (see chapter 9 for more information). For MRSA cases, demographic and epidemiological information is registered. Based on the epidemiological information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO), import (IMP) and livestock-associated (LA) MRSA. For CA, HACO and LA, classification was separated into known and not known exposure.

Surveillance of bacteraemia

In 2018, altogether 2,276 *S. aureus* bacteraemia cases corresponding to 39.4 cases per 100,000 inhabitants were reported from the departments of clinical microbiology (DCMs)

in Denmark. This is an increase after approximately 2,000 reported cases in 2014-2016 and 2,104 cases in 2017. Thirty-seven (1.6%) of the bacteraemia cases were caused by MRSA. This proportion is almost identical to the previous years, and remains below most other European countries participating in EARS-Net [EARS-Net 2017]. Eight of the 37 MRSA cases were caused by LA-MRSA CC398 (4 LA-MRSA CC398 in 2017).

Within 30 days from the bacteraemia onset, 510 (22%) patients died (all cause mortality). The mortality for the MRSA bacteraemia cases was 19% (n = 7, of which 2 were due to CC398 MRSA).

A total of 504 representative isolates was susceptibility tested. Results from antimicrobial resistance testing in *S. aureus* bacteraemia isolates from 2009-2018 is presented in Table 8.16. Resistance to penicillin was 72%, showing a decreasing trend from 77% in 2009. The highest frequency of resistance to other antimicrobials than penicillin was observed for fusidic acid (17%), erythromycin (5%), clindamycin (4%) and norfloxacin (4%). For most antimicrobial agents, the susceptibility remained at the same level as the previous years. However, resistance to fusidic acid increased for the second consecutive year (14% in 2017 and 12% in 2016).

Typing revealed 630 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 34% of the total number, are presented in Table 8.17 together with *spa* types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The PVL toxin was present in 29 (1.3%) cases of which five were MRSA. The 29 PVL presenting isolates were distributed among 25 different *spa* types and 11 different CCs.

Table 8.16 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark

DANMAP 2018

Antimicrobial agent	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
	%	%	%	%	%	%	%	%	%	%
Methicillin	1.6	1.4	1.4	1.2	1.7	2.9	1.5	2.1	2.2	1.6
Penicillin	77	75	77	74	76	77	71	71	72	72
Erythromycin	7	5	7	6	7	8	7	7	6	5
Clindamycin	6	4	6	6	6	8	7	6	5	4
Tetracycline	2	3	2	2	3	5	4	3	3	3
Fusidic acid	9	13	13	14	15	15	16	12	14	17
Rifampicin	<1	<1	<1	<1	0	<1	<1	<1	<1	<1
Norfloxacin	2	3	4	4	5	6	6	4	4	4
Kanamycin	1	1	<1	1	2	2	3	1	1	2
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	<1	0	<1	0
Trimethoprim-sulfamethoxazole	nt	nt	<1	1	1	1	<1	<1	<1	0
Numbers tested	1479	1416	1515	1523	962	381	502	560	551	504

nt = not tested. In web annex Table A8.1 the distribution of MICs and resistance for all tested antimicrobial agents are shown.

Table 8.17 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2018

DANMAP 2018

SAB			MRSA			
<i>spa</i> type	CC group	No. of cases	<i>spa</i> type	CC group	No. of cases	No. causing infections (%)
t127	CC1	137	t304	CC8	234	78 (33)
t002	CC5	101	t223	CC22	213	70 (33)
t084	CC15	105	t002	CC5	177	76 (43)
t230	CC45	81	t127	CC1	142	72 (51)
t091	CC7	74	t008	CC8	97	60 (62)
t012	CC30	70	t044	CC80	62	29 (47)
t021	CC30	61	t019	CC30	61	40 (66)
t008	CC8	53	t4549	CC8	58	43 (74)
t015	CC45	49	t005	CC22	50	27 (54)
t701	CC8	40	t437	CC59	48	33 (69)

CC = Clonal complex, SAB = *S. aureus* bacteraemia

Surveillance of methicillin-resistant *S. aureus*

In 2018, 3,669 MRSA cases were detected (63.5 per 100,000 inhabitants). This was a slight increase compared to 2017 (3,579; Figure 8.19) and followed the increasing trend registered since 2009. A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation only).

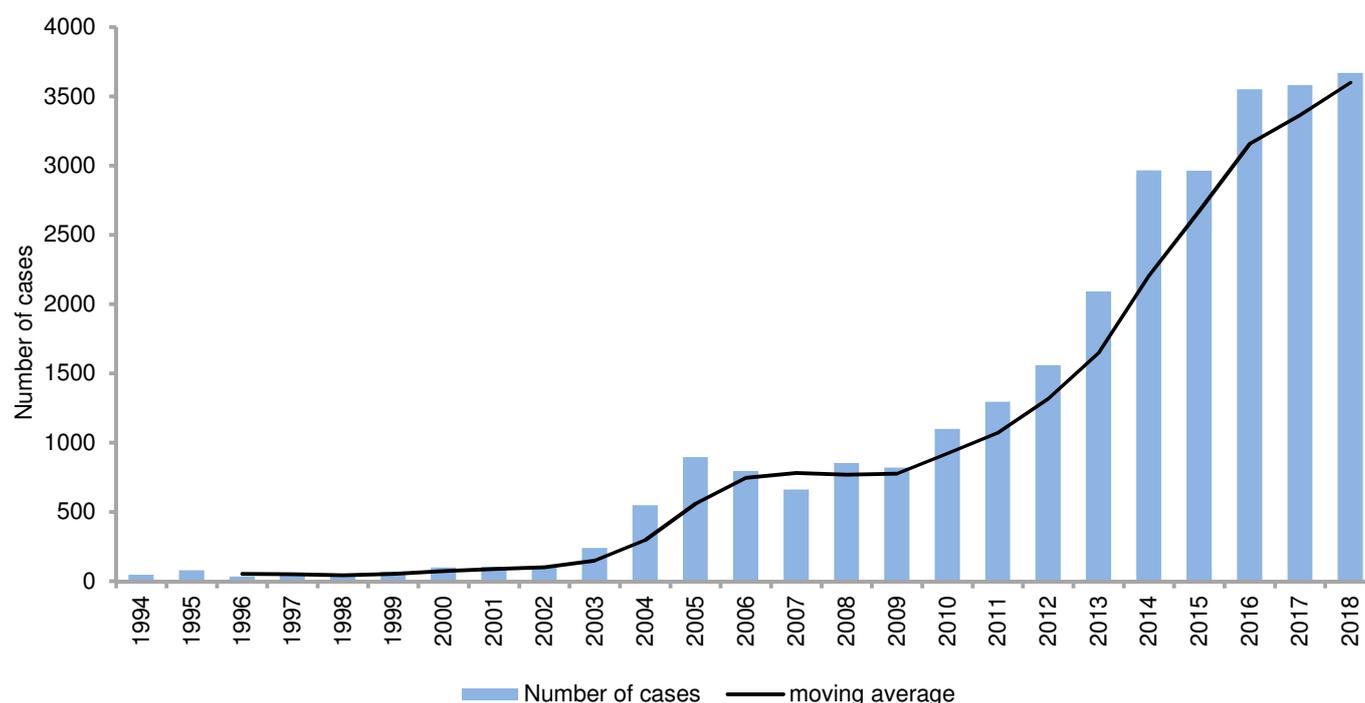
CC398 cases constituted 34% ($n = 1,250$) of new MRSA cases, of which 1,215 belonged to the LA-MRSA CC398 and the remaining 35 to a human adapted variant harbouring the PVL encoding genes. The number of LA-MRSA CC398 is at the same level as the previous four years. The levelling in number

of cases may be influenced by the fact that only new cases are registered in the surveillance program. Many people in contact with livestock have already been examined and tested positive at an earlier stage and also cases where the clinical situation changes from colonisation to infection will thus not be registered as new cases.

MRSA isolates carrying *mecC* were detected in 52 cases (1.4%). Twenty-two of the cases (42%) had infections at the time of diagnosis. Three patients reported contact to horses, which previously have been shown to be reservoirs for *mecC* MRSA. The remaining 27 patients reported no known contact to any livestock.

Figure 8.19 Number of new MRSA cases with a three years moving average, Denmark

DANMAP 2018



The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.18. Most of the cases (84%) were acquired in Denmark. At the time of diagnosis, 40% (n = 1,478) of cases had infection, which was similar to 2017.

The trend of MRSA infections for 2009-2018 based on their epidemiological classification is shown in Figure 8.20. For the first time in almost a decade the number of CA infections did not increase in 2018 and infections caused by LA-MRSA

CC398 seemed to level off, whereas imported and HACO infections continued to increase. Imported cases presented with infections in 58% of the cases and the number of infections in this category has been increasing from less than 100 cases in 2007 to 341 cases in 2018. HA-MRSA infections remains at a low level with only 100 new cases registered in 2018. It should be noted that the average time patients are hospitalised has decreased over the years to approximately three days, which means that HA-MRSA cases may not be recognized before patients are discharged.

