

DANMAP 2012

DANMAP 2012 - Use of antimicrobial agents and occurrence
of antimicrobial resistance in bacteria from food animals,
food and humans in Denmark



Statens Serum Institut
National Veterinary Institute, Technical University of Denmark
National Food Institute, Technical University of Denmark

DANMAP 2012

Editors:

Helle Korsgaard (hkor@food.dtu.dk)
Birgitte Borck Høg (bibo@food.dtu.dk)
Yvonne Agersø (yvoa@food.dtu.dk)
National Food Institute,
Technical University of Denmark
Mørkhøj Bygade 19, DK - 2860 Søborg

Anette M. Hammerum (ama@ssi.dk)
Line Skjøl-Rasmussen (lbs@ssi.dk)
Microbiology and Infection Control,
Statens Serum Institut
Artillerivej 5, DK - 2300 Copenhagen

Authors:

National Food Institute

Yvonne Agersø, Vibe Dalhoff Andersen, Birgitte Helwich, Birgitte Borck Høg,
Lars Bogø Jensen, Vibeke Frøkjær Jensen, Helle Korsgaard, Lars Stehr Larsen,
Karl Pedersen, Anne Mette Seyfarth

Statens Serum Institut

Tine Dalby, Anette M. Hammerum, Steen Hoffmann, Katrin Gaardbo Kuhn,
Anders Rhod Larsen, Maja Laursen, Eva Møller Nielsen, Stefan S. Olsen,
Andreas Petersen, Line Skjøl-Rasmussen, Robert L. Skov,
Hans-Christian Slotved, Mia Torpdahl

DANMAP board:

National Food Institute

Yvonne Agersø, Flemming Bager

National Veterinary Institute

Sven Erik Jorsal

Statens Serum Institut

Anette M. Hammerum, Robert L. Skov

Layout:

National Food Institute

Susanne Carlsson

Photos: Colourbox and Mikkel Adsbøl

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This report is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of monitoring the antimicrobial use and antimicrobial resistance in food animals, food and humans in 2012. The report is produced in collaboration between the National Food Institute, Technical University of Denmark and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Science, Innovation and Higher Education and the Ministry of Food, Agriculture and Fisheries.

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STATENS
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National Veterinary Institute



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1. Introduction

1.1 About DANMAP

Antimicrobial resistance is considered a major threat to human health. While antimicrobial agents are essential for treating disease in humans and in animals, they are also the main contributors to the selection and spread of antimicrobial resistance. It is, therefore, essential to monitor trends in antimicrobial resistance and consumption of antimicrobial agents, in order to identify the risk factors that contribute to the dissemination of resistance, and the interaction between the risk factors.

Humans and animals constitute over-lapping reservoirs of resistance and an integrated approach that takes this into account is therefore needed. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, has implemented the One Health approach, comprising the entire chain from farm to fork to sickbed, since 1995. The organisation and collection of DANMAP data is presented in Figure 1.1.

DANMAP was established at the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries. The programme participants are the National Food Institute and the National Veterinary Institute, both at the Technical University of Denmark (DTU), as well as Statens Serum Institut (SSI). The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Science, Innovation and Higher Education, and the Ministry of Food, Agriculture and Fisheries.

The objectives of DANMAP are:

- to monitor the consumption of antimicrobial agents in food animals and humans;
- to monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin (e.g. meat) and humans;
- to study associations between antimicrobial consumption and antimicrobial resistance; and
- to identify routes of transmission and areas for further research studies.

The monitoring of antimicrobial resistance is based on three categories of bacteria:

- Human and animal pathogens that cause infections and are thought to reflect resistance caused by the use of antimicrobial agents in the respective reservoirs;
- Zoonotic bacteria that can develop resistance in the animal reservoir, which may subsequently compromise treatment effect when causing infection in humans;
- Indicator bacteria (enterococci and *E. coli*) due to their ubiquitous nature in animals, food and humans, and their ability to readily develop or transfer antimicrobial resistance in response to selective pressure in both reservoirs.

All pathogens may be considered reservoirs of resistance determinants – genes – that may be disseminated independently of the bacterial hosts.

A web annex presenting Minimum inhibitory concentrations (MIC) distributions, detailed tables of antimicrobial consumption and other additional data are available for download at www.danmap.org. Current and previous DANMAP reports are also available at the website (PDF versions).

Public health risks

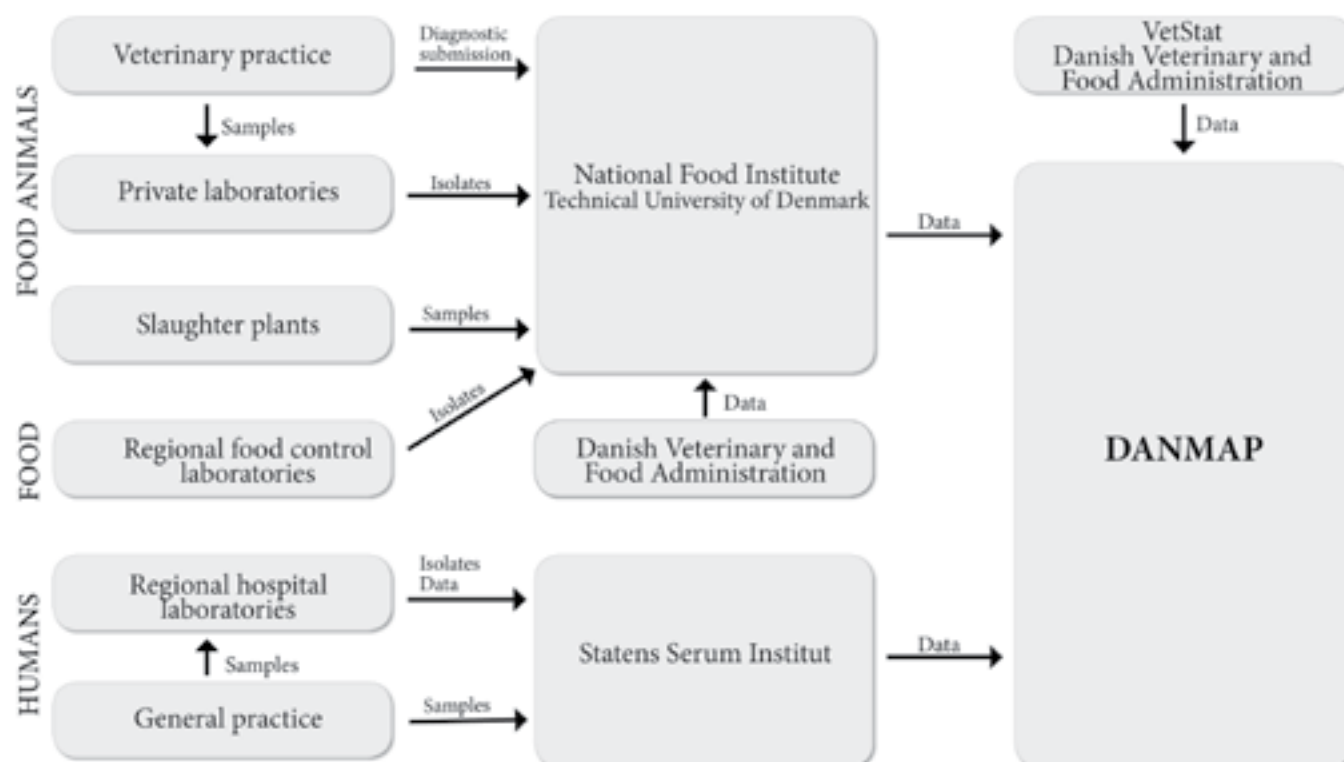
Bacteria become resistant either by spontaneous mutation or by transfer of resistance genes from other bacteria. Resistant strains are favoured when use of antimicrobial agents provide a selective pressure. This occurs in humans as well as in animals undergoing antimicrobial treatment. Resistant bacteria 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, leading to life-threatening illness.

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 the restriction of critical antimicrobial agents for use in humans only, as well as the elimination of over-use, i.e. only humans and animals suffering from an infection responsive to antimicrobial treatment should be exposed to antimicrobial agents.

Figure 1.1. Organisation of DANMAP



1.2 Acknowledgements

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1.3 DANRES

The Danish Study Group for Antimicrobial Resistance Surveillance provides data from the Departments of Clinical Microbiology (DCM) in Denmark.

DCM, Hvidovre Hospital:

Alice Friis-Møller
Jenny Dahl Knudsen
Elly Kristensen
Pia Littauer
Kristian Schönning
Henrik Westh

DCM, Rigshospitalet:

Maria Kristin Björnsdóttir
Michael Tvede

DCM, Herlev Hospital:

Magnus Arpi
Hanne Wiese Hallberg
Tina Larsen

DCM, Hillerød Hospital:

Dennis Schrøder Hansen
Ellen Larsen
Lisbeth Nielsen

DCM, Slagelse Hospital:

Ram Dessau
Ole Heltberg
Bent Røder

DCM, Odense University Hospital:

Bente Gahrn-Hansen
Anette Holm
Thøger Gorm Jensen
Ulrik Stenz Justesen

DCM, Esbjerg Hospital:

Esad Dzajic
Susanne Hartvig Hartzen

DCM, Vejle Hospital:

Jens Kjølseth Møller

DCM Midt-Vest, Herning Hospital:

Ingrid Astrup
Helga Schumacher
Marianne Hedegaard Søndergaard

DCM Midt-Vest, Viborg Hospital:

Berit Have Kallesøe
Birgitte Tønning

DCM, Skejby Hospital:

Svend Ellermann-Eriksen
Lars Erik Lemming
Marianne Bøgild Pedersen
Marianne Kragh Thomsen

DCM, Aalborg Hospital:

Lena Mortensen
Henrik C. Schönheyder



2. Summary

2.1 Sammendrag

DANMAP (Danish Antimicrobial Resistance Monitoring and Research Program) har siden 1995 beskrevet det årlige forbrug af antibiotika og forekomsten af antibiotikaresistens hos dyr og mennesker i Danmark. Denne udgave beskriver udviklingen i 2012.

Antibiotikaforbrug til dyr

Siden 2001 er al anvendelse af receptordineret medicin til dyr registreret i det offentlige register VetStat.

I DANMAP 2012 introducerer vi to nye enheder som beskriver udviklingen i antibiotikaforbruget til dyr; DADD (defined animal daily dose) som er standard dosis pr dyr pr dag og DAPD som angiver DADD pr 1.000 dyr pr dag. DAPD er en statistisk måleenhed, der anslår andelen af en population (i tusinder), som dagligt behandles med en standard dosis antibiotikum. Disse nye enheder bliver primært introduceret for at sikre robusthed i analyserne således, at forbrugsdata er sammenlignelige over tid samt mellem den veterinære og humane sektor.

I 2012 blev der brugt 112,3 tons antibiotika (aktivt stof) til dyr i Danmark. Størstedelen blev anvendt i svineproduktionen (76 %) og en mindre andel i kvæg (11 %), pelsdyr (5 %), fisk (3 %) og fjerkræproduktionen (1 %). De resterende 4 % blev blandt andet brugt til kæledyr og heste. Det totale forbrug (kg aktivt stof) til dyr i 2012 var 4 % højere end i 2011.

De overordnede ændringer i antibiotikaforbruget til dyr er primært styret af ændringer i forbrugsmønstret til svin. Svineproduktionen står for 80 % af den danske kødproduktion, men kun 43 % af den totale levende biomasse. Kvæg – primært malkekøer – står for 40 % af den levende biomasse. I det følgende benytter vi DAPD som enhed, når vi sammenligner antibiotikaforbruget mellem dyrearter, da denne enhed tager højde for forskelle i biomasse og levetid.

Svin: Det totale antibiotikaforbrug til svin i 2012 var på 86 tons aktivt stof. Dette er en stigning på 4 tons i forhold til 2011 og svarer til en stigning på 10 %, når forbruget udregnes i DAPD. Antibiotikaforbruget til svin har været faldende i perioden efter "Gult kort-ordningen" blev bekendtgjort den 1. juli 2010. Ordningen, som har til hensigt at reducere forbruget, er rettet specifikt mod de svinebesætninger, som har det højeste antibiotikaforbrug pr svin. Uagtet stigningen, var forbruget i 2012 16 % lavere end i 2009 og på niveau med forbruget i 2007.

Stigningen i antibiotikaforbrug (DAPD) skyldtes især et øget forbrug af tetracykliner (15 %) og makrolider (19 %), som primært tilsættes til foder eller vand i forbindelse med mave-tarm lidelser. Visse typer antibiotika – især kinoloner og cefalosporiner – er kritisk vigtige i behandling af sygdom hos mennesker. I svin var forbruget af 3. og 4. generations cefalosporiner meget lavt (1 kg), hvilket er et resultat af, at svinebranchen i 2010 frivilligt indførte stop for brugen af disse stoffer. Brugen af fluorkinoloner var på det samme lave niveau (7 kg), som det har været siden 2003, hvor lovgivning om begrænsning af fluorkinolonforbruget trådte i kraft.

Kvæg: Det totale antibiotikaforbrug til kvæg har ligget relativt stabilt på ca. 14 tons aktivt stof om året siden 2005. Forbruget af beta-laktamase følsomme penicilliner udgjorde som tidligere år størstedelen af forbruget.

Forbruget af 3. og 4. generations cefalosporiner til systemisk behandling af malkekvæg steg med 5 % til 47 kg i 2012, mens forbruget til intramammær behandling var 7 kg, et fald på 13 % i forhold til 2011. Forbruget af fluorkinoloner har siden 2003 været stort set nul. Det totale forbrug målt i standard doser (DADD) til intramammær behandling var på samme niveau som i 2011, men det dækker over ændringer i behandlingsmønstret, hvor forbruget til gouldyrbehandling steg med 20 %, hvorimod antallet af DADD til behandling af klinisk mastitis faldt med 9 %.

Fjerkræ: Antibiotikaforbruget til fjerkræ var cirka 809 kg aktivt stof i 2012, hvilket er 5 % lavere end i 2011. Antibiotikaforbruget i den danske fjerkræproduktion, inklusiv avl og opdræt, er generelt meget lavt. Forbruget til slagtekyllinger (2 DAPD) faldt med hele 61 % efter nogle år med højt forbrug som følge af en række sygdomsproblemer i perioden 2008-2011. Bemærk dog, at en del af forklaringen på dette fald kan være en ufuldstændig rapportering af ordinationer til slagtekyllinger i 2012, men omfanget heraf er ikke klarlagt. I æglæggerproduktionen forblev antibiotikaforbruget lavt (0,4 DAPD), mens forbruget til kalkuner steg med 20 % til 21 DAPD. Det rapporterede forbrug af fluorkinoloner har været lavt siden 2006, og har ikke været brugt til slagtekyllinger, æglæggere eller kalkuner i 2012, ligeledes er der ikke rapporteret brug af cefalosporiner til fjerkræ i mere end 10 år.

Fisk: Det totale antibiotikaforbrug til fisk i akvakultur var på 2.900 kg aktivt stof i 2012. Forbruget i ferskvandsdambrug steg med 7 % til 11 DAPD, mens forbruget i havbrug steg med 28 % til 29 DAPD. Antibiotikaforbruget til fisk er meget afhængig af vandtemperaturen, og den relativt kolde danske sommer i 2012, og især i 2011, har medført et lavere forbrug end de foregående år. Desuden har udbredt brug af vaccine i den marine produktion siden 2006 begrænset brugen af antibiotika.

Kæledyr: Oplysningerne i VetStat vedrørende antibiotikaforbrug til kæledyr er mindre detaljerede end oplysningerne vedrørende produktionsdyr. Derfor har vi i 2012 kun estimeret forbruget af antibiotika til oral behandling af kæledyr (primært katte og hunde), hvilket udgjorde ca. 12 DAPD. I 2011 blev det estimeret, at den orale behandling udgjorde cirka 70 % af det totale forbrug i kæledyr. Den stigning, som er set i det orale antibiotikaforbrug til kæledyr siden 2005, fortsatte ikke i 2012 til trods for, at forbruget af 'kombinationspenicillinet' amoxicillin i kombination med clavulansyre fortsatte med at stige. Det orale forbrug af fluorkinoloner steg med 7 % til 0,6 DAPD. Forbruget af 3. og 4. generations cefalosporiner til kæledyr var på 0,7 DAPD, et niveau som er højere end for nogen af produktionsdyrene.

Forbrug af bredspektrede antibiotika, som er kritisk vigtige til behandling af alvorlige infektionssygdomme hos mennesker, er højt i kæledyr sammenlignet med produktionsdyr og mennesker og giver stadig anledning til øget bevågenhed.

Det totale forbrug til svin målt i kg aktivt stof steg med 5 % i 2012, hvilket svarer til en 10 % stigning i DAPD.

Brugen af kritisk vigtige antibiotika i den danske svineproduktion er fortsat lav. Brugen af især bredspektret antibiotika til kæledyr er derimod høj sammenlignet med forbruget til produktionsdyr, og stigningen i forbruget af disse stoffer til kæledyr fortsatte i 2012.

Antibiotikaforbrug til mennesker

Forbruget af receptordineret medicin på patientniveau er blevet overvåget siden begyndelsen af 1990'erne.

Totalforbrug: I 2012 faldt det totale forbrug af antibiotika til systemisk brug (primærsektoren og hospitalssektoren sammenlagt) til mennesker med 2 % (18,90 DDD pr 1000 indbyggere pr dag (DID) i 2011 sammenholdt med 18,48 DID i 2012). Primærsektoren udgjorde 90 % af forbruget. Fra 2003 til 2012 er det totale forbrug af antibiotika i Danmark steget med 3,44 DID (23 %).

I denne DANMAP rapport beskriver vi for første gang forbruget af antibiotika samt incidensen af multiresistente bakterier i Grønland og på Færøerne (**Textbox 1 og Textbox 2**).

Primærsektor: Det totale antibiotikaforbrug i primærsektoren faldt (3 %) sammenlignet med 2011 (17,06 DID i 2011 og 16,47 DID i 2012). Dog steg forbruget for nogle grupper af antibiotika. De mest udtalte stigninger fra 2011–2012 blev observeret for 'kombinationspenicilliner' (0,16 DID) og tetracykliner (0,12 DID).

I **Textbox 5** beskrives forbruget af tetracykliner til henholdsvis unge og den samlede befolkning. Det viste sig, at størstedelen af tetracykliner i stor udstrækning bruges af unge, og mange af recepterne på tetracykliner var til behandling af hudlidelser som f.eks. akne.

Som i de tidligere år udgjorde beta-laktamase følsomme penicilliner den største gruppe af antibiotika (29 %), efterfulgt af penicilliner med udvidet spektrum (21 %) og makrolider (14 %). Penicilliner udgjorde 65 % af det totale forbrug i praksis i 2012. Forbruget af bredspektrede antibiotika steg med 0,3 DID (4 %) i forhold til 2011.

I det seneste årti er forbruget af antibiotika i primærsektoren steget med 22 %, fra 13,53 DID i 2003 til 16,47 DID i 2012. I 2012 udgjorde bredspektrede antibiotika 42 % (6,85 DID) af det samlede antibiotikaforbrug i primærsektoren, hvilket er en stigning på 72 % sammenlignet med 2003 (3,98 DID, 29 %). Denne stigning skyldes sandsynligvis til dels et øget antal DDD pr behandlet patient (definerede dagsdoser) og et øget antal DDD pr udskrevet medicinpakning. Sidstnævnte kan afspejle ændrede retningslinjer for behandling til kortere behandlingstider med højere doser.

På den Europæiske Antibiotikadag i november 2012 lanceredes en borgerrettet hjemmeside (www.antibiotikaellerej.dk) om anvendelse af antibiotika (**Textbox 4**).

Hospitaller: På somatiske hospitaler steg det totale antibiotikaforbrug opgjort i DDD pr 100 sengedage (DBD) med 3 % (2,28 DBD) fra 2011 til 2012.

Fra 2011 til 2012 steg forbruget af 'kombinationspenicilliner' (3,49 DBD, 41 %), beta-laktamase resistente penicilliner (1,07 DBD, 15 %) og beta-laktamase sensitive penicilliner (0,81 DBD, 9 %), mens der sås et fald i forbruget af 2. generations cefalosporiner (1,99 DBD, 12 %), 3. generations cefalosporiner (0,32 DBD, 23 %), carbapenemer (0,30 DBD, 7 %) og fluorkinoloner (0,68 DBD, 6 %). Ændringerne var i overensstemmelse med den nationale vejledning om ordination af antibiotika udgivet af Sundhedsstyrelsen, som er beskrevet i **Textbox 3**.

Fra 2003–2012 steg det totale antibiotikaforbrug med 39,4 DBD (73 %). Denne stigning skyldtes en kombination af stigning i DDD og et fald i antallet af sengedage. I løbet af det seneste årti er forbruget af bredspektret antibiotika på somatiske hospitaler steget med 133 %, fra 27,11 DBD i 2003 til 63,14 DBD i 2012.

I 2012 faldt det totale forbrug af antibiotika til systemisk behandling af mennesker (primær- og hospitalssektoren sammenlagt) (2 %) sammenlignet med 2011. Forbruget i primærsektoren udgjorde 90 % af det totale forbrug, mens forbruget på hospitalerne udgjorde de resterende 10 %. Over en 10-årig periode fra 2003 til 2012 steg det totale forbrug af antibiotika til mennesker i Danmark med 23 %. Generelt steg antibiotikaforbruget i hospitalssektoren med 3 % fra 2011 til 2012, mens forbruget af 2. og 3. generations cefalosporiner, fluorkinoloner og carbapenemer faldt, hvilket stemmer overens med den vejledning om ordination af antibiotika, som Sundhedsstyrelsen udstedte i november 2012.

Resistens i zoonotiske bakterier

Zoonotiske bakterier som *Salmonella* og *Campylobacter* er sygdomsfremkaldende bakterier, som kan overføres fra dyr til mennesker. Udvikles der resistens i disse bakterier i husdyrproduktionen, kan resistens overføres til mennesker via fødevarer og kan i visse tilfælde medføre behandlingssvigt ved sygdom.