Table 8.18 Epidemiological classification of new MRSA cases, 2018, Denmark

DANMAP 2018

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		588 (16)	341 (58)
Hospital-acquired (HA)		100 (3)	39 (39)
Health-care associated, community onset (HACO)		244 (7)	
	with known exposure	18	8 (44)
	without known exposure	226	178 (79)
Health care worker		42 (1)	20 (48)
Community-acquired (CA)		1457 (40)	
	with known exposure	797	109 (14)
	without known exposure	660	527(80)
LA-MRSA CC398		1215 (33)	
	with known exposure	1057	169 (16)
	without known exposure	158	87 (55)
Unknown/missing		23	

Numbers shown in bold are totals

Figure 8.20 Number of MRSA infections according to epidemiological classification, Denmark

DANMAP 2018

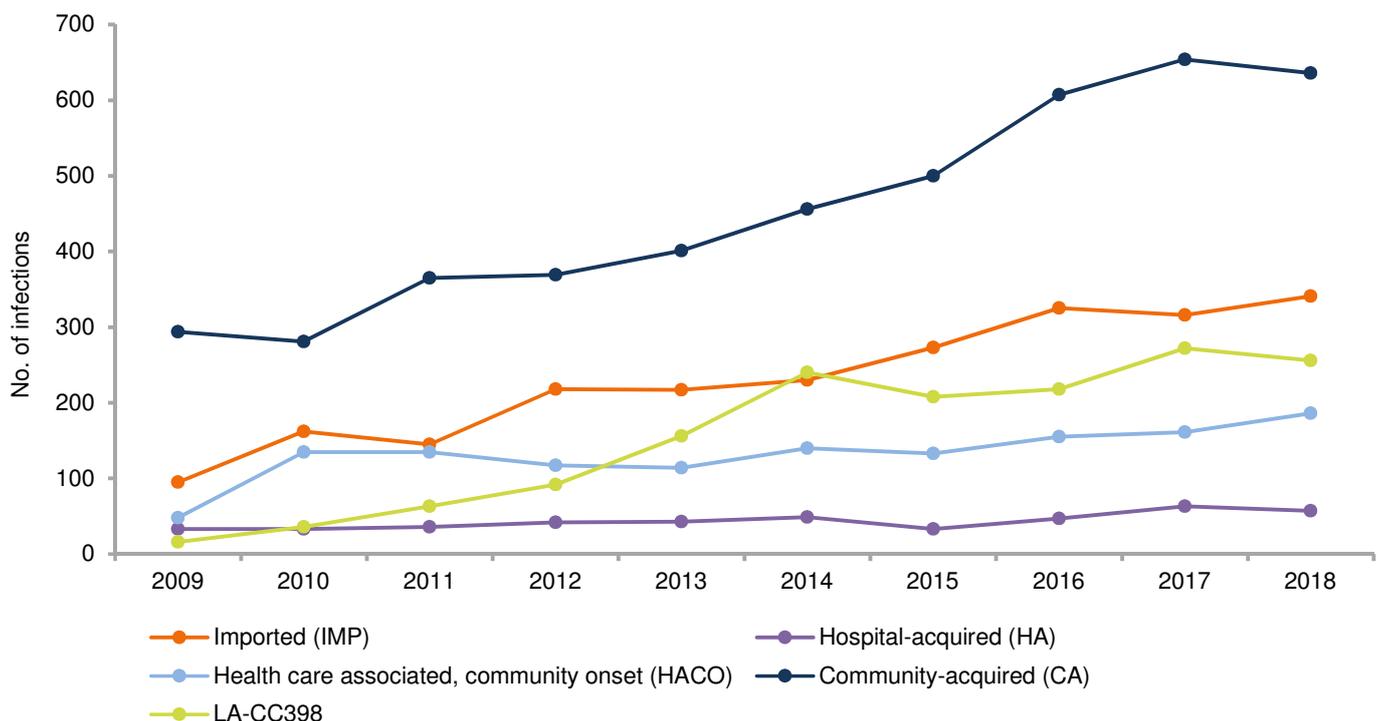


Table 8.19 Resistance (%) in non LA-CC398 MRSA isolates, 2018, Denmark

DANMAP 2018

	% non-CC398
Erythromycin	33
Clindamycin	28
Tetracycline	26
Fusidic acid	18
Rifampicin	1
Norfloracin	21
Kanamycin	28
Linezolid	<1
Mupirocin	<1
Trimethoprim-sulfamethoxazole	3
Number of tested isolates	1233

Molecular typing of the MRSA strains.

In total, *spa* typing revealed 363 different strain types, not including isolates belonging to LA-CC398. Among the infections, 278 *spa* types were demonstrated. The 10 dominating non-LA-CC398 *spa* types isolated in 2018 are listed in Table 8.17. They constituted 43% of the total number of non-LA-CC398 MRSA isolates. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016 and can be linked to the refugee crisis following the civil war in Syria. Ten years ago, t024/CC8 was among the five most prevalent *spa* types, but has since decreased in prevalence. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC.

The *pvl* gene was detected in 29% of the infections and in 12% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 66), t019 (n = 57), t044 (n = 56), t437 (n = 37) and t002 (n = 36).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.19. Every other non-LA-CC398 isolate received in 2018 was tested (n = 1,233). Resistance prevalences were similar to previous years.

Andreas Petersen and Anders Rhod Larsen
For further information: *Andreas Petersen, aap@ssi.dk*

Textbox 8.2

LA-MRSA CC398 in animals and humans

LA-MRSA CC398 infections in humans

The annual number of registered LA-MRSA CC398 infections in people with livestock contact has been relatively constant since 2014, with 121-174 new cases per year (Figure 1). Similarly, the number of LA-MRSA CC398 infections in people with no livestock contact, i.e. the general population, seems to have reached a plateau, with 87-98 cases during 2016-2018 (Figure 1). It should be noted that these numbers do not include people who have been found positive for LA-MRSA CC398 in previous years. This is particularly relevant for people who have been working in the sector for some time, as many of them have already been screened and found positive for LA-MRSA CC398.

Figure 1 LA-MRSA CC398 in conventional pig production herds and as a cause of human infections DANMAP 2018

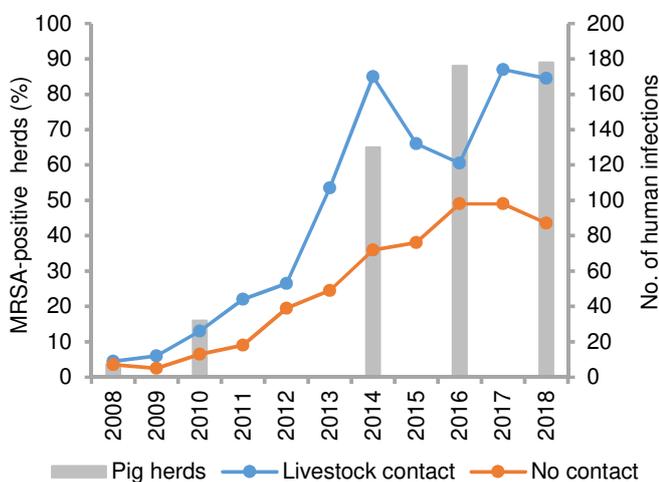


Table 1 Occurrence of MRSA in various animal populations, 2018, Denmark DANMAP 2018

Farm/herd type	MRSA-positive (%)
Conventional production pigs	89% (116/130)
Conventional breeding pigs	83% (34/41)
Organic/free-range pigs	20% (21/104)
Mink	25% (31/122)
Horses	8% (10/123)
Dairy cows	6% (8/132)
Layer hens	3% (4/124)
Turkeys	0% (0/19)

MRSA surveys in animals

In 2018, the Danish Veterinary and Food Administration (DVFA) conducted MRSA surveys in conventional pig herds (breeding and production), free-range pig herds (both organic and non-organic), mink farms, horse herds, dairy cattle herds, layer hen and turkey flocks. For pigs, mink, horses, and dairy cattle, 25 individual animals were tested per herd/farm and the samples analysed as pools of five samples, whereas sampling of layer hen and turkey flocks was performed by collecting five sock samples per flock. Thus, the results described below represent prevalence estimates at the farm level (between-farm prevalence) rather than at the animal level (within-farm prevalence). The results of the MRSA surveys are summarised in Table 1.

MRSA in conventional pig herds

A total of 130 randomly selected production herds and 41 breeding herds were tested; The overall prevalence of LA-MRSA was 89%, which is similar to the results obtained in 2016 (88%) but higher than in previous years (Figure 1). Among the breeding herds, 83% tested MRSA-positive, which is an increase compared to 2016 (66%). All 150 isolates had *spa*-types associated to CC398, with *spa*-types t034 (n = 109) and t011 (n = 28) being the most prevalent, while 10 other *spa*-types were found in one to three isolates.

MRSA in organic and free-range pigs

Testing of 104 organic and non-organic free-range pig herds in 2018 revealed that 20% were positive for LA-MRSA, compared with 6% in 2015. It should be noted, however, that the 2015 survey only included organic herds and also differed in size and sample selection procedure, which makes it difficult to compare the results. It has been speculated that the lower frequency of MRSA in organic and non-organic free-range pigs compared to conventionally raised pigs is due to differences in animal density, access to open air, and antimicrobial use. A study will be conducted in 2020-2021 to determine the impact of these factors. All 21 MRSA isolates had *spa*-types associated to CC398, most of which belonged to t034 (n = 15) and t011 (n = 4).

continued ... Textbox 8.2

MRSA in mink

Previous studies have revealed that LA-MRSA in mink is most often located on the paws and in the pharynx, probably reflecting that mink acquire MRSA from contaminated food, which contains slaughter offal including by-products from the Danish pig production. Testing of paws from mink from 122 mink farms revealed that 25% were positive for MRSA. This is similar to the prevalence of 29% found in a 2015 survey [Hansen et al., *Vet. Microbiol.* 2017; 207:44-9]. Of the 31 MRSA isolates, 30 had CC398-associated *spa*-types, with t034 (n = 19) and t011 (n = 6) being the predominant *spa*-types, while a single isolate had *spa*-type t13790 associated with CC1. In a recent study, it was shown that most of the LA-MRSA CC398 isolates obtained from mink resembled isolates found in the Danish pig production, supporting the hypothesis that mink become colonized through contaminated mink feed [Fertner et. al, *Vet. Microbiol.* 2019; 231: 80-86].