Salmonella Typhimurium er en af de mest almindeligt forekommende serotyper i danske svin, dansk svineköd og i humane *Salmonella* infektioner. Blandt *S. Typhimurium* fra svin var 65–67 % af isolaterne resistente overfor ampicillin, streptomycin, sulfonamid og tetracyklin, hvilket er en stigning i forhold til 2011. Dette kan primært tilskrives den stigende forekomst af monofasiske varianter, som ofte er multiresistente. I 2012 fandt vi, at 56 % af *S. Typhimurium* isolaterne fra svin var monofasiske. Vi fandt også høj forekomst af resistens blandt *S. Typhimurium* isolater fra dansk svineköd. Generelt fandt vi højere forekomst af multiresistens blandt *S. Typhimurium* isolater fra danske svin (66 %) og svineköd (59 %), end i *Salmonella* generelt (*Salmonella* spp.), hvor hhv.

34 % og 32 % var multiresistente. Der blev ikke påvist resistens overfor cefalosporiner (cefotiofur og cefotaxim) eller kinoloner (ciprofloxacin og nalidixansyre) blandt *Salmonella* isolaterne fra svin eller dansk svinekød.

Ligesom for svin og svinekød steg den relative forekomst af de monofasiske *S. Typhimurium* varianter blandt isolater fra patienter, som havde erhvervet infektionen i Danmark (både sporadiske og udbrudstilfælde). Siden 2008 har forekomsten af multiresistens været stigende, og i 2012 blev der påvist multiresistens i 62 % af *S. Typhimurium* isolater fra sporadiske tilfælde erhvervet i Danmark. Blandt disse tilfælde steg andelen af isolater med resistens overfor fluorkinoloner (ciprofloxacin) og amfenikoler (fluorfenikol) også.

Resistensforekomsten var højere for 7 ud af de 16 stoffer, som indgik i testpanelet, når man sammenligner isolater fra patienter med rejse-relaterede infektioner og isolater fra sporadiske infektioner erhvervet i Danmark. Det gælder også for fluorkinoloner, som bruges til behandling af patienter med alvorlige mave-tarm infektioner forårsaget af både *Salmonella* og *Campylobacter*.

Forekomsten af multiresistente *S. Typhimurium* er steget i både svin, svinekød og blandt humane infektioner. Dette kan hovedsageligt forklares med en øget forekomst af monofasiske *S. Typhimurium*, som har tendens til at være multiresistente. Tilsvarende er der set en stigning i niveauet af resistens overfor fluorkinoloner, som bruges til behandling af alvorlige mave-tarm infektioner. Resistensforekomsten for 7 ud af de 16 stoffer, som indgik i testpanelet, var højere blandt de rejserelaterede tilfælde end blandt de hjemligt erhvervede infektioner, heriblandt for fluorkinoloner.

Resistensforekomsten i *Campylobacter jejuni* isolater fra kyllinger og kvæg og i *Campylobacter coli* isolater fra svin var på samme niveau som i 2011. Det bør bemærkes, at den stigende forekomst af fluorkinolon (ciprofloxacin) og tetracyklin-resistens, som er observeret i *C. jejuni* fra slagtekyllinger de seneste ti år, ikke fortsatte i 2012. Dette har muligvis en sammenhæng med, at brugen af tetracyklin til slagtekyllinger faldt i 2012.

Siden 2007 har vi set en stigende forekomst af tetracyklin-resistens i *C. coli* fra svin, og i 2012 var 15 % af isolaterne tetracyklin resistente. Dette stemmer overens med et generelt stigende forbrug af tetracyklin til svin i perioden.

Set i en europæisk sammenhæng er resistensforekomsten i *C. jejuni* fra danske slagtekyllinger og kyllingekød samt *C. coli* fra svin blandt Europas laveste. Fluorkinolon resistens i *C. jejuni* var også højere blandt isolater fra importeret kyllingekød (46 %) end fra dansk kyllingekød (29 %).

Som i de foregående år var forekomsten af fluorkinolon resistens i *C. jejuni* isolater fra patienter med rejse-relaterede infektioner (80 %) højere end i isolater fra patienter, hvor infektionen var erhvervet i Danmark (35 %). Både blandt patienter med rejse-relaterede og hjemligt erhvervede infektioner er der sket et fald i resistens overfor tetracyklin.

Fluorkinolon resistens i *C. jejuni* er fortsat højere blandt isolater fra importeret kyllingekød sammenlignet med dansk kyllingekød, og blandt *C. jejuni* fra patienter med rejse-relaterede infektioner i forhold til patienter, hvor infektionen var erhvervet i Danmark.

Clostridium difficile forårsager tarminfektioner hos mennesker og har forårsaget hospitalsudbrud både i Danmark og i udlandet [DANMAP 2009], hvorfor forekomsten af *C. difficile* i kvæg, svin og kød blev undersøgt. Fra 2010 til 2011 faldt forekomsten af *C. difficile* i svinebesætninger, hvilket muligvis kan forklares ved det kraftigt reducerede forbrug af cefalosporiner til svin i denne periode (til næsten nul) samt i nogen grad et generelt fald i forbruget til svin. Forekomsten af *C. difficile* var generelt lavt i kød (0 % – 7 %) og de mest virulente typer, som kan producere binært toksin, blev slet ikke fundet i kød, selv om de blev observeret i både kvæg og svin. Den højeste forekomst fandt vi i kyllingekød (7 %), men hvorvidt dette skyldes slagteprocessen, som adskiller sig meget fra slagteprocesserne for kvæg og svin, eller forskelle i forekomsten i de forskellige dyrearter kræver nærmere undersøgelse. Selvom der ikke blev påvist nogle af de mest virulente typer i prøverne fra kød, kan typer observeret med *tcdA* og *tcdB* potentielt forårsage infektioner hos mennesker, så den zoonotiske vigtighed af disse typer bør undersøges yderligere (Textbox 7).

Resistens i indikatorbakterier

Indikatorbakterier er inkluderet i DANMAP overvågningen for at kunne give et indblik i den generelle forekomst af resistens i raske husdyr og i kød.

Blandt *Enterococcus faecium* fra slagtekyllinger fandt vi høje forekomster af salinomycin-resistens (71 %). Salinomycin er et coccidiostika, som bruges i kyllingeproduktionen, men det bruges ikke til human behandling, hvorfor salinomycin-resistens i sig selv ikke udgør en sundhedsrisiko for mennesker. Derimod kan kryds-resistens til andre antibiotika være af betydning, da 27 % af de salinomycin resistente isolater også var resistente overfor andre antibiotika, især erythromycin, som tilhører gruppen af antibiotika, som anses for kritisk vigtige i den humane behandling. I *E. faecalis* fra slagtekyllinger steg tetracyklin resistens til 43 %, selv om tetracyklinforbruget til slagtekyllingerne faldt i perioden.

Tetracyklin resistens blandt *E. faecium* og *E. faecalis* fra svin var høj (hhv. 62 % og 87 %), og tetracykliner har været de mest benyttede antibiotika i den danske svineproduktion i mange år. Erythromycin resistens i *E. faecium* fra svin har været faldende siden 2007 og var i 2012 24 %. I *E. faecalis* har erythromycin resistens derimod været stigende og nåede i 2012 op på 56 %. Disse modsatte tendenser kunne ikke forklares ud fra de tilgængelige data.

I lighed med de foregående år var resistensforekomsten af flere testede antibiotika højere i både *E. faecium* og *E. faecalis* fra importeret kyllingekød end fra dansk produceret kyllingekød.

Resistensforekomsten i *E. faecium* og *E. faecalis* fra dansk og importeret svinekød var på samme niveau, på nær for tetracyklin i *E. faecalis*, som var højere i importeret svinekød.

Set i et 'One Health' perspektiv er der en direkte sammenhæng mellem antibiotikaforbruget i kyllingeproduktionen og forekomsten af antibiotikaresistente enterokokker i det danske kyllingekød. Derimod finder vi, at enterokokkerne fra svin er betragtelig mere resistente end isolaterne fra det danske svinekød. Mulige forklaringer kan være, at nogle af de mere følsomme typer er bedre til at overleve slagteprocessen end de resistente typer, og/eller at kødet krydskontamineres på slagteriet eller i opskæringsvirksomheden.

Vi fandt et højt niveau af resistens mod salinomycin – et coccidiostatika - blandt enterokok isolater fra kyllinger. Flertallet af disse isolater var fuldt følsomme for de fleste andre antibiotika i testpanelet, men det skal bemærkes, at 22 % af de salinomycin resistente isolater også var resistente for andre antibiotika, særligt erythromycin (18 %).

Indikator *Escherichia coli* fra slagtekyllinger var ofte resistente overfor sulfonamid (21 %) og ampicillin (20 %), som typisk bruges til slagtekyllinger. Vi påviste fluorkinolon (ciprofloxacin) resistens i 8 % af isolaterne, og resistens overfor 3. generations cefalosporiner (ceftiofur) blev påvist i to isolater fra slagtekyllinger. Resistensforekomsten i *E. coli* fra slagtekyllinger og dansk kyllingekød var sammenlignelig. Resistensforekomsten i isolater fra kvæg og dansk oksekød var som foregåede år ganske lav. Resistens i *E. coli* fra svin var den højeste blandt produktionsdyrene og forblev på samme høje niveau som i 2011, på nær et mindre fald i spectinomycin-resistens.

Blandt isolaterne fra kød havde isolaterne fra importeret kyllingekød generelt de højeste resistensforekomster – også når det gjaldt de kritisk vigtige antibiotika. Sammenlignet med dansk kyllingekød havde *E. coli* isolater fra importeret kyllingekød højere resistens overfor 13 af de 16 testede antibiotika. For svinekød havde *E. coli* isolater fra importeret kød højere resistensforekomst overfor ampicillin, chloramfenicol, ciprofloxacin og tetracyklin end isolaterne fra dansk svinekød.

ESBL-producerende bakterier er et af de hurtigst voksende resistensproblemer verden over. Flere nyere studier finder de samme ESBL gener, plasmider og kloner af *E. coli* isolater i både dyr og i mennesker med infektioner, hvilket tyder på et zoonotisk link. Forekomsten af *Enterobacteriaceae* bakterier, der er resistente overfor carbapenemer, er også en voksende trussel, idet carbapenemer er sidste mulighed for antibiotikabehandling af mennesker med infektioner, der skyldes multiresistente Gram-negative bakterier.

Otte procent af slagtesvinene havde ESBL-producerende *E. coli*, hvilket var højere end i 2011, men lavere end i 2010. Den højeste prævalens af ESBL-producerende *E. coli* i kødprøver blev fundet i importeret kyllingekød (61 %), hvilket også udgør en stigning i forhold til 2011. Forekomsten af ESBL-producerende *E. coli* i det importerede kyllingekød var også højere end i det danske kyllingekød, hvor vi fandt ESBL-producerende *E. coli* i 36 % af prøverne, hvilket er samme niveau som i 2011. I de andre kødtyper var forekomsten lav (0–2 %). ESBL-producerende *E. coli* fra produktionsdyr og kød fra 2011 og 2012 blev testet for carbapenem resistens, og ingen af de testede isolater var resistente overfor carbapenemer. Derfor er der ikke noget der

tyder på at kød eller produktionsdyr i Danmark er kilde til carbapenemase gener i human kliniske bakterier (**Textbox 8**).

Indikator *E. coli* fra svin havde de højeste resistensforekomster blandt isolater fra produktionsdyr. I kød påviste vi de højeste resistensforekomster i isolater fra importeret svine- og kyllingekød. Forekomsten af ESBL-producerende *E. coli* i svin steg, men var stadig under niveauet i 2010. Kyllingekød anses stadig for at være den vigtigste kilde til ESBL-producerende *E. coli*. Der blev ikke påvist carbapenem-resistente *E. coli* fra de undersøgte produktionsdyr eller kødprøver.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Rapporteringen af antibiotikaresistens i bakterier fra diagnostisk indsendelser fra mennesker er baseret på frivillig indsendelse af data fra DANRES-gruppen, som dækker de Klinisk Mikrobiologiske Afdelinger (KMA) i Danmark. Undtagelser omfatter methicillin-resistente *Staphylococcus aureus* (MRSA) og invasive *Streptococcus pneumoniae*, som er anmeldeligt. Data vedr. disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt ***Escherichia coli* isolater fra blod** var forekomsten af 3. generations cefalosporin resistens 7 % i 2012, hvilket er det samme niveau som i 2011, men højere end i de andre nordiske lande i 2011. Resistens for 3. generations cefalosporiner er kun rapporteret siden 2008; i denne periode er resistensen steget. Ciprofloxacin resistens var 14 % i 2012, dette var det samme niveau som i 2011. I løbet af det seneste årti er resistens for cefuroxim, ciprofloxacin og gentamicin steget. Ampicillin resistens faldt fra 48 % i 2011 til 45 % i 2012.

Blandt ***E. coli* isolater fra urin fra patienter på hospital** faldt forekomsten af ciprofloxacin resistens fra 13 % i 2011 til 12 % i 2012, hvilket er et brud med den stigende tendens set siden 2003, hvor 2 % af isolaterne var ciprofloxacin resistente. Aminoglykosid (gentamicin) resistens steg fra 4 % i 2011 til 5 % i 2012. Sulfonamid resistens faldt fra 35 % i 2011 til 33 % i 2012.

Blandt ***E. coli* isolater fra urin fra patienter i almen praksis** steg forekomsten af ciprofloxacin resistens støt fra 2 % i 2003 til 10 % i 2012. Sulfonamid resistens faldt fra 35 % i 2011 til 33 % i 2012, og ampicillin resistens faldt fra 41 % i 2011 til 40 % i 2012.

I 2012 havde 2.511 patienter en urinvejsinfektion med en 3. generations cefalosporin resistent *E. coli*. Blandt *E. coli* urin-isolater fra patienter på hospital steg forekomsten af både 2. og 3. generations cefalosporin resistens fra 5 % i 2011 til 6 % i 2012, og blandt *E. coli* urin-isolater fra patienter i praksis steg forekomsten af både 2. og 3. generations cefalosporin resistens fra 3 % i 2011 til 4 % i 2012. Den stigende forekomst af 3. generations cefalosporin resistens blandt *E. coli* urin-isolater er beskrevet i **Textbox 9**.

Carbapenem (meropenem) resistens blev observeret i *E. coli* blod-isolater (n = 1) og urin-isolater fra både hospitaler (n = 6) og praksis (n = 9). Blandt disse isolater blev identificeret den første NDM-4 producerende *E. coli* i Danmark samt to

VIM-4 producerende *E. coli*. Forekomsten af carbapenemase-producerende bakterier i Danmark i 2012 er beskrevet i **Textbox 11**. Forekomsten af carbapenem resistens er ikke anmeldeligt.

Blandt *Klebsiella pneumoniae* isolater fra blod var forekomsten af 3. generations cefalosporin resistens (9 %) og aminoglykosid (gentamicin, 6 %) resistens på samme niveau som i 2011, mens ciprofloxacin resistens faldt fra 12 % i 2011 til 9 % i 2012. Niveauet var højere end i de andre nordiske lande og på samme niveau som i flere europæiske lande i 2011.

Blandt *K. pneumoniae* isolater fra urin var forekomsten af 3. generations cefalosporin resistens 8 % i isolater fra hospitaler og 5 % i isolater fra praksis, hvilket er samme niveau som i 2011. Ciprofloxacin resistens faldt fra 11 % i 2011 til 9 % i 2012 i urin-isolater fra hospitaler; i urin-isolater fra praksis var ciprofloxacin resistens 8 %, hvilket er samme niveau som i 2011. Mecillinam resistens faldt fra 12 % i 2011 til 11 % i 2012 i isolater fra praksis; i urin-isolater fra hospitaler var mecillinam resistens også 11 %, hvilket er samme niveau som i 2011. Sulfonamid resistens faldt fra 33 % i 2011 til 24 % i 2012 i urin-isolater fra hospitaler og fra 35 % i 2011 til 26 % i 2012 i urin-isolater fra praksis.

Carbapenem (meropenem) resistens blev observeret i *K. pneumoniae* blod-isolater (n = 2) og urin-isolater fra både hospitaler (n = 3) og praksis (n = 2). Forekomsten af carbapenemase-producerende bakterier i Danmark i 2012 er beskrevet i **Textbox 11**.

I perioden januar–maj 2012 blev der udført en national undersøgelse af *Bacteroides fragilis* gruppe isolater fra bloddyrkninger fra 11 af landets 13 KMA'er. I alt blev der inkluderet 118 blod-isolater fra *B. fragilis* gruppen. Ingen af isolaterne var resistente for metronidazol. Der sås en høj frekvens af nedsat følsomhed for piperacillin-tazobactam og clindamycin i *B. thetaiotaomicron* isolater og for meropenem i *B. fragilis* isolater (**Textbox 10**).

Blandt *Pseudomonas aeruginosa* blod-isolater var niveauet af resistens for alle testede antibiotika ikke signifikant forskelligt fra niveauet i 2011, men der sås en stigende tendens for gentamicin resistens i perioden 2007–2012 og for meropenem resistens i perioden 2007–2011. Meropenem resistens blev observeret i 4 % af *P. aeruginosa* blod-isolater i 2012. I løbet af 2012 blev der identificeret fire VIM-producerende *P. aeruginosa* isolater på SSI (**Textbox 11**).

I 2012 var forekomsten af resistens for penicillin og erythromycin stadig lav blandt *Streptococcus pneumoniae* og gruppe A, B, C og G streptokokker.

Forekomsten af ampicillin resistens i *Enterococcus faecium* isolater fra blod var 94 % i 2012, hvilket er samme niveau som i 2011. Vancomycin resistens var 1,8 % i *E. faecium* og 0,2 % i *E. faecalis* blod-isolater. I 2012 rapporterede to KMA'er udbrud med vancomycin resistente *E. faecium* (*vanA*). To andre KMA'er testede enterokokker fra blodinfektioner for højniveau gentamicin resistens (HLGR); 27 % af de testede *E. faecalis* isolater og 62 % af de testede *E. faecium* isolater var HLGR.

I løbet af det seneste årti er ciprofloxacinresistens i *Neisseria gonorrhoeae* steget støt fra 30 % i 2003 til 75 % i 2009, efterfulgt af et fald til 57 % i 2012. Penicillinase produktion blandt gonococcus isolater svingede mellem 12–24 % fra 2003–2012, og der blev ikke rapporteret hverken ceftriaxon resistente isolater eller tilfælde af ceftriaxon behandlingssvigt i denne periode. Fra 2009–2012 faldt azithromycin resistens fra 46 % til 12 %, cefixim resistens svingede mellem 11–21 %, og der blev ikke detekteret spectinomycin resistente isolater (**Textbox 12**).

I 2012 blev der indrapporteret 1.528 tilfælde af *Staphylococcus aureus* bakteræmi svarende til en incidens på 27,4 tilfælde pr 100.000 indbyggere. Heraf var antallet af methicillin-resistente *S. aureus* (MRSA) 19 (1,2 %), hvilket er på samme niveau som tidligere år og blandt de laveste incidenser observeret i Europa. Den højeste resistensforekomst udover penicillin resistens var resistens for fusidinsyre (14 %), erythromycin (6 %), clindamycin (6 %) og norfloxacin (4 %). Niveauet af resistens for de testede antibiotika var det samme som i 2011.

Antallet af nye MRSA tilfælde (både koloniserede og inficerede personer) steg i 2012 til 1.556 sammenlignet med 1.292 i 2011. Den stigning, der startede i 2010, fortsatte således i 2012. Stigningen blev primært set i tilfælde kategoriseret som samfundserhvervede, 596 i 2011 vs. 726 i 2012. Andelen af patienter med infektion var på samme niveau i 2012 som i 2011 (54 % vs. 53 %). Antallet af hospitalserhvervede tilfælde var fortsat lavt og udgjorde kun 4 % af det totale antal MRSA tilfælde i 2012.

Landbrugs-associerede MRSA (LA-MRSA) tilhører primært klonal kompleks CC398. Antallet af MRSA påvisninger hos mennesker tilhørende CC398, som er associeret med svin, steg fra 164 i 2011 til 232 i 2012. Den mest almindelige *spa* type relateret til CC398 var type t034 (n = 185), hvoraf 75 af t034-tilfældene havde en egentlig MRSA infektion. MRSA isolater med den nye *mecA* homolog *mecC* blev fundet i 24 tilfælde (9 i 2009, 21 i 2010 og 37 i 2011).

Svin ved slagtning og tankmælksprøver fra mælkeproducenter blev testet for MRSA. Prævalensen af MRSA hos svin ved slagtning var 77 %, hvilket er signifikant højere end tidligere år (13 % i 2009 og 44 % i 2011, **Textbox 13**). Som i de tidligere år var t034 og t011 de mest almindelige *spa* typer. Der blev for første gang påvist MRSA i tankmælk. I alt 4 prøver var positive (2 %), og alle isolater var af MRSA *mecA* typen med *spa* typer korresponderede til CC398 og CC1, typer der tidligere er fundet hos svin. MRSA med *mecC* typen blev ikke fundet blandt svin eller i tankmælk.

I 2012 steg det totale antal af nye MRSA tilfælde til 1,556. Stigningen sås primært i tilfælde kategoriseret som samfundserhvervede. Antallet af hospitalserhvervede tilfælde var fortsat lavt og udgjorde kun 4 % af det totale antal MRSA tilfælde i 2012. Antallet af MRSA af typen CC398, som er associeret med svin, steg fra 164 i 2011 til 232 i 2012. CC398 er nu den anden oftest forekommende CC gruppe blandt humane MRSA cases. Antallet af MRSA isolater, der indeholder *mecC* hos mennesker, ser ud til at være stabiliseret.

Prævalensen af MRSA hos svin ved slagting steg kraftigt, men hvorvidt flere besætninger er MRSA positive vides ikke. Svin er stadig det vigtigste reservoir for MRSA CC398, men LA-MRSA CC398 blev også påvist i tankmælk, hvilket kan skyldes en spredning fra svineproduktionen.