MRSA in horses

Of the 123 horse herds tested, 8% were found positive for MRSA, which is similar to the prevalence of 9% found in a previous study from 2015 [Islam et al., *Front. Microbiol.* 2015; 8:543]. Of the 10 MRSA isolates, nine had *spa*-types t011 or t034 associated with CC398, whereas a single isolate had *spa*-type t843 associated with CC130 and carried the methicillin resistance gene *mecC* rather than the typical *mecA* gene found in LA-MRSA CC398 and CC1.

MRSA in dairy cows

In 2018, 132 dairy herds were tested for LA-MRSA, of which 6% were positive. Previous surveys of bulk milk showed a lower percentage of MRSA positive samples, which may indicate that sampling using nasal swabs is more sensitive. All MRSA isolates had *spa*-type t034 associated with CC398, except from a single isolate with *spa*-type t267 associated with CC97. MRSA with *spa*-type t267 has previously been described in bovine samples.

MRSA in layer hens

In 2018, a total of 124 layer hen flocks were examined, of which 3% tested positive. The four MRSA isolates had *spa*-types t011 or t034 associated with CC398. All 19 tested turkey farms were MRSA-negative.

MRSA in turkeys

In 2015, a single turkey flock tested positive, whereas all broiler and layer hen flocks were negative.

Conclusions

The results presented above show that conventionally raised pigs are still the primary reservoir for LA-MRSA CC398, and that LA-MRSA CC398 is capable of spreading to other animal species, such as cattle, mink, horses, and poultry, and to people in contact with those animals. The prevalence of LA-MRSA CC398-positive pig farms and the number of LA-MRSA CC398 infections in the general population seem to have reached a maximum in the last couple of years (Figure 1), which suggests that the occurrence of LA-MRSA CC398 in humans is due to a constant spillover from pig farms. Nonetheless, the continued spread of LA-MRSA CC398 to people with no livestock contact is worrisome because they include a higher proportion of elderly and immunocompromised people with an elevated risk of developing serious and life-threatening infections. In addition, there are international concerns about the possibility that LA-MRSA CC398 can adapt to humans, which would result in increased spread in the human population and an increasing number of infections in people with no livestock contact. It is therefore important to continue the ongoing surveillance and efforts to prevent spillover of LA-MRSA CC398 from the farm through people or via the environment.

*Anders Rhod Larsen, Andreas Petersen, Jesper Larsen, Øystein Angen.
For further information: Anders Rhod Larsen, arl@ssi.dk*

8.3.9 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhoea, which is usually located in the urethra in males and cervix in females. *N. gonorrhoeae* (gonococci) may sometimes be demonstrated in specimens from the pharynx or rectum in either gender. In females, the finding of gonococci in rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it is due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

Complications of gonorrhoea include e.g. salpingitis, epididymitis, orchitis, and prostatitis. Furthermore, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct inoculation.

Surveillance

Since 1962, the departments of clinical microbiology in Denmark have voluntarily submitted isolates of gonococci to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

At the NSR laboratory ceftriaxone, ciprofloxacin and azithromycin MICs are determined. Both resistant and intermediate susceptible isolates are categorized as resistant in this report.

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, 110 - 120 gonococcus isolates are collected consecutively each year and investigated for susceptibility to an expanded panel of antimicrobial agents. This panel includes cefixime and in selected years also spectinomycin and sometimes gentamicin.

Results and discussion

Most of the received isolates are from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

The NSR laboratory received isolates from 1067 unique cases of gonorrhoea diagnosed in 2018. The annual number increased considerably from 2011 through 2016 (Figure 8.21), partly because the widespread use of combined nucleic acid amplifications tests for *Chlamydia trachomatis* and *N. gonorrhoeae* has identified unexpected cases of gonorrhoea (sometimes followed by culture), and partly due to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion are women. A decrease in the annual number of unique cases has been observed in 2017, continuing in 2018.

Figure 8.21 The number of submitted gonococcus isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark

DANMAP 2018

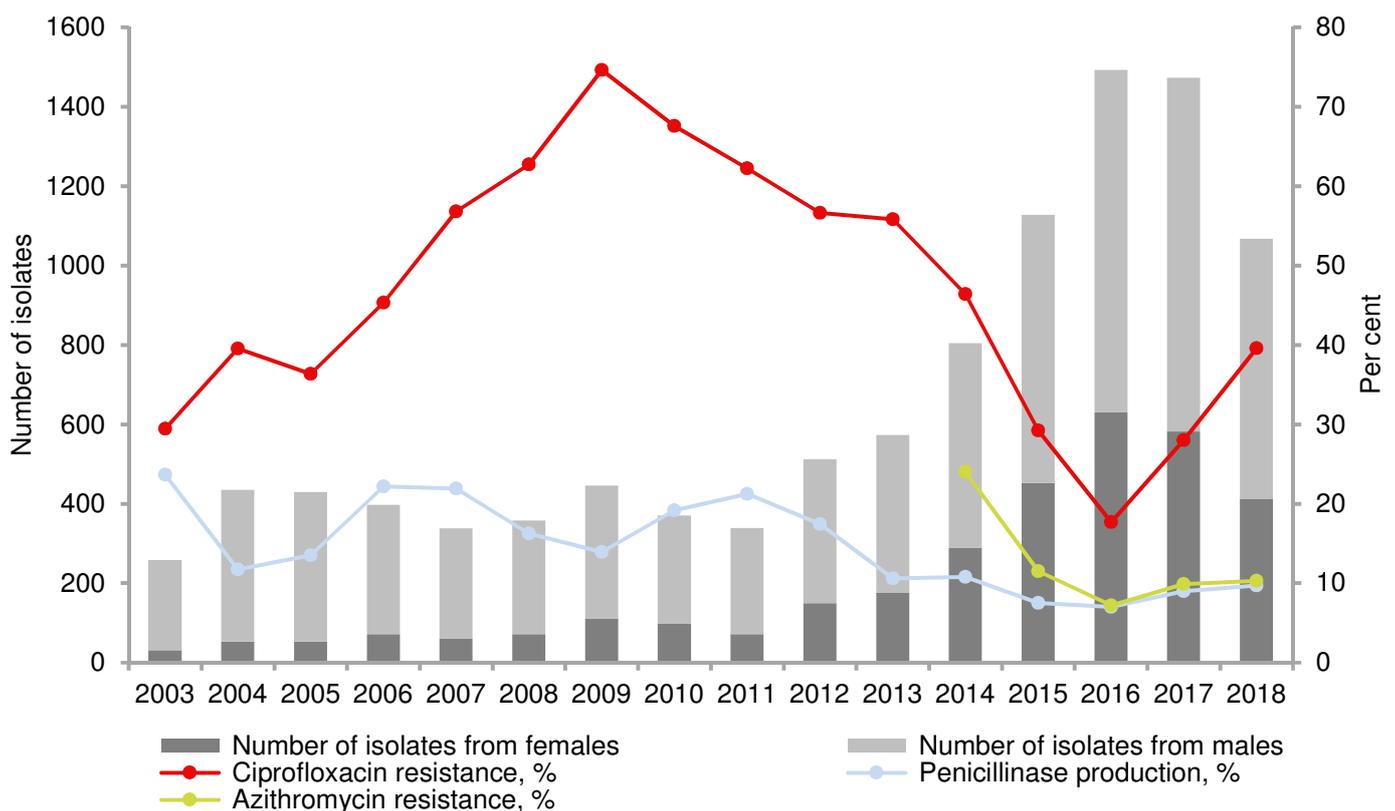
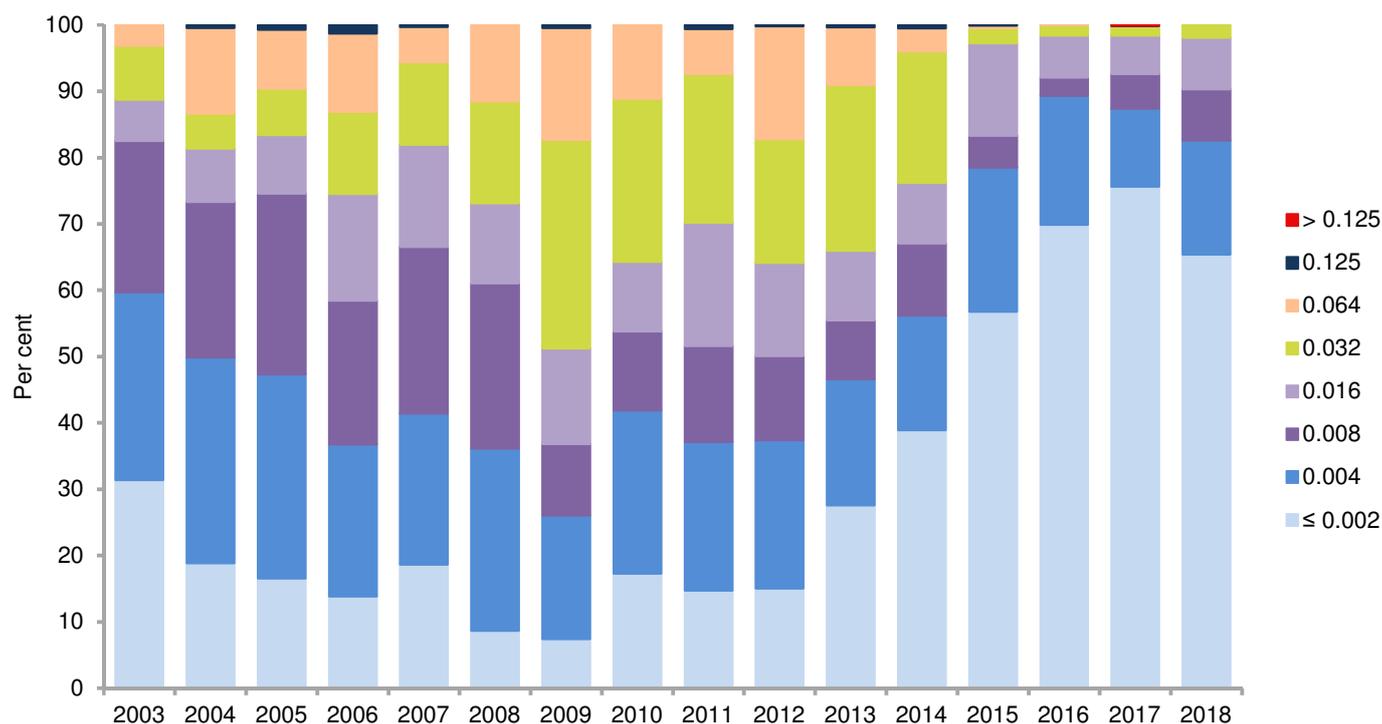