I 2012 havde 2,511 patienter en urinvejsinfektion med en 3. generations cefalosporin resistent *E. coli*. I *E. coli* urin-isolater fra patienter på hospital steg resistens for 3. generations cefalosporiner til 6 % i 2012, og i *E. coli* urin-isolater fra patienter i praksis steg resistens for 3. generations cefalosporiner til 4 % i 2012.

Der blev observeret høj frekvens af nedsat følsomhed for piperacillin-tazobactam og clindamycin i isolater fra *Bacteroides fragilis* gruppen.

Der blev identificeret fire nye tilfælde af carbapenemase-producerende *Enterobacteriaceae* (CPE) og fire carbapenemase-producerende (VIM) *P. aeruginosa* i 2012. Endvidere sås et udbrud med carbapenemase-producerende (OXA-23) *A. baumannii* i 2012.

I 2012 rapporterede to KMA'er udbrud med vancomycin resistente *E. faecium* (*vanA*).

2.2 Summary

DANMAP (Danish Antimicrobial Resistance Monitoring and Research Program) has monitored antimicrobial resistance and consumption of antimicrobial agents in food animals and in humans in Denmark since 1995. This report describes changes and trends in 2012.

Antimicrobial consumption in animals

Data on all medicines prescribed by veterinarians have been registered at the farm and species level by the official VetStat programme since 2001.

In DANMAP 2012, we have introduced two new metrics to follow trends in antimicrobial consumption – defined animal daily doses (DADD) and DADD per 1,000 animals per day (DAPD). DAPD is a statistical measure, providing an estimate of the proportion of animals (in thousands) treated daily with a particular antimicrobial agent. These new metrics have been introduced primarily to ensure the robustness of the analyses over time and to facilitate comparisons between animal species, as well as comparisons between the veterinary and human sectors.

The total consumption of antimicrobial agents in 2012 amounted to 112.3 tonnes of active compounds, a 4% increase compared with 2011. Pigs accounted for 76%, cattle for approximately 11%, fur animals for 5%, aquaculture for 3%, and poultry for 1% of the total veterinary consumption of antimicrobials measured in kg active compounds. The remaining 4% was used in companion animals and others.

The overall changes in veterinary consumption are generally driven by changes in consumption in pig production. Pigs account for approximately 80% of the meat production in Denmark, but only 43% of the total live biomass, while adult cattle – mainly dairy cows – constitute another 40% of the total live biomass.

Pigs: The total consumption of veterinary antimicrobial agents in Danish pig production was 86 tonnes. This was an increase of 4 tonnes (5%) compared with 2011, corresponding to a 10% increase when measured in DAPD. This follows a decrease in 2010–2011, which was probably the result of the introduction of legislation to reduce overuse of antimicrobials in the pig production. Even though antimicrobial consumption increased in 2012, it remained at a level 16% lower than in 2009, and similar to the 2007 level.

In 2012, the increase in consumption (DAPD) was attributed mainly to an increase in prescriptions of tetracyclines (15%) and macrolides (19%) that are mostly used in feed or water medication for gastrointestinal disease. The use of 3rd and 4th generation cephalosporins in pigs remained close to zero (1 kg), as a result of a voluntary ban on cephalosporins introduced by the Danish pig industry in 2010. The use of fluoroquinolones remained at the same low level (7 kg) as has been observed since legal restrictions were enforced in 2003.

Cattle: Overall, the antimicrobial consumption in cattle has remained stable at around 14 tonnes since 2005, and beta-lactamase sensitive penicillins account for the majority of the consumption. For critically important antimicrobials, the use of fluoroquinolones has been close to zero since 2003. In

contrast, use of 3rd and 4th generation cephalosporins for systemic treatment increased by 5% to 47 kg, whereas the intramammary use decreased by 13% to 7 kg. The overall use of antimicrobials for intramammary treatment was unchanged, however, use of drying-off treatment increased by 20%, while therapeutic treatment of mastitis decreased by 9%.

Poultry: In 2012, the overall consumption of antimicrobial agents in poultry was approximately 809 kg active compound, which represents a 5% decrease compared with 2011. The usage of antimicrobial agents in the broiler and layer production, including rearing and breeding, is generally very low. In 2012, the consumption in broilers (2 DAPD) decreased by 61%, following an increased consumption due to disease problems in 2008–2011. A part of this decrease may be explained by incomplete reporting of use in broilers during 2012, although the magnitude of underreporting is uncertain. In layers, the consumption remained very low (0.4 DAPD), whereas the antimicrobial consumption in turkeys increased by 20% (21 DAPD). The reported use of fluoroquinolones in poultry has been low since 2006, and they were not used in the production of broilers, layers or turkeys in 2012. Furthermore, use of cephalosporins has not been reported in Danish poultry production for more than a decade.

Aquaculture: The overall antimicrobial consumption in aquaculture was 2,900 kg in 2012. The consumption in fresh water fish increased by 7% to 11 DAPD, while the consumption in marine aquaculture increased by 28% to 29 DAPD, assuming an unchanged production. However, the consumption in marine aquaculture 2011–2012 was very low compared to previous years probably due to cold summers in 2011–2012 and effects of improved vaccination schemes since 2006.

Pet animals: The information available on antimicrobial consumption in pet animals is not as detailed as for production animals. Therefore, we only estimated the consumption of antimicrobial agents used for oral treatment in pet animals (mainly cats and dogs), which amounted to 12 DAPD in 2012. In 2011, we estimated oral use to account for about 70% of the total consumption in pet animals. The increasing trend in oral treatment of pet animals observed since 2005 did not continue, however the use of the broad-spectrum combination penicillins (amoxicillin with clavulanic acid) continued to increase. The oral use of fluoroquinolones increased by 7% to 0.6 DAPD in 2012. The consumption of 3rd and 4th generation cephalosporins in pet companion animals was 0.7 DAPD, which is higher than for any production animal species.

The consumption in pet animals of broad-spectrum antimicrobials and the use of antimicrobial agents critical for treatment of human infections is high compared with both production animals and humans and are still a matter of concern.

The total consumption (kg active compound) in pigs increased by 5.4% in 2012 corresponding to a 10% increase in DAPD.

Use of critically important antimicrobials in the pig production remains low. Use of critically important and broad-spectrum antimicrobial agents in pet animals is high compared with other species, and continued to increase in 2012.

Antimicrobial consumption in humans

The use of prescription medicines at the level of the individual patient has been monitored since the early 1990s.

Total consumption: In 2012, the total consumption of antimicrobial agents for systemic use (primary health care and hospital care) decreased by 2% (from 18.90 DDD per 1,000 inhabitants per day (DID) in 2011 compared to 18.48 DID in 2012). Primary health care contributed with 90% of the overall consumption. Since 2003, the total consumption of antimicrobial agents in humans in Denmark has increased by 3.44 DID (23%).

For the first time, we report consumption of antimicrobial agents and incidence of multi-resistant bacteria in Greenland and Faroe Islands (**Textbox 1** and **Textbox 2**).

Primary health care: In 2012, the total consumption of antimicrobial agents for systemic use in primary health care decreased slightly by 3% compared with 2011 (from 17.06 DID in 2011 to 16.47 DID in 2012). However, consumption of some classes of antimicrobial agents increased. The most pronounced increases from 2011–2012 were for ‘combination penicillins’ (0.16 DID) and tetracyclines (0.12 DID).

In **Textbox 5**, the consumption of tetracyclines from 2008–2011 in young adults and the population as a whole is described. We found that tetracyclines are used to a great extent among adolescents, and many of the prescriptions with tetracyclines were given for skin disorders such as acne.

As in previous years, beta-lactamase sensitive penicillins represented the largest therapeutic group of antimicrobial agents consumed in 2012 (29%), followed by penicillins with extended spectrum (21%) and macrolides (14%). Penicillins accounted for 65% of the total consumption in 2012. Consumption of broad-spectrum agents increased by 0.3 DID (4%) compared with 2011.

During the past decade, antimicrobial consumption in primary health care has increased by 22%, from 13.53 DID in 2003 to 16.47 DID in 2012. In 2012, broad-spectrum agents accounted for 42% (6.85 DID) of the total antimicrobial consumption which, compared with 2003 (3.98 DID, 29%), represents an increase of 72%. This increase is most likely driven by rises in DDDs per treated patient and number of DDDs per prescribed package. The latter may reflect a change in guidelines advising shorter treatment regimens at higher dosages.

A citizen-centered website on how and when to use antimicrobial agents (www.antibiotikaellerej.dk) was launched on the European Antibiotic Awareness Day in November 2012 (**Textbox 4**).

Hospitals: In somatic hospitals, the consumption of antimicrobial agents expressed in DDDs per 100 occupied bed-days (DBD) increased by 2.28 DBD (3%) from 2011 to 2012.

From 2011 to 2012, consumption increased for ‘combination penicillins’ (3.49 DBD, 41%), beta-lactamase resistant penicillins (1.07 DBD, 15%), and beta lactamase sensitive penicillins (0.81 DBD, 9%). In contrast, consumption decreased for 2nd generation cephalosporins (1.99 DBD, 12%), 3rd generation cephalosporins (0.32 DBD, 23%), carbapenems (0.30 DBD,

7%), and fluoroquinolones (0.68 DBD, 6%). The changes were in agreement with the national guidelines on prescribing antibiotics from the Danish Health and Medicines Authority described in **Textbox 3**.

From 2003–2012, the consumption of antimicrobial agents by humans in hospitals increased by 39.4 DBD (73%). This increase was due to a combination of increased DDDs and a decreased number of hospital bed-days. During the past decade, the consumption of broad-spectrum antimicrobial agents in somatic hospitals has increased by 133%, from 27.11 DBD in 2003 to 63.14 DBD in 2012.

In humans, the overall consumption in 2012 of antimicrobial agents for systemic use decreased (2%) compared with 2011. Antimicrobial consumption in the primary health care sector represented 90% of the total consumption and the hospital sector accounted for the remaining 10%. From 2003 to 2012, the total consumption of antimicrobial agents by humans in Denmark increased by 23%. In the hospital sector, the consumption of antimicrobial agents increased by 3% from 2011 to 2012, however a decreased consumption was observed for 2nd and 3rd generation cephalosporins, fluoroquinolones and carbapenems, which is in agreement with the national guidelines on prescribing antibiotics issued by the Danish Health and Medicines Authority in November 2012.

Resistance in zoonotic bacteria

Zoonotic bacteria such as *Salmonella* and *Campylobacter* can develop resistance in the animal reservoir. The resistant bacteria may be transferred to humans via food and may subsequently compromise treatment effect when causing infection in humans.

***Salmonella* Typhimurium** is one of the most common serovars in Danish pigs and pork. Among *S. Typhimurium* from pigs, 65–67% of the isolates were resistant to ampicillin, streptomycin, sulfonamide, and tetracycline; an increase compared with 2011. This can mainly be attributed to an increasing prevalence of monophasic *S. Typhimurium* that have a strong tendency to be multi-resistant. In 2012, 56% of the *S. Typhimurium* isolates from pigs were of the monophasic variants. We also found high levels of resistance in the tested *S. Typhimurium* isolates from pork. Generally, we found higher levels of multi-resistance among *S. Typhimurium* isolates from Danish pigs (66%) and pork (59%) compared with other *Salmonella* spp. isolates, where 34% and 32% were multi-resistant, respectively. Notably, no resistance to cephalosporins (ceftiofur or cefotaxim) or quinolones (ciprofloxacin or nalidixic acid) was observed among *Salmonella* from Danish pigs or pork.

As in isolates from pigs and pork, the occurrence of monophasic variants of *S. Typhimurium* in humans increased in prevalence among both domestic sporadic cases and outbreaks. This was also reflected in an increase in multi-resistance that has been seen since 2008. In 2012, multi-resistant isolates were recovered from 62% of the domestic sporadic cases. In *S. Typhimurium*

from human domestic cases, resistance to fluoroquinolones (ciprofloxacin) and amphenicols (florfenicol) also increased.

Resistance to 7 of the 16 tested compounds was higher among travel-associated cases than domestic human cases, including resistance to fluoroquinolones, which are used for empiric treatment of adults with severe bacterial gastroenteritis caused by both *Salmonella* and *Campylobacter*.

The level of multi-resistant *S. Typhimurium* increased in pigs, pork and humans. This can mainly be attributed to an increasing prevalence of monophasic *S. Typhimurium* that have a strong tendency to be multi-resistant. In human sporadic cases, resistance to fluoroquinolones, which is used for empiric treatment of adults with severe bacterial gastroenteritis, also increased. Resistance to 7 of the 16 tested compounds was higher among travel-associated cases than domestic human cases, including resistance to fluoroquinolones.

Resistance among *Campylobacter jejuni* isolates from Danish broilers and cattle and *Campylobacter coli* from Danish pigs remained at the same levels as in 2011. It is however noteworthy, that the increasing trend in fluoroquinolone (ciprofloxacin) and tetracycline resistance, which has been observed in *C. jejuni* from broilers for almost a decade, did not continue in 2012. This corresponds to the decrease in consumption of tetracycline in the broiler production.

We have seen an increasing trend in tetracycline resistance in *C. coli* from pigs since 2007, and in 2012, 15% of the isolates were tetracycline resistant. This trend generally matches the growth in consumption of tetracycline in the pig production over the past years.

In a European context, Denmark reports the lowest levels of antimicrobial resistance among *C. jejuni* from broilers and broiler meat and for *C. coli* isolates obtained from pigs.

For several years, the level of fluoroquinolone resistance in *C. jejuni* has been higher among isolates from imported broiler meat (46%), compared with isolates from Danish broiler meat (29%).

In 2012, the *C. jejuni* isolates from the travel-associated cases continued to have a significantly higher level of fluoroquinolone resistance (80%) compared with domestic cases (35%). It is worth noting that the level of tetracycline resistance decreased in *C. jejuni* isolates from both domestic cases and cases associated with travel.

The level of fluoroquinolone (ciprofloxacin) resistance in *C. jejuni* is still higher among isolates from imported broiler meat compared with isolates from Danish broiler meat, and among *C. jejuni* from travel-associated cases compared with domestic cases.

Clostridium difficile is causing intestinal infections in humans and has caused outbreaks in hospitals in Denmark and in other countries [DANMAP 2009]. Therefore, the occurrence of *C. difficile* in cattle, pigs and meat was investigated. The occurrence of *C. difficile* decreased in pig farms from 2010 to 2011, and this may be explained by a reduction close to zero of cephalosporin consumption and to some extent reduction in the total consumption in pigs during the same period. The prevalence of *C. difficile* was generally low in meat (0%-7%) and none of the most virulent types containing the binary toxin were observed in the meat, although present in cattle and pigs. The highest prevalence was observed in the broiler meat (7%), but whether this was due to differences in the slaughter processes for broilers compared to pigs and cattle or differences in occurrence in the animals requires further investigation. Although none of the most virulent types were detected in the meat samples, the types observed with *tcdA* and *tcdB* may contribute to human infections, so the zoonotic importance of these types should be further investigated (Textbox 7).

Resistance in indicator bacteria

Indicator bacteria, **enterococci** and *Escherichia coli*, are included in the DANMAP programme to provide information about the general levels of resistance in healthy production animals and meat.

In *Enterococcus faecium* from Danish broilers, we observed high levels of salinomycin resistance (71%). Salinomycin is not used for treating humans, so salinomycin resistance in itself does not pose a public health problem. However, co-resistance with other antimicrobial agents can be of importance, and in 2012, 27% of the salinomycin-resistant isolates were also resistant to other antimicrobial agents, especially erythromycin, which belongs to the group of antimicrobial agents considered of critical importance in human medicine. In *Enterococcus faecalis* from broilers, resistance to tetracycline increased to 43%, in contrast to the reduced usage of tetracyclines in broilers.

Among *E. faecium* and *E. faecalis* from pigs, the highest occurrence of resistance was to tetracycline (62% and 87%, respectively). Tetracycline has been the most widely used antimicrobial agent in the Danish pig production for a decade. Erythromycin resistance in *E. faecium* from pigs was 24% and has been declining since 2007. However, in *E. faecalis*, resistance has increased, reaching 56% in 2012. These contrasting results could not be explained from the data available.

Among isolates from broiler meat, the highest level of resistance to several compounds was observed in imported broiler meat, similar to previous years. In general, resistance to antimicrobial agents of critical importance for human treatment was low, but fluoroquinolone (ciprofloxacin) resistance was observed in two *E. faecalis* isolates from imported broiler meat. Occurrences of resistance were similar among *E. faecium* and *E. faecalis* from Danish and imported pork, except for a higher level of tetracycline resistance among *E. faecalis* from imported pork.

A high level of resistance to the coccidiostat salinomycin was observed in enterococci from broilers. Although most of these isolates were susceptible to all other compounds, 22% of the salinomycin-resistant isolates were also resistant to other antimicrobial agents, especially erythromycin (18%).

In a One Health perspective, the usage of antimicrobial agents in the broiler production can be directly linked to the prevalence of antimicrobial resistance in the Danish broiler meat. But Danish pork enterococcal isolates were in general more susceptible when compared with isolates from pigs, possibly due to better survival of more susceptible subtypes or due to cross contamination in the meat processing chain.

Indicator *Escherichia coli* from broilers were most often resistant to sulfonamide (21%) and ampicillin (20%), which can be explained by the usage pattern. Resistance to fluoroquinolones was observed in 8% of the isolates and ceftiofur (3rd generation cephalosporin) resistance was observed in two *E. coli* isolates from broilers. Resistance in isolates from Danish broiler meat reflected the findings in the broilers. Resistance in isolates from cattle and beef was generally low. The highest occurrence among production animals was observed in pigs, and resistance was at the same level as in 2011 except for a decrease in resistance to spectinomycin. In isolates from meat, the highest occurrence of resistance, including resistance to critically important antimicrobials, was found in imported broiler meat. Compared with Danish broiler meat, we found higher levels of resistance for 13 of 16 tested antimicrobial agents from imported broiler meat. For *E. coli* from pork of domestic origin, resistance to ampicillin, chloramphenicol, ciprofloxacin and tetracycline was significantly lower than among isolates from imported pork.

Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide. Lately, several studies have found the same ESBL genes, plasmids and clones of *E. coli* isolates originating from animals and isolates involved in human infections, suggesting a zoonotic link. The occurrence of *Enterobacteriaceae* resistant to carbapenems is a growing threat in human medicine because carbapenems are the last line of defence for treatment of infections caused by multidrug resistant Gram-negative bacteria in humans.

Eight percent of pigs at slaughter had ESBL-producing *E. coli*, which was higher than in 2011, but lower than in 2010. From meat samples, the highest occurrence of ESBL-producing *E. coli* was found among imported broiler meat (61%); an increase compared with 2011. The occurrence of ESBL-producing *E. coli* was higher in imported broiler meat when compared with Danish broiler meat. The occurrence of ESBL-producing *E. coli* in Danish broiler meat (36%) was at the same level as in 2011. In pork and beef, occurrence was generally low (0%–2%). ESBL-producing *E. coli* obtained from production animals and meat in 2011 and 2012 was tested for **carbapenem resistance**, and none of the tested isolates were resistant to carbapenems. So at present, we have no indication that meat or food-producing animals in Denmark represent a source of carbapenemase genes found in human clinical bacteria (Textbox 8).

The highest level of resistance in *E. coli* from production animals was observed in pigs. In meat, the high level of resistance was observed in imported pork and imported broiler meat. Occurrence of ESBL-producing *E. coli* in pigs increased in 2012, but was still significantly lower than in 2010. Broiler meat still seems to be the most important meat source for ESBL-producing *E. coli*. No carbapenem resistant *E. coli* were detected from production animals and meat.

Resistance in human clinical bacteria

Data on antimicrobial resistance in bacteria from diagnostic submissions from human patients were gathered by voluntary reporting from the DANRES group which covers the Departments of Clinical Microbiology (DCM) in Denmark. Exceptions were methicillin-resistant *Staphylococcus aureus* (MRSA) and invasive *Streptococcus pneumoniae* that are notifiable. Data on these bacteria were obtained from the reference laboratories at SSI.

In *Escherichia coli* blood isolates, resistance to 3rd generation cephalosporins was 7% in 2012, the same level as reported in 2011, but above the 2011 level in the other Nordic countries. Resistance to 3rd generation cephalosporins has only been reported since 2008; in this period the resistance has increased. In 2012, ciprofloxacin resistance was 14%, the same level as in 2011. Over the last decade, resistance to cefuroxime, ciprofloxacin and gentamicin has increased. Ampicillin resistance decreased from 48% in 2011 to 45% in 2012.

In *E. coli* urine isolates from hospital patients, ciprofloxacin resistance decreased from 13% in 2011 to 12% in 2012, which is a change to the steady increase seen in ciprofloxacin resistance from 2% in 2003. Aminoglycoside (gentamicin) resistance increased from 4% in 2011 to 5% in 2012. Sulfonamide resistance decreased from 35% in 2011 to 33% in 2012.

In *E. coli* urine isolates from primary health care, ciprofloxacin resistance has increased steadily from 2% in 2003 to 10% in 2012. Sulfonamide resistance decreased from 35% in 2011 to 33% in 2012, and ampicillin resistance decreased from 41% in 2011 to 40% in 2012.

In 2012, 2,511 patients had a urinary tract infection with 3rd generation cephalosporin resistant *E. coli*. In *E. coli* urine isolates from hospital patients, resistance to both 2nd and 3rd generation cephalosporins increased from 5% in 2011 to 6% in 2012, and in *E. coli* urine isolates from primary health care, resistance to both 2nd and 3rd generation cephalosporins increased from 3% in 2011 to 4% in 2012. The increased occurrence of 3rd generation cephalosporin resistance among *E. coli* urine isolates is described in **Textbox 9**.

Carbapenem (meropenem) resistance was observed in *E. coli* blood ($n = 1$) and urine isolates from both hospitals ($n = 6$) and primary health care ($n = 9$). Among these, the first NDM-4 producing *E. coli* in Denmark was identified as well as two VIM-4 producing *E. coli*. The occurrence of carbapenemase-producing bacteria in Denmark in 2012 is described in **Textbox 11**. The occurrence of carbapenem resistance is not mandatory reportable.