Figure 8.22 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark

DANMAP 2018



The ciprofloxacin resistance rate was 40% in 2018 (28% in 2017 and 18% in 2016), thus still considerably lower than the peak of 75% in 2009 (Figure 8.21). The percentage of strains producing penicillinase was 10%. It has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance was found in 6% (10% in 2017) and intermediate susceptibility in 4% of the isolates (4% in 2017).

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. During 2003 through 2009, the proportion of isolates with ceftriaxone MIC \geq 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.22). During recent years this trend has nearly reversed, the proportion being 44% in 2014, 11% in 2016 and 17% in 2018.

The Danish National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g) is recommended for the treatment of gonorrhoea. Ciprofloxacin (500 mg p.o.) may be used for

treatment if the strain is fully susceptible. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L and azithromycin MIC of 0.25 mg/L.

In a subset of 120 isolates, resistance against cefixime (MIC > 0.125 mg/L) was 0% in 2018 (0.8% in 2017 and 0% in 2016 and 2015). Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2018 as well as in the years 2015 through 2017. MIC values for gentamicin were 1 to 4 mg/L, but no break-points are defined for this agent against gonococci.

Conclusions

The ciprofloxacin resistance rate among gonococci increased in 2018. Although resistance problems are still not present in Denmark, the emerging ceftriaxone treatment failures in other countries underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

Steen Hoffmann

For further information: Steen Hoffmann, hof@ssi.dk

9

MATERIALS AND METHODS



9. Materials and methods

9.1 General information

For the DANMAP 2018 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined at the individual farm level, meaning that only one isolate per bacterial species per farm was included in the report. The individual flock of broilers was defined as the epidemiological unit, and for food, the epidemiological unit was defined as the individual meat sample.

For humans, the epidemiological unit was defined as the individual patient and the first isolate per species per patient per year was included. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 3.2.

9.2 Data on antimicrobial consumption in animals

9.2.1 Data

In Denmark, all antimicrobial agents used for treatment are available on prescription only. Until 2007, antimicrobial agents were exclusively sold by pharmacies or as medicated feed from the feed mills. However, since April 2007, the monopoly was suspended and private companies (four in 2018) were given license to sell prescribed veterinary medicinal products for animals, when following strict guidelines, identical to those applied to pharmacies. Furthermore, in 2007 price setting of antibiotic was liberalised, which allowed for discounts to veterinarians, when buying larger quantities.

A pharmacy or company either sells the medicine to veterinarians for use in their practice or for re-sale to farmers, or sells the medicine directly to the animal holder on presentation of a prescription. By law, veterinarians are allowed only very small profits on their sale of medicine (5%), to limit the economic incentive to overprescribe.

In 2018, 97% of antimicrobial agents were purchased through pharmacies and the drug trading companies, while 3% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 83% of antimicrobial agents used in 2018 were purchased from pharmacies, whereas 10 years ago two thirds of the antimicrobial agents used in cattle was purchased through the veterinarian. In aquaculture, approximately two thirds is purchased through the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database called VetStat, which

is hosted by the Danish Veterinary and Food Administration. Prior to 2001, all data on antimicrobial sales were derived from pharmaceutical companies. Veterinarians are required by law to report all use of antibiotics and prescriptions for production animals to VetStat monthly. For most veterinarians, the registration of data is linked to the writing of invoices. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and type of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at SSI and to VetStat. However, VetStat does not have any validation on data entry and slight typing errors from vets may occur.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) have also been collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark for the past twenty years. In very rare instances, medicines are prescribed on special license and will not be included in VetStat (i.e. medicines not approved for marketing in Denmark).

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age group, disease category and code for farm-identity (CHR Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" (DADD) a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no]. The data presented in DANMAP 2018 were extracted from VetStat on 3th March 2019.

9.2.2 Methods

In DANMAP, we report use of antimicrobials dispersed in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound, to enable an overall crude comparison of consumption in different animal species and in the veterinary and human sectors.

A more detailed comparison of antimicrobial use is performed, taking into account their potency, formulation, route of administration and the age of the animals (where relevant), by generating defined animal daily doses (DADDs). For these calculations, we select data with relevant animal and age group codes, e.g. pigs and weaners and relevant codes for dispensation. For example, when calculating the antimicrobial use for systemic treatment in pigs, we exclude antimicrobials dispensed as tablets, products for topical use, intramammarys and gynaecologicals. This is described in the footnotes for figures and tables in chapter 4.

Numerator - DADD

Defined animal daily dose (DADD) is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species and when appropriate, also age group. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The following principles are applied when setting the DADDs:

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products is given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group is maintained as the common DADD even if a new product is approved with a higher dosage;
4. If the dosage for a group shows large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] are applied;
5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age groups, indications or formulations.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - biomass

Trends in antimicrobial use in pigs are presented in DADD per 1,000 animals per day (DAPD). The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species.

Pig production: The estimation was based on the number of pigs produced, including exports at different ages, productivity data for each year [Statistics Denmark; Danish Agriculture and

Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) was estimated from the annual productivity numbers [Danish Agriculture and Food Council]. For DANMAP 2018, productivity data from 2017 were used to estimate the biomasses for pigs, since the 2018 productivity data were not available when estimates were calculated. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this could not be accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark 2019] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Broiler (Gallus gallus): The live biomass was estimated based on number of broilers produced [Statistics Denmark; Danish Agriculture and Food Council], an average live weight at slaughter of 1.97 kg after an estimated average life span of 30 days. The mean live biomass per broiler is assumed to be half of the weight at slaughter.

Turkey production: The live biomass is estimated based on the number of turkeys produced [Statistics Denmark; Danish Agriculture and Food Council] and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Fur animals: The live biomass of mink is estimated from production data [Kopenhagen Fur] and carried out as described by Jensen et al., 2016 [Prev Vet Med. 26:170].

Pet animals: Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The population is based on census data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture: The estimation is based on data from the Danish AgriFish Agency (Ministry of Environment and Food) on produced amounts in each subtype of production, and information

on the typical lifespan, entrance and exit body weights. The estimation was calculated in cooperation with Danish Aquaculture [N.H. Henriksen, Danish Aquaculture].

Treatment proportion - DAPD

The treatment proportion is calculated as the number of DADDs administered to an animal species during a year (in thousands) divided by the number of standard animals at risk per day (DADD per 1000 animals per day). The number of standard animals at risk per day takes into account species differences in average body-mass and lifespan. When relevant, the numbers of DADDs and standard animals at risk are estimated for specific age groups, or simply as number of doses (DADDs) used to treat one kg of animal divided with the total estimated biomass (in tonnes).

DAPD is a statistical measure, which provides a rough estimate of the proportion of animals treated daily with an average maintenance dose of a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. The DAPD is also referred to as the treatment proportion or treatment intensity. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, defined daily dose per 1000 inhabitants per day (DID), see section 9.8.2.

In 2018, DAPD calculations were carried out for pigs, cattle and fur animals.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of the average antimicrobial use in all age groups was calculated (DAPD_{adj}). The adjustment is based on the assumption that pigs exported at 30 kg, on average, would have received the same amount of antimicrobial agents as other pigs from farrowing to slaughter.

Antimicrobial use per pig produced (adjusted) is calculated as: $[DADDs + DADDw + (1+Q)*DADDf] / (\text{biomass-days-total} + Nw * AN_{BDW} (\text{kg*days}))$, where

DADDs= the amount of antimicrobial agents used in sows
 DADDw = the amount of antimicrobial agents used in weaners
 DADDf = the amount of antimicrobial agents used in finishers
 Q is the proportion of weaning pigs exported around 30 kg
 Nw= the number of pigs exported at 30 kg bodyweight
 AN_{BDW}=Average number of biomass days contributed by a weaner pig

9.3 Collection of bacterial isolates - animals and meat

9.3.1 Animals

Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. The legislation requires, in addition to sampling for the national *Salmonella* control programmes

in poultry farms, sampling of broilers and fattening turkeys at slaughter in even years (2014-2020) and sampling of fattening pigs and cattle <1 year at slaughter in odd years (2015-2019).

In 2018, most of the sampling for DANMAP was allocated to the mandatory sampling of broilers (examined for *Campylobacter jejuni*, indicator *E. coli* and ESBL/AmpC/Carbapenemase-producing *E. coli*). Additionally, sampling of slaughter pigs (examined for *Salmonella* and indicator *E. coli*) and cattle <1 year (examined for *Campylobacter jejuni* and indicator *E. coli*) was carried out (Table 9.1).

Meat inspection staff or abattoir personnel at the slaughterhouses collected caecal samples from healthy pigs, cattle (<1 year) and broilers. For broilers, the samples were collected throughout 2018, but the majority of samples were taken during June to November; in order to collect isolates during the expected high-prevalence period of *Campylobacter*. For broilers, the sampling took place at the two major Danish slaughterhouses and at one minor slaughterhouse. For pigs and cattle, the samples were collected throughout 2018. The sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse.

Four intact caeca from each broiler flock were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal. All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted, including antimicrobial susceptibility testing and *Salmonella* serotyping.

Salmonella from layers, broilers, turkeys and cattle are not included in DANMAP 2018 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2018].

9.3.2 Meat

The EU harmonised monitoring requires, in addition to sampling for the national *Salmonella* control programmes at slaughter, sampling at retail of broiler meat in even years (2014-2020) and sampling of pork and beef in odd years (2015-2019) for detection of ESBL/AmpC-producing *E. coli* [Decision 2013/652/EU].

In 2018, ESBL/AmpC/Carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled broiler meat collected in Danish wholesale and retail outlets throughout the year by the regional DVFA officers (Table 9.1). Products with added saltwater or other types of marinade were excluded. Most the packages of broiler meat (n = 293) were selected without pre-selecting based on the country of origin as requested for the harmonised EU monitoring. In 2018, an additional 131 samples of imported meat was collected. The number of establishments

and samples selected by each regional DVFA control unit was proportional to the number of establishments in the region in relation to the total number of establishments in the country. One unit of product (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory.

The *Salmonella* isolates from pork originate from the national control programme at the slaughterhouses (Table 9.1), where the carcasses are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). The numbers of swabs collected depend on the slaughterhouse capacity. All samples were processed at Industry laboratories. Isolates from all *Salmonella* positive samples were sent to the DVFA laboratory, where one isolate per sample was serotyped and susceptibility tested.

Salmonella from broiler meat and beef are not included in DANMAP 2018 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2017]. *Campylobacter* from broiler meat for DANMAP originate from the national control program: Intensified Control of *Salmonella* and *Campylobacter* in fresh meat. However, very few *Campylobacter* isolates from domestically produced broiler meat was susceptibility tested in 2018 and therefore not included in DANMAP 2018.

9.4 Microbiological methods - isolates from animals and meat

9.4.1 *Salmonella*

Salmonella was isolated in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] or ISO 6579-1 [ISO6579-1:2017]. Serotyping of isolates was performed by whole genome sequencing using the Illumina MiSeq platform, paired-end sequencing 2x250 cycles. For bioinformatics, a CGE service (Centre for Genomic Epidemiology, DTU) for *Salmonella* serotyping was applied based on the genetic background for antigenic formulas given by the White-Kauffmann-Le Minor scheme. Only one isolate per serotype was selected from each herd, flock or slaughter batch.

9.4.2 *Campylobacter*

Campylobacter from broilers and cattle was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] followed by species-determination by BAX® rtPCR assay. Pre-enrichment in Bolton broth was used for cattle samples, whereas direct spread of caecal sample on to selective agar was used for broiler samples. Only one *Campylobacter jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

9.4.3 Indicator *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread of caecal sample material onto violet red bile

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2018

DANMAP 2018

Bacteria	Origin of isolates	Legislative reporting frequency (2013/652/EU)	Number of tested and positive samples in 2018
<i>Salmonella spp.</i>	On-farm samples from laying hens (production flocks)	Even years	4,245 flocks (35 positive)
	On-farm samples from broilers (production flocks)	Even years	454 flocks (12 positive)
	Caecal samples from fattening pigs		553 animals (87 positive)
	Neck skin samples from broilers	Even years	249 flocks (1 positive)
	Carcass swabs from fattening pigs (a)	Odd years	18,994 animals (0.8% positive)
	Carcass swabs from cattle <1 year (a)	Odd years	6,356 animals (0.2% positive)
<i>Campylobacter jejuni</i>	Caecal samples from broilers	Even years	836 flocks (256 positive)
	Caecal samples from cattle <1 yr		154 animals (111 positive)
Indicator <i>E. coli</i>	Caecal samples from broilers	Even years	186 flocks (174 positive)
	Caecal samples from fattening pigs	Odd years	154 animals (150 positive)
	Caecal samples from cattle <1 yr	Odd years	198 animals (187 positive)
Specific monitoring of ESBL/AmpC and Carba – producing <i>E. coli</i>	Caecal samples from broilers	Even years	837 flocks (124 positive)
	Fresh broiler meat at retail (domestic origin)	Even years	244 units (36 positive)
	Fresh broiler meat at retail (Imports)	Even years	180 units (82 positive)
	WGS data for collected ESBL/AmpC isolates		189 isolates

Note: Carcasses swabs collected at slaughterhouse slaughtering more than 30,000 pigs or 7,500 cattle, swab samples are analysed in pools of 5 samples. When estimating the prevalence of *Salmonella*, both the loss of sensitivity and the probability of more than one sample being positive in each pool are taken into consideration

Testing for carbapenemase producing *E. coli* is voluntary according to regulation 2013/652/EU. Carbapenemase producing *E. coli* was not detected in any of the analysed sample. Most the packages of broiler meat (n = 293) were selected without pre-selecting based on the country of origin as requested for the harmonised EU monitoring. In 2018, an additional 131 samples of imported meat was collected and analysed for ESBL/AmpC and Carbapenemase-producing *E. coli*

agar incubated for 24h at 44°C. Presumptive *E. coli* was identified on TBX agar incubated at 44°C o/n. Only one indicator *E. coli* isolate per flock or herd was selected. For specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* from meat and caecal samples, the present EURL-AR laboratory protocols describing the selective enrichment procedures was applied in accordance with the EU harmonised monitoring. Only one ESBL/AmpC-producing *E. coli* isolate per broiler flock and meat sample was selected (no isolates of carbapenemase-producing *E. coli* was detected).

9.5 Susceptibility testing - isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and *E. coli* was performed as Minimum Inhibitory Concentration (MIC) determination using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/ EU about the EU harmonised monitoring of antimicrobial resistance.

The relevant quality control strains were used at the laboratories: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Campylobacter jejuni* ATCC 33560 and *Pseudomonas aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted that is accredited by DANAK (the national body for accreditation).

Table 9.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli* DANMAP 2018

Antimicrobial classes	<i>Salmonella</i> and <i>E. coli</i>
Beta-lactam penicillins	Ampicillin
Macrolides	Azithromycin
Cephalosporins	Cefotaxime and/or ceftazidime
Phenicols	Chloramphenicol
Quinolones	Ciprofloxacin and/or nalidixic acid
Polymycins	Colistin
Aminoglycosides	Gentamicin
Carbapenems	Meropenem
Sulfonamides	Sulfonamides
Tetracyclins	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

Note: An isolates is considered multidrug-resistant if resistant to three or more of the defined antimicrobial classes and fully sensitive if susceptible to all antimicrobial agents included in the test panel

9.6 Whole genome sequencing - isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (section 9.4.1), whole genome sequencing (WGS) and in silico bioinformatic tools were also used to detect the genetic background of the ESBL/AmpC and carbapenemase-producing *E. coli*. At the DVFA laboratory in Ringsted, strains were sequenced using the Illumina MiSeq platform followed by bioinformatics analysis at DTU National Food Institute from Centre for Genomic Epidemiology (www.genomicepidemiology.org; <https://cge.cbs.dtu.dk/services/all.php>) including:

1. De novo assembly using SPAdes [Bankevich et al. 2012. J Comput Biol. 19(5):455];
2. MLST using MLST Finder 2.0 [Larsen et al. 2012. J Clin Microbiol. 50(4):1355];
3. Detection of antimicrobial resistance genes using ResFinder 3.1 which includes chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari et al. 2012. J Antimicrob Chemother. 67(11):2640; Zankari et al. 2017. J Antimicrob Chemother. 72(10):2764]; and
4. Detection of plasmid replicons using PlasmidFinder 2.0 [Carattoli et al. 2014. Antimicrob Agents Chemother. 58(7):3895].

9.7 Data handling - isolates from animals and meat

For the samples processed at the DVFA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were sent to National Food Institute at DTU (Excel sheets). Here data were harmonised and one isolate per epidemiological unit was selected for reporting. All data are stored in an Oracle database at isolate level (9i Enterprise Edition®). The database contains all antimicrobial data reported in DANMAP or to EFSA since 2007 (partial dataset from 2001-2006). Variables include: Bacterial species (subtype where applicable), date of sampling, animal species or food type, herd identifier and country of origin whenever possible.

9.7.1 Interpretation of MIC values

MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant cut-off from 2018 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at www.danmap.org/downloads/reports. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals.