In *Klebsiella pneumoniae* blood isolates, resistance to 3rd generation cephalosporin (9%), and aminoglycoside (gentamicin 6%) was the same level as reported in 2011, whereas ciprofloxacin resistance decreased from 12% in 2011 to 9% in 2012. The level was above the level reported by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2011.

In *K. pneumoniae* urine isolates, 3rd generation cephalosporin resistance was 8% in isolates from hospitals and 5% in isolates from primary health care, the same level as in 2011. Ciprofloxacin resistance decreased from 11% in 2011 to 9% in 2012 in urine isolates from hospitals; in urine isolates from primary health care, ciprofloxacin resistance was 8%, the same level as in 2011. Mecillinam resistance decreased from 12% in 2011 to 11% in 2012 in isolates from primary health care; in urine isolates from hospitals, mecillinam resistance was also 11%, the same level as in 2011. Sulfonamide resistance decreased from 33% in 2011 to 24% in 2012 in urine isolates from hospitals and from 35% in 2011 to 26% in 2012 in isolates from primary health care.

Carbapenem (meropenem) resistance was observed in *K. pneumoniae* blood ($n = 2$) and urine isolates from both hospitals ($n = 3$) and primary health care ($n = 2$). The occurrence of carbapenemase-producing bacteria in Denmark in 2012 is described in **Textbox 11**.

A national study of *Bacteroides fragilis* group isolates from blood cultures at 11 of the 13 DCM was performed January–May 2012. In total, 118 blood isolates were included from the *B. fragilis* group. None of the isolates were resistant to metronidazole. High rates of reduced susceptibility towards piperacillin-tazobactam and clindamycin were seen in *B. thetaiotaomicron* and towards meropenem in *B. fragilis* (**Textbox 10**).

In *Pseudomonas aeruginosa* blood isolates, resistance to all the tested antimicrobial agents was not significantly different from the level in 2011, but an increasing trend has been observed for gentamicin resistance during 2007–2012 and for meropenem resistance during 2007–2011. Meropenem resistance was observed in 4% of the *P. aeruginosa* blood isolates in 2012. During 2012, four VIM-producing *P. aeruginosa* isolates were identified at SSI (**Textbox 11**).

Resistance to penicillin and erythromycin in *Streptococcus pneumoniae* and in group A, B, C and G streptococci remained low in 2012.

In 2012, resistance to ampicillin was 94% in *Enterococcus faecium* isolates from blood, the same level as in 2011. Vancomycin resistance was 1.8% in *E. faecium* and 0.2% in *Enterococcus faecalis* blood isolates. During 2012, two DCM reported outbreaks with vancomycin resistant *E. faecium* (vanA). Two other DCM tested all enterococci from bloodstream infections for high-level gentamicin resistance (HLGR); 27% of the *E. faecalis* isolates and 62% of *E. faecium* isolates were HLGR.

Through the last decade, ciprofloxacin resistance in *Neisseria gonorrhoeae* increased steadily from 30% in 2003 to 75% in 2009, followed by a decrease to 57% in 2012. Penicillinase production among gonococcus isolates fluctuated between 12%–24% from 2003–2012, and no ceftriaxone-resistant isolates, or cases of

ceftriaxone treatment failure, were reported in this period. From 2009–2012, azithromycin resistance decreased from 46% to 12%, cefixime resistance fluctuated between 11%–21%, and no spectinomycin resistant isolates were detected (**Textbox 12**).

In 2012, 1,528 cases of *Staphylococcus aureus* bacteraemia were reported, corresponding to 27.4 cases per 100,000 citizens. Of these cases the number of methicillin-resistant *S. aureus* (MRSA) was 19 (1.2%), a level similar to previous years and among the lowest incidences recorded in Europe. The highest frequency of resistance in addition to penicillin was observed for fusidic acid (14%), erythromycin (6%), clindamycin (6%) and norfloxacin (4%). Susceptibility to the tested antimicrobial agents was at the same level as in 2011.

The number of new cases of MRSA (both infected and colonized persons) increased in 2012 to 1,556 compared with 1,292 in 2011. Thus, the increase starting in 2010 continued into 2012. The increase was primarily seen in cases categorised as community-acquired (CA), 596 in 2011 vs. 726 in 2012. The proportion of cases presenting with infection was similar in 2012 to 2011 (54% vs. 53%). The number of hospital-acquired (HA) cases continued to be low and constituted only 4% of the total number of MRSA cases in 2012.

Livestock associated MRSA primarily belong to clonal complex 398 (CC398). The number of MRSA belonging to CC398, which is associated with pigs, increased in humans from 164 in 2011 to 232 in 2012. The most frequent *spa* type related to CC398 was type t034 (n = 185). Seventy-five t034 cases represented infections. MRSA isolates carrying the new *mecA* homologue *mecC* were demonstrated in 24 cases (9 in 2009, 21 in 2010, and 37 in 2011).

Pigs at slaughter and bulk milk samples from dairy farms were tested for MRSA. The prevalence of MRSA in pigs at slaughter was estimated to be 77% which was higher than in previous years (13% in 2009 and 44% in 2011) (**Textbox 13**). As in previous years, *spa* types t034 and t011 were the most common types. For the first time, MRSA was detected in bulk milk from dairy farms. The four (2%) MRSA isolates found were of the *mecA* type with *spa* types corresponding to CC398 and CC1, types that have previously been detected in pigs. MRSA isolates of *mecC* type were not found among pigs or bulk milk.

In 2012, the total number of new human MRSA cases increased to 1,556. This increase was primarily seen in community-acquired cases. The number of hospital-acquired cases remained low and accounted for only 4% of the total number of MRSA cases in 2012. The number of MRSA type CC398, which is associated with pigs, increased from 164 cases in 2011 to 232 in 2012. CC398 now constitutes the second most common CC group among human MRSA cases. The number of MRSA isolates in humans carrying the *mecC* gene seems to have stabilised.

The prevalence of MRSA in pigs at slaughter increased dramatically, but whether more herds are MRSA positive compared to previous years is unknown. Pigs still seem to be the most important reservoir for MRSA CC398, but detection of LA-MRSA CC398 in bulk milk depicts a spread possibly from the pig production.

In 2012, 2,511 patients had a urinary tract infection with 3rd generation cephalosporin resistant *E. coli*. In *E. coli* urine isolates from hospital patients, resistance to 3rd generation cephalosporins increased to 6% in 2012, and in *E. coli* urine isolates from primary health care, resistance to 3rd generation cephalosporins increased to 4% in 2012.

High rates of reduced susceptibility towards piperacillin-tazobactam and clindamycin in isolates from the *Bacteroides fragilis* group were observed.

Four new cases of carbapenemase producing *Enterobacteriaceae* (CPE) and four carbapenemase producing (VIM) *P. aeruginosa* isolates were identified in 2012. Furthermore, an outbreak of carbapenemase producing (OXA-23) *A. baumannii* was seen in 2012.

During 2012, two DCM reported outbreaks with vancomycin resistant *E. faecium* (*vanA*).



3. Background information

The following sections present general information about the human population in Denmark in 2012, and the production of food animals and the amount of meat available for human consumption in Denmark, over the past decade. It also provides an overview of the antimicrobial agents for systemic and intramammary therapeutic use in humans and animals in 2012.

3.1 Populations

The distribution of the Danish human population, which could potentially have received antimicrobial treatment in 2012, is displayed in Figure 3.1, together with the 5 healthcare regions and the 13 Departments of Clinical Microbiology (DCM).

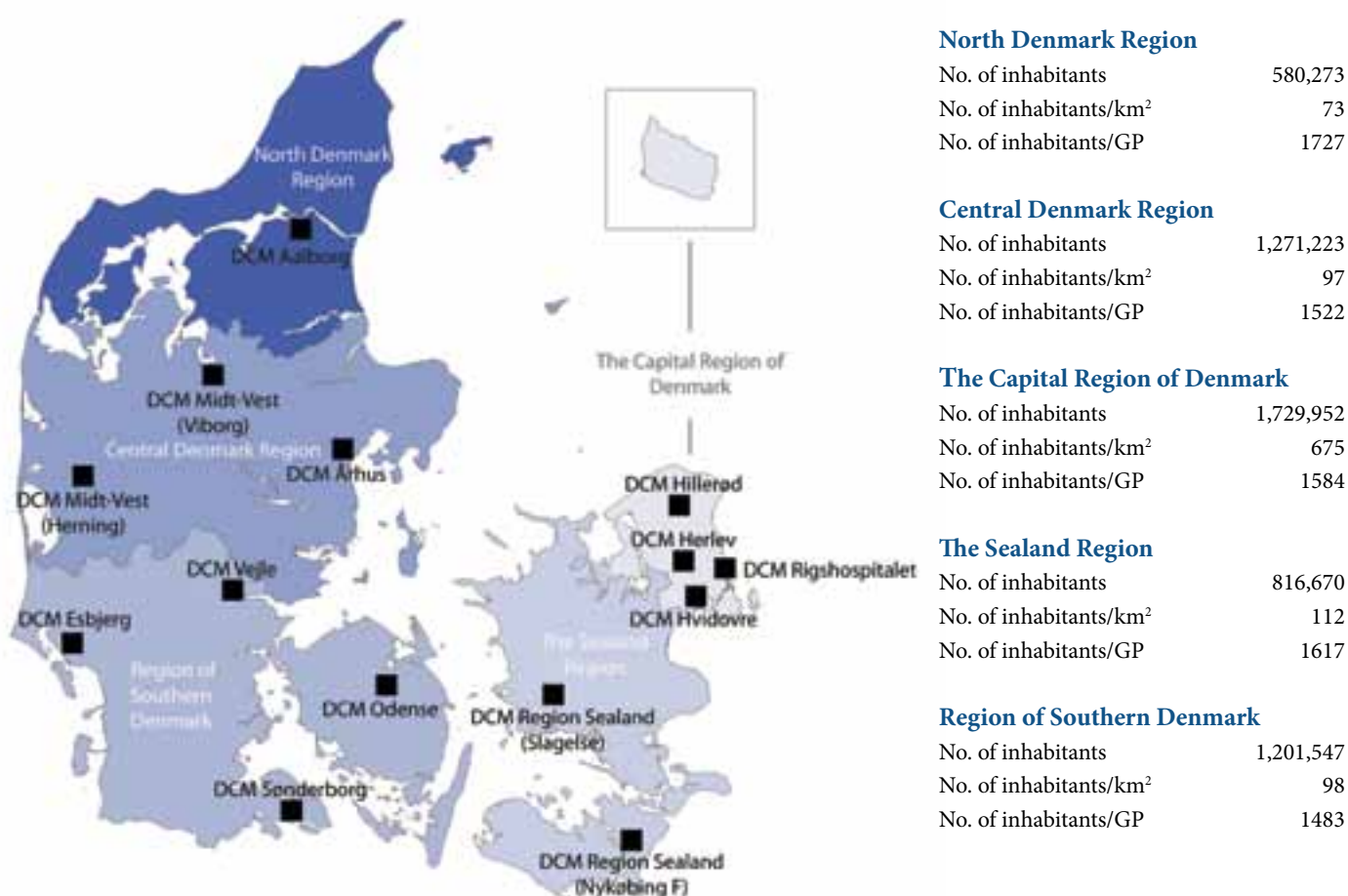
In 2012, the number of slaughtered cattle and pigs was 1%–2% lower than in 2011; however, the volumes (kg) of beef and pork produced were reduced by 5% (Table 3.1). The number of fattening pigs (15–50 kg) exported increased by 15%, and the export has increased by five-fold since 2004. As in the previous years, an increase in the number of dairy cows (1%) and milk produced (3%) occurred.