Table 9.2 Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values and the corresponding EUCAST clinical breakpoints

DANMAP 2018

	<i>Salmonella</i>		<i>E. coli</i>		<i>C. jejuni</i>		<i>Staphylococcus aureus</i>
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8	>8	>8	>8			
Azithromycin	>16 (a)		>16 (a)				
Cefepime	>0.125 (a)	>4	>0.125	>4			
Cefotaxime	>0.5	>2	>0.25	>2			
Cefotaxime/clavulansyre	>0.5 (a)		>0.25 (a)				
Cefoxitin	>8		>8				>4
Ceftaroline							>1
Ceftazidime	>2	>4	>0.5	>4			
Ceftazidime/clavulansyre	>2 (a)		>0.5 (a)				
Ceftobiprole							>2
Chloramphenicol	>16	>8	>16	>8			
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>0.5	>0.5	
Clindamycin							>0.5 (c)
Colistin	>2 (b)	>2	>2	>2			
Daptomycin							>1
Ertapenem	>0.064	>1	>0.064	>1			
Erythromycin					>4	>4	>2
Fusidic acid							>1
Gentamicin	>2	>4	>2	>4	>2		>1
Imipenem	>1	>8	>0.5	>8			
Kanamycin							>16
Linezolid							>4
Meropenem	>0.125	>8	>0.125	>8			
Mupirocin							>2
Nalidixic acid	>16		>16		>16		
Norfloxacin							>4
Penicillin							>0.125
Rifampicin							>0.5
Streptomycin					>4		
Sulfamethoxazole/Trimethoprim							>4
Sulfonamide	>256 (a)		>64				
Temocillin	>32 (a)		>32 (a)				
Tetracycline	>8		>8		>1	>2	>2
Tigecycline	>1	>2	>1	>2			
Trimethoprim	>2	>4	>2	>4			

Note: EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted

a) No current EUCAST ECOFF is available, apply complementary interpretative thresholds as suggested by EFSA [EFSA Supporting publication 2019:EN-1559]

b) The EUCAST ECOFF (>2) for colistin was applied for *S. Typhimurium* and other serotypes, except for *S. Enteritidis* and *S. Dublin* where ECOFF >8 was applied according to investigations presented in DANMAP 2011

c) Inducible clindamycin resistance is included

An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 9.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

9.7.2 ESBL/AmpC phenotypes

Classification of CPE, ESBL and AmpC phenotypes was done according to the scheme provided by EFSA [EFSA 2018, EFSA Journal 16(2):5182]:

1. CPE phenotype if meropenem MIC >0.12 $\mu\text{g/ml}$;
2. ESBL phenotype if cefotaxime/ceftazidime MIC >1 $\mu\text{g/ml}$ and meropenem MIC ≤ 0.12 $\mu\text{g/ml}$ and ceftazidime MIC ≤ 8 $\mu\text{g/ml}$ and synergy (clavulanic acid and cefotaxime/ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 $\mu\text{g/ml}$ and meropenem MIC ≤ 0.12 $\mu\text{g/ml}$ and ceftazidime MIC >8 $\mu\text{g/ml}$ and synergy (clavulanic acid and cefotaxime/ceftazidime);
4. AmpC phenotype if cefotaxime/ceftazidime MIC >1 $\mu\text{g/ml}$ and meropenem MIC ≤ 0.12 $\mu\text{g/ml}$ and ceftazidime MIC >8 $\mu\text{g/ml}$ and no synergy (clavulanic acid and cefotaxime/ceftazidime);
5. Other phenotype if not in 1-4.

Synergy is defined as ≥ 3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS® Software, SAS Enterprise Guide 6.1 using univariable 2x2 Chi-square, or Fisher's Exact Tests as appropriate. All changes and differences yielding $p < 0.05$ were commented on in the text, whereas the remaining data was visualised in figures or tables only.

Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test. The significance levels were calculated using SAS Enterprise Guide 6.1. A p -value of < 0.05 is generally considered significant. Presented in this report are results from trend analysis for five years- and ten years trend, respectively. The test was applied to several bacteria's resistance to a broad range of antimicrobials. One-sided tests were chosen because of preliminary expected trend directions.

Some types of resistances were looked for, but not found by the DANMAP monitoring system, yielding a prevalence of zero. It is not possible for surveys to prove freedom from diseases or resistances in populations, but with a defined confidence, surveys can identify the maximum possible prevalence given that the survey failed to find any positives [Textbox 6.2, DANMAP 2016]. This maximum prevalence was calculated for the report using 95% confidence and assuming a perfect test by a probability formula to substantiate freedom from disease [Cameron and Baldock 1998, Prev. Vet. Med].

Link to calculation example: <http://epitools.ausvet.com.au/content.php?page=HerdSens5&Sens=1&SampleSize=100&Population=&TargetSeH=0.95>.

9.8 Data on antimicrobial consumption in humans

9.8.1 Data registration

All antimicrobial consumption in Denmark has since 1997 been reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Until 2012, data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. Since 2013, all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at SSI.

Reportings on human antimicrobial consumption in Denmark exist from before 1997. These were performed through the Association of Medicine Importers (Medicinimportørforeningen, MEDIF) and the Association of Danish Medicinal Factories (Foreningen af danske Medicinfabrikker, MEFA) based on whole sales data to the pharmacies. This reporting became less reliable over time, since there was an increasing amount of parallel imported drugs from the late 1980's, which were not covered by this registration.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported through the pharmacies by a code relating to the defined package. The information from the code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The sale also reports the age, gender and regional residence of the patient. Since 2004, the sales registration has included the indication code as well. Still, for the treatment of infectious diseases the clinical indications given were often quite unspecific, such as "against infection". Since 2016, the use of more specific indication codes has become more feasible through the implementation of the "common medicine card" (fælles medicinkortet, FMK), mandatory to be used by all medical doctors. In 2018, indication codes were available for 93% of prescriptions, but specific indication codes still accounted for only 69%.

For hospitals, reporting is based on deliveries from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between different medical suppliers and Amgros, a private company under agreement with the regions, responsible for harmonisation of prices and for ensuring deliveries to all hospitals. Amgros works closely together with the Regions' Joint Procurement. Detailed information is given on the different drugs delivered on ATC5 level. For surveillance purposes, it has to be assumed that the amount of delivered antimicrobials is similar to the consumption at the different departments. In reality, antimicrobials may be exchanged between different specialties and departments belonging to the same trust, which makes precise calculations of the consumption on specialty level difficult. In DANMAP, reporting

of data on hospital consumption is therefore kept at a national or regional level. Data on hospital level can be supplied upon request.

In case of production failures and shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency. These special deliverances are reported separately to DANMAP through the Danish Health Authority. An example is the shortages in deliverance of piperacillin with tazobactam and pivmecillinam as well as mecillinam in 2017. The shortage in piperacillin with tazobactam had significant impact on the amount used, while the shortage in (piv)mecillinam could not be clearly tracked in changes in consumption. For 2018, no significant shortages or special deliverances were reported.

Data on treatment at patient level is available at very few of the hospitals and has so far been used in local quality assurance only but has not been available to the national surveillance system. Thus a national account of the prudence of use of antimicrobials at hospitals has so far not been possible.

9.8.2 Method

Primarily somatic hospitals were included in the DANMAP reporting. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP in most calculations since their activity and functions are not comparable to public, acute care hospitals and therefore may skew the data. Their consumption accounts for approximately 3% of the antimicrobial consumption at hospitals.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2017 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary health care and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and oral preparations of vancomycin (A07AA09). As recom-

mended by the World Health Organization (WHO), consumption of antibacterial agents in primary healthcare is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitant-days).

The consumption in hospital healthcare is expressed as the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days or DBD). Since reporting in DBD does not necessarily reflect changes in hospital activity and production, consumption at hospitals is also presented as DAD (the number of DDDs per 100 admissions) and crude DDD. Finally, the consumption of antibacterial agents at hospitals has also been calculated in DIDs, primarily for comparison with primary healthcare.

9.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whocc.no/atcddd/ index database).

Per January 2019 the WHO updated the DDDs for seven main antimicrobial agents, based on recommendations from an expert working group in collaboration with the European Center for Disease Control (ECDC). New and former DDDs are presented in Table 9.4.

9.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the date of discharge minus the date of admission and rounded up to nearest 24 hours, e.g. one day. Every new admission to a new hospital department counts as a new bed-day. Number of admissions was extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

Table 9.4 New DDDs assigned by WHO Collaborating Centre per January 2019

DANMAP 2018

ATC5 code	ATC level name	Previous DDD			New DDD		
		Weight	Unit	Route of administration	Weight	Unit	Route of administration
J01CA01	Ampicillin	2.0	g	Parenteral	6.0	g	Parenteral
J01CA04	Amoxicillin	1.0	g	Oral	1.5	g	Oral
J01CA04	Amoxicillin	1.0	g	Parenteral	3.0	g	Parenteral
J01CA17	Temocillin	2.0	g	Parenteral	4.0	g	Parenteral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.0	g	Oral	1.5	g	Oral
J01DE01	Cefepime	2.0	g	Parenteral	4.0	g	Parenteral
J01DH02	Meropenem	2.0	g	Parenteral	3.0	g	Parenteral
J01MA02	Ciprofloxacin	0.5	g	Parenteral	0.8	g	Parenteral
J01XB01	Colistin	3.0	MU	Parenteral	9.0	MU	Parenteral

9.9.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific ward (i.e. one patient can be registered as admitted multiple times if transferred between wards during the same hospital stay). The admissions were extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

9.8.6 DaDDD

Danish adjusted daily dose. This unit was developed for DANMAP 2018 as an attempt to better picture the actual dosages used for antibiotic treatment in Denmark. DaDDD units were made by combining recommended dosages in Danish treatment guidelines with data from the prescription register, thus defining a Danish maintenance dose for each given drug. The work with DaDDD was initiated by an expert group under the Danish Regional Learning and Quality teams (LKT) developing

measurable units for consumption at Danish hospitals. The units were developed for monitoring progress in a nationwide project on introducing antibiotic stewardship principles at emergency departments and internal medicine wards. The units covered only intravenously applied drugs. The DANMAP group further developed these units to also apply to drugs given orally. DaDDD, their counterparts DDD and the conversion factors are presented in Table 9.5 and Table 9.6 for the primary and hospital sector, respectively.

For further information regarding the LKT initiative please go to <https://kvalitetsteams.dk/laerings-og-kvalitetsteams/lkt-rationelt-antibiotikaforbrug-paa-hospitaler>, (only available in Danish). The LKT report with results from the project can be found at https://kvalitetsteams.dk/media/9049/lkt-ab_status_2018-06-01.pdf.

Table 9.5 Danish adjusted DDD for penicillins in the primary sector, 2019

ATC5 code	Antimicrobial agent	WHO DDDs in grams	Danish adjusted DDDs in grams	Conversion factor	Primary indication
J01CA02	Pivampicillin	1.05	2.10	0.50	Urinary tract infection
J01CA04	Amoxicillin	1.50	1.50	1.00	Otitis media
J01CA08	Pivmecillinam	0.60	1.20	0.50	Urinary tract infection
J01CE02	Phenoxymethylpenicillin	2.00	1.90	1.05	Upper respiratory tract infection
J01CF01	Dicloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CF05	Flucloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CR02	Amoxicillin and beta-lactamase inhibitors	1.50	1.50	1.00	Upper respiratory tract infection

Note: Solely per oral administration routes

Table 9.6 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019

ATC5 code	Antimicrobial agent	WHO DDDs in gram	Danish adjusted DDDs	Conversion factor	Route of administration
J01CA01	Ampicillin	6.00	8.00	0.75	Parenteral
J01CA02	Pivampicillin	1.05	2.10	0.50	Oral
J01CA04	Amoxicillin	1.50	1.50	1.00	Oral
J01CA08	Pivmecillinam	0.60	1.20	0.50	Oral
J01CE01	Benzympenicillin	3.60	4.80	0.75	Parenteral
J01CE02	Phenoxymethylpenicillin	2.00	2.67	0.75	Oral
J01CF01	Dicloxacillin	2.00	4.00	0.50	Oral
J01CF05	Flucloxacillin	2.0	4.0	0.5	Oral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.50	1.50	1.00	Oral
J01CR05	Piperacillin and beta-lactamase inhibitor	14.00	11.97	1.17	Parenteral
J01DC02	Cefuroxim	3.00	4.48	0.67	Parenteral
J01DH02	Meropenem	3.00	3.00	1.00	Parenteral
J01FA09	Clarithromycin	0.50	1.00	0.50	Oral
J01FA10	Azithromycin	0.30	1.00	0.30	Oral
J01GB03	Gentamicin	0.24	0.35	0.69	Parenteral
J01MA02	Ciprofloxacin	0.80	0.80	1.00	Parenteral

Figures based on DaDDD can be found in chapter 5, antimicrobials in humans, Figure 5.4b and Figure 5.13b, presenting data from primary sector and hospital sector, respectively.

9.9 *Salmonella* and *Campylobacter* in humans

9.9.1 Data source

Antimicrobial susceptibility was performed on human clinical isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all clinical microbiology laboratories in Denmark and *Campylobacter* isolates were submitted from clinical microbiology laboratories representing the island of Zealand excluding the capital region, Funen, and Northern Jutland. As in previous years, SSI collected information on travel history from the patients. Cases were categorised as “domestically acquired” if the patients had not travelled abroad within the week prior to the onset of disease.

9.9.2 Microbiological methods

Salmonella isolates were analysed by whole genome sequencing and the serotypes were derived from the DNA sequences. In a few cases, the DNA information was supplemented with slide agglutination according to the Kauffman-White Scheme. *Campylobacter* species identification was performed by the use of MALDI-TOFF.

9.9.3 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* was performed as Minimum Inhibitory Concentration (MIC) determination using the Sensititre broth microdilution system (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

9.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

9.10 *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter* spp., *E. faecium* and *E. faecalis* in human patients

9.10.1 Data source

The surveillance of invasive isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* was based on data from routine diagnostics at the 10 departments of clinical microbiology (DCMs) in Denmark. For 2018, all these data were extracted directly from the Danish Microbiology Database (MiBa) (<https://miba.ssi.dk/Service/English.aspx>). Before 2018, data were reported from the individual DCMs to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1.

9.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCMs. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST break-points and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs where local rules were applied on the susceptibility interpretations in specific cases - e.g. susceptibility to mecillinam in invasive cases.

9.10.3 Data handling

Cases were identified in MiBa and susceptibility results extracted. Before 2018, cases were identified based on the reported data from the individual DCMs.

The case definition has been harmonised with the definition by the European Antimicrobial Resistance Surveillance Network (EARS-Net): The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates from the same patient, within the year of observation, were removed. Thereby only resistance data on the first isolate per patient per specimen per year were included. Resistance data from the individual DCMs were excluded if not tested or registered in MiBa routinely (minimum 75% of the specific species/antimicrobial agent combination). Samples were either invasive (including blood cultures or cerebrospinal fluid) or urinary samples from hospital patients or from primary healthcare patients.

9.10.4 Statistical test

Significance tests for trends in rates of resistance in human bacteria were performed by applying Cochran-Armitage test. The significance levels were calculated using the DescTools v0.99.19 package in R version 3.5.0. A p-value of < 0.05 was considered significant. Presented in this report are results from trend analysis for five years and ten years trends, respectively.

The test was applied to several bacterias resistance to a broad range of antimicrobials. One-sided tests were chosen because of preliminary expected trend directions. Cochran-Armitage test calculates probability in binomial proportions across one single, levelled variable. In this report, the test has been performed on susceptibility data from the past 10 years containing numbers of resistant and susceptible/intermediate cases, respectively. The resulting p-values are reported in chapter eight, supplied by an arrow indicating trend direction. Note that the significance levels serve to support the graphs and thus should be interpreted with caution.

9.11 ESBL-producing bacteria in human patients

9.11.1 Data source

Since 2014, the Danish DCMs have on a voluntary basis submitted 3rd generation cephalosporin resistant *Escherichia coli* isolates from bloodstream infections for verification and genotyping at SSI. Since January 1st 2018, 3rd generation cephalosporin resistant *Klebsiella pneumoniae* isolates from

bloodstream infections have been included as well (data not available at this point).

9.11.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and *in silico* bioinformatics analysis have been applied for isolates predicted to carry ESBL and/or AmpC genes based on initial phenotypic tests, to characterize the genetic background of the ESBL and/or AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.11.3 Data handling

The Bifrost QC and analysis pipeline (<https://github.com/ssi-dk/bifrost>) was used for the *in silico* detection of acquired ESBL genes, pAmpCs, carbapenemase genes and MLST from assembled WGS data. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate chromosomal AmpC by the use of myDb-Finder version 1.2 (<https://cge.cbs.dtu.dk/services/myDbFinder-1.2/>). Further subtyping of isolates by core genome MLST (cgMLST) were performed using the SeqSphere+ software from Ridom GmbH (<https://www.ridom.de/seqsphere/>).

9.12 CPO in human patients

9.12.1 Data source

The Danish DCMs have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at SSI. Since September 5th 2018, CPO has had mandatory notification in Denmark (<https://www.retsinformation.dk/Forms/R0710.aspx?id=202889>).

9.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were subjected to WGS. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases.

9.12.3 Data handling

The Bifrost QC and analysis pipeline (<https://github.com/ssi-dk/bifrost>) was used for the *in silico* detection of acquired CPO genes and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types where such schemes are available (*E. coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*).

9.13 VRE in human patients

9.13.1 Data source

The Danish DCMs have on a voluntary basis, submitted VRE for species identification, genotyping and surveillance to SSI.

9.13.2 Microbiological methods

All clinical VRE isolates (i.e. excluding screening samples) have been whole-genome sequenced. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.13.3 Data handling

The Bifrost QC and analysis pipeline (<https://github.com/ssi-dk/bifrost>) was used for the *in silico* detection of genes related to vancomycin resistance in enterococci and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types.

9.14 Invasive *Streptococcus pneumoniae* in humans

9.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark, and all invasive isolates nationwide were sent to SSI for identification or confirmation as well as serotyping and susceptibility testing.

9.14.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* was based on: visual evaluation of colonies, positive optochin test and test with either latex omni test (ImmuLex™ *S. pneumoniae* Omni, SSIDiagnostica, Denmark) or Neufeld based Omni serum (SSIDiagnostica, Denmark). If challenging results occurred, MALDI-TOF and bile solubility test were performed to further confirm the correct species identification.

Serotype identification of invasive *S. pneumoniae* were performed by using latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Denmark) and serotype specific antisera by the Neufeld test (SSI Diagnostica, Denmark).

9.14.3 Susceptibility testing

Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates, that were found non-susceptible by screening were further analysed for penicillin and erythromycin MICs by broth microdilution using the STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST (Eucast Clinical Breakpoint Tables v.8.0). Both resistant and intermediate susceptible isolates were counted as non-susceptible.

9.14.4 Data handling

Data on susceptibility testing of isolates were stored as zone diameters and/or as MICs in a Microsoft® Access database linked to a SQL server at SSI. Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.15 Invasive beta-haemolytic streptococci (group A, B, C and G streptococci) in humans

9.15.1 Data source

Isolates of beta-haemolytic streptococci from normally sterile sites (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) were submitted to SSI from the departments of clinical microbiology on a voluntary basis.

9.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark).

9.15.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin resistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Denmark) on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with E-test (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Müller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 8.0). Both resistant and intermediate susceptible isolates were categorised as resistant.

9.15.4 Data handling

Data on susceptibility testing of isolates were stored as inhibition zone diameters and if indicated also as MICs in a Microsoft® Access database linked to a SQL server at SSI. Only one isolate from each unique case of invasive beta-haemolytic streptococci were included in the DANMAP report. A new case was defined if there were 30 days or more between consecutive isolates.

9.16 Invasive *Haemophilus influenzae* in humans

9.16.1 Data source

Invasive infections with *Haemophilus influenzae* type b (Hib) is a notifiable disease in Denmark and all invasive isolates nationwide are sent to the reference laboratory at SSI. By tradition, invasive isolates of other serotypes have also been submitted on a voluntary basis, and thus a broader picture of invasive *H. influenzae* in Denmark can be obtained. All cases were identified through MiBa and registered in the surveillance database at SSI. Cases, where isolates were not submitted to the reference laboratory were registered as "unknown serotype".

9.16.2 Microbiological methods

At SSI the isolates were serotyped and bityped. Identification or confirmation of the species *H. influenzae* was based on: visual evaluation of colonies, the satellitism test and biochemical reactions. Serotypes were determined by latex agglutination (ImmuLex™ *H. influenzae*, SSI Diagnostika, Denmark). Biotypes were determined by a series of biochemical reactions.

9.16.3 Susceptibility testing

Susceptibility-testing was not performed at the reference laboratory for the 2018 isolates. The presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 were found through whole-genome sequencing. Data from antimicrobial susceptibility testing at the departments of clinical microbiology were extracted from MiBa.

9.16.4 Data handling

Data on all invasive cases of *H. influenzae* were stored in a Microsoft® Access database linked to a SQL server at SSI. A case was defined as isolation of *H. influenzae* from normally sterile sites (e.g. blood, spinal fluid, pleura). Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.17 *Staphylococcus aureus* including MRSA in humans

9.17.1 Data source

Blood isolates were referred on a voluntary basis by all DCMs to the National reference laboratory for antimicrobial resistance at SSI. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types were sent to the reference laboratory.

9.17.2 Microbiological methods

At SSI, all isolates were initially tested using a multiplex PCR detecting the *spa*, *mecA*, *hsd*, *scn* and *pvl* (LukF-PV) genes [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400]. *Spa* was used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing [Harmsen et al. 2003. J Clin Microbiol. 41: 5442-5448], *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to CC398, respectively. All bacteremia cases and *mecA* negative presumed MRSA were tested for presence of the *mecC* gene.

spa-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the *spa* type and known association with MLST typing, each isolate was assigned to a clonal complex (CC).

9.17.3 Susceptibility testing

Antimicrobial susceptibility testing of *Staphylococcus aureus* was performed by Minimum Inhibitory Concentration (MIC) determination using a custom-made panel (DKSSP2, Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonised monitoring of antimicrobial resistance.

Staphylococcus aureus ATCC 29213 was included as quality control for each batch of resistance determination.

9.17.4 Data handling

For blood isolates a case was defined as a patient with a positive blood culture. Subsequent isolates from the same patient was only included if the positive blood cultures were obtained at least one month apart (new episode).

For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

9.18 Gonococci in humans**9.18.1 Data source**

Isolates of gonococci (*Neisseria gonorrhoeae*) were submitted from the departments of clinical microbiology to SSI on a voluntary basis. The isolates were obtained by culture of specimens from a variety of anatomical locations, e.g. urethra, cervix, rectum, throat, eyes, joint fluid, and blood.

9.18.2 Microbiological methods

The bacteriological identification of the submitted isolates were performed by MALDI-TOF.

9.18.3 Susceptibility testing

For all isolates the MIC was determined with E-test (Bio-mérieux) with azithromycin, ceftriaxon and ciprofloxacin on chocolate agar. The MIC of approximately 110 consecutive isolates was determined with cefixime, gentamicin, and spectinomycin. The breakpoints used were those defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 8.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

The susceptibility data were stored as MIC values in a Microsoft® Access database linked to a SQL server at SSI. Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens were considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.

10

TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined animal daily dose
DaDDD	Danish adjusted defined daily doses
DAPD	Defined animal daily dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1000 inhabitant days)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	Extended spectrum beta-lactamase
GP	General Practitioner
HAI	Hospital-acquired infections
HCAI	Health care associated infections
HACO	Health care associated community onset
HAIBA	Hospital-acquired infections database
HLGR	High-level gentamicin resistance
MiBa	The Danish Microbiology Database
MIC	Minimum inhibitory concentration
MDR	Multidrug-resistant
MRSA	Methicillin-resistant Staphylococcus aureus
OIE	World Organisation for Animal Health
RFCA	Regional Veterinary and Food Control Authorities
SEGES	Knowledge Centre for Agriculture
SSI	Statens Serum Institut
ST	Serotype/Sequence type
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin resistant enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification.

An international classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) (www.whooc.no/atcddd/indexdatabase/). The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whooc.no/atcvet/database/).

Antibacterial agents. Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. In the section on human consumption, 'antibacterial agents' are referred to as 'antimicrobial agents' (see below).

Antimicrobial agents. The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is generally used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only). In the chapter on human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system) including metronidazole and vancomycin, which are used for systemic treatment but registered under the ATC code P01AB01 and A07AA09, respectively.

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.51 kg.

Central Husbandry Register (CHR). This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined daily dose (DDD). This is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that the Defined Daily

Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whooc.no/atcddd/indexdatabase/).

Defined daily dose per 100 admissions (DAD). DAD measures the amount of daily doses consumed per 100 admitted patients at hospitals during a given timeframe (one year). It is used for benchmarking consumption related to the hospital activity and will typically be compared to the consumption measured in DBD (see below). DAD and DBD will generally be applied to comparing individual hospitals and consumption of individual drug classes over time. In DANMAP DAD cover all patients attended to at somatic hospitals only. Admission-days are extracted from the National Patient Registry (Landspatientregistret, LPR). Time of reporting differs between hospitals and closing time for reporting is later than extraction time for the DANMAP report. The current report is the first to update data 10 years back.

Defined animal daily dose (DADD). DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC). The DADD is defined as mg active compound per kg live biomass for each antimicrobial agent, administration route and animal species. In DANMAP 2012, the DADD replaced the ADD (as defined in VetStat and assigned at product level). For more details, see section 9.2, Materials and Methods and the applied DADD's are listed in the web annex.

DADD per 1,000 animals per day (DAPD). Trends in veterinary consumption, both within and across species, are presented in DAPD, which allows for comparison between sectors and adjustment for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight and lifetime. This may also be referred to as the 'standard-animals-at-risk' and takes into account species differences in body-mass and lifespan. The DAPD is a statistical measure, providing an estimate of the proportion of animals (in thousands) treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day (see section 9.2, Materials and Methods).

Defined daily doses per 100 occupied bed-days (DBD).

DBD is the consumption calculated in defined daily doses at hospitals, divided through the number of bed-days. This allows comparison of hospitals related to the length of patient stays. The number of bed-days is extracted from The National Patient Registry (Landspatientregistret, LPR). Time of reporting differs between hospitals and closing time for reporting is later than extraction time for the DANMAP report. The current report is the first to update data retrospectively for 10 years. Every patient admitted to hospital accounts for one bed-day, independent of the actual length of stay within every 24 hours. This corresponds to the actual hours at hospital divided by 24 hours and rounded up to the next whole number. For patients transferred between wards each transfer will count as a new bed day. Non-ended hospital stays are not included.

DDD per 1,000 inhabitants per day (DID). Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, allowing for comparison between sectors and for illustration of the consumption in hospital care without taking hospital activity (discharges and length of stays) into account. Data presented in DID provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DIDs indicate that 1% of the population on average gets a certain treatment daily. In figures presented as DDD/1,000 inhabitant-days.

ESBL. In the DANMAP report, 'ESBL' describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC and OXA-ESBLs [Giske et al. 2009. J. Antimicrob. Chemother. 63: 1-4].

Finishers. Pigs from 30-100 kg live weight from after the weaner stage to time of slaughter.

Fully sensitive. An isolate will be referred to as fully sensitive if susceptible to all antimicrobial agents included in the test panel for the specific bacteria.

Intramammaries. Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Layer. A hen raised to produce eggs for consumption.

Minimum inhibitory concentration (MIC). This is the lowest concentration of antimicrobial agent in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

Multidrug-resistant. A *Salmonella* or *E. coli* isolate is assumed multidrug-resistant if it is resistant to three or more of the main antimicrobial classes as defined in section 9.7, Material and Methods.

Pets or pet animals. Dogs, cats, birds, mice, Guinea pigs and more exotic species kept at home for pleasure, rather than one kept for work or food. Horses are not included as pet animals. The live biomasses of Danish pets used for estimating veterinary consumption only include dogs and cats.

Piglet. The new-born pig is called a piglet from birth till they are permanently separated from the sow at 3-4 weeks of age. The weight of the piglet at weaning is approximately 7 kg.

Poultry. The major production species are fowl *Gallus gallus* (broilers, layers, including breeding and rearing) and turkey. Regarding antimicrobial consumption, 'poultry' also includes domesticated ducks, geese, game birds and pigeons.

Sow. Any breeding female pig on the farm.

Weaner. Any pig of 7-30 kg live weight after it has been weaned (dry diet and water only).



DANMAP 2018