There was a 4% reduction in the Danish broiler production from 2011 to 2012 (Table 3.1). The export of live broilers for slaughter has increased markedly; from 0.1% in 2003 to 10% of the broilers produced in 2012. The annual production of turkeys has fluctuated considerably over the last decade. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus all turkey meat available in Denmark is listed as imported.

The amounts of meat available for consumption in Denmark during 2008–2012 are presented in Table 3.2. The amount of domestically produced meat available for consumption in Denmark is estimated as production minus export, and includes chilled and frozen fresh meat as well as natural-marinated broiler meat. Approximately 3.1 million tonnes of pork and 1.7 million tonnes of beef meat were available for consumption in Denmark during 2012, where 72% of the pork and 48% of the beef was of Danish origin. Statistics on export of broiler meat was incomplete, for 2011 and 2012, thus, the correct amount of Danish broiler meat available for consumption in Denmark could not be accurately calculated, however import of broiler meat continued to increase.

Figure 3.1 The five health care regions and 13 Departments of Clinical Microbiology (DCM) of Denmark

DANMAP 2012



Source: Statistics Denmark (www.dst.dk) and the Danish Medical Association (www.laeger.dk) GP=general practitioner

Table 3.1. Production of food animals and the production of meat and milk, Denmark

DANMAP 2012

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish ^(a)	
	1,000 heads	mill. kg ^(b)	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	Export 1,000 heads ^(c)	mill. kg	Fresh water mill. kg	Marine mill. kg
2003	129861	197	777	11.2	625	161	596	4540	24434	-	1898	34	8
2004	130674	198	1086	19.6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	183	1237	17.4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	161	785	11.3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	163	1009	14.4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	163	1068	12.3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	165	1175	11.1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	178	1184	14.0	519	142	574	4830	28505	7074	1974	28	10
2011	115454	175	960	9.4	551	145	575	4801	29399	7632	2008	28	11
2012	111080	168	1103	12.4	539	138	580	4928	29047	8794	1902	-	-
Increase ^(d)	-4%	-4%	15%	31%	-2%	-5%	1%	3%	-1%	15%	-5%	-	-

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Production data for farmed fish was not available for 2012. Live animals exported prior to slaughter are included in number of animals and amount of meat produced. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg live pigs from Danish Agriculture and Food

a) The production of farmed fish includes fish transferred from one production facility to another. In 2011, this included 4 tonnes transferred between freshwater facilities (14% of the freshwater production), and 2.9 tonnes transferred from freshwater to marine facilities, corresponding to 25% of the marine aquaculture production

b) Assume a final slaughtered weight of 1.51 kg per broiler produced (Danish Agriculture and Food, 2013)

c) Export of 15-50 kg live pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside of Denmark

d) Increase from 2011 to 2012

Table 3.2. Danish and imported chilled and frozen fresh meat available for consumption (mill kg), Denmark

DANMAP 2012

	Pork		Beef		Broiler meat ^(a)		Turkey meat
	Danish	Import	Danish	Import	Danish	Import	Import
2008	216	83	83	81	48	32	8
2009	187	83	84	89	51	30	7
2010	185	90	97	101	56	44	9
2011	105	85	85	92	-	51	8
2012	230	88	84	91	-	60	7

Note: Source Danmarks Statistik (www.dst.dk). Data from 2011 and 2012 are extracted on March 25th 2013. Data from 2012 are preliminary and will be updated in the 2013 report. The amount of Danish meat for consumption is estimated as production minus export

a) Natural-marinated broiler meat included. Statistics on export of broiler meat was incomplete for 2011 and 2012, and the amounts of Danish broiler meat available for consumption in Denmark could therefore not be accurately calculated

3.2 Marketed antimicrobial agents

Table 3.3 shows the antimicrobial agents that are registered to treat bacterial infections in humans and animals. Some of these antimicrobial agents are considered critically important for humans by WHO. An antimicrobial agent is considered critically important if it is the only compound, or one of limited available therapy, to treat serious human disease. Critically important antimicrobial agents are also used in food animals and pets to treat veterinary diseases, and bacteria that are resistant to these critically important agents may be transmitted to humans.

Bacteria that cause human disease may acquire resistance genes from bacteria of animal origin. Fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides and glycopeptides are among the antimicrobial agents considered critically important for humans [AGISAR, WHO 2009].

Growth promoters, which are no longer used for animals in Denmark, are shown in parentheses in Table 3.3. Most of the antimicrobial agents used for growth promotion in Denmark had effects on Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance to growth promoters.

3. BACKGROUND INFORMATION

Table 3.3. Antimicrobial agents marketed for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2012

DANMAP 2012

ATC / ATCvet codes ^(a)	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, oxytetracycline, tetracycline, tigecycline
J01BA / 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, incl. beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanate, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	First-generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DD	Third-generation cephalosporins	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, imipenem/cilastatin, doripenem
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	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycin, roxithromycin, clarithromycin, azithromycin
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
J01FG / QJ01XX ^(b)	Streptogramins	(Virginiamycin)	
J01G / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ofloxacin, ciprofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ ^(b)	Quinoxalines	(Carbadox, olaquinox)	
J01XA,A07AA / Not in ATCvet ^(b,c)	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
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
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet ^(b)	Oligosaccharides	(Avilamycin)	
Not in ATCvet ^(b)	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes starts with a Q

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

c) Although intestinal antiinfectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) are used to treat human patients, they are not reported by DANMAP



4. Antimicrobial consumption in animals

4.1 Introduction

The use of antimicrobial agents in humans and animals has been monitored by the DANMAP programme since 1995. Since the early 1990s there has been both political and public focus on the use of antimicrobial agents in the Danish animal production. This has resulted in discontinued use of several antimicrobial agents used for growth promotion from 1994–1999, and recently a voluntary ban of use of cephalosporins in the pig production and regulatory legislation regarding therapeutic use [DANMAP 2010].

Since the end of the 1990s, an increase in the antimicrobial consumption for both humans and animals has been observed. While the increase in consumption for humans has been gradual throughout the period, the consumption in animals has fluctuated notably. The increase in veterinary consumption can partly be explained by the increase in pork production, which constitutes approximately 80% of the meat production in Denmark (Table 3.1). Figure 4.1 shows the total antimicrobial consumption in animals and humans since 1994 and 1997, respectively.

The prescription pattern has been clearly influenced by implemented legislation. For example, a steep decrease in antimicrobial consumption from 1994 to 1995 was likely the result of 1) limitation of veterinarians profit from sales of medicine [Directive (DK) 60/1995], and 2) enforcement of the so called “cascade rule” [Order (DK) 142/1993], which limits the use of (cheaper) extemporaneously produced medicines - this particularly affected the use of tetracyclines from 1994. Another important intervention was the restriction on the use of fluoroquinolones in production animals, through legislation implemented in 2002 and 2003. Furthermore, in July 2010, the pig industry imposed a voluntary ban on use

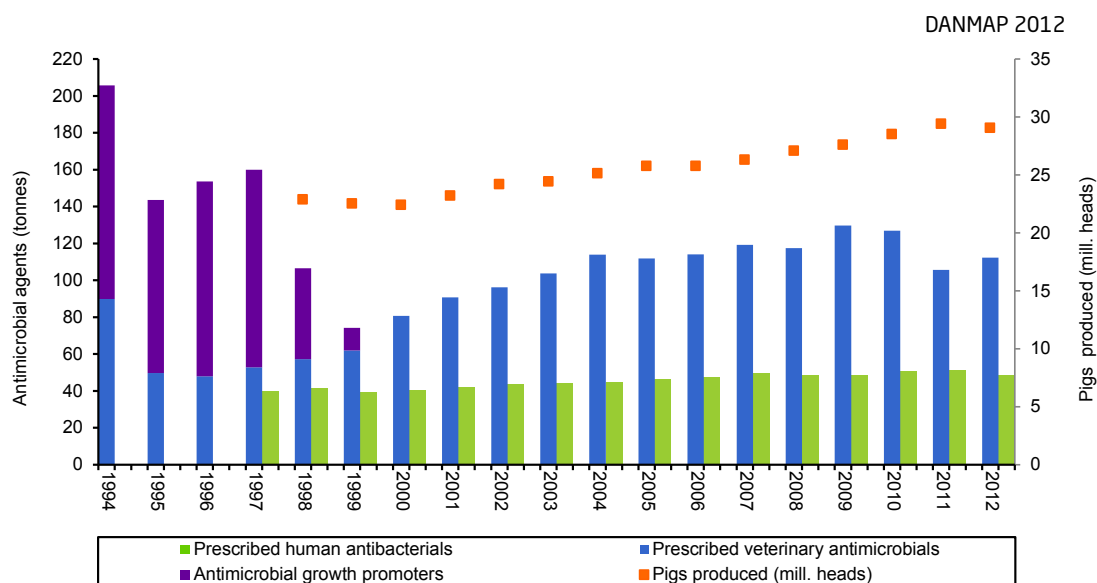
of cephalosporins, due to concerns regarding extended beta-lactamase resistance (ESBL).

From 2010 to 2011, consumption decreased following the introduction of threshold values for antimicrobial consumption adopted within the “yellow card initiative”. This enforces legal actions on pig farmers with high antimicrobial agent use per pig [DANMAP 2010].

Effects from other parts of the legislation may be less obvious, but are important to keep in mind, when interpreting the veterinary prescription patterns.

Official guidelines for the selection of antimicrobial agents that veterinarians may choose from for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for the selection of the appropriate antimicrobial agents for treatment of all common indications in major production animal species. Initially, guidelines were developed by the National Veterinary Serum Laboratory (presently, DTU National Veterinary Institute). Since 2005, the guidelines have been updated by the Danish Veterinary and Food Administration (DVFA) in collaboration with DTU National Veterinary Institute, DTU National Food Institute, the Practising Veterinarians Organization, university experts, the Danish Association of the Veterinary Pharmaceutical Industry and the Danish Agriculture and Food Council. The latest update was in 2010, when new dynamic evidence-based treatment guidelines for pigs were launched [DANMAP 2010, www.fvst.dk]. In 2012, the Danish Veterinary Association published treatment guidelines to promote prudent use of antimicrobials in dogs and cats, prepared by clinical specialists and expert scientists from the Veterinary Faculty at Copenhagen University and DTU National Food Institute.

Figure 4.1. Prescribed antimicrobial agents for humans and for animals compared to the number of pigs produced, Denmark



Sources: Human therapeutics: The Danish Medicines Agency. Veterinary consumption: Until 2001, data are based on reports from the pharmaceutical industry of total annual sales from the Federation of Danish pig producers and slaughterhouses (1994–1995) and Danish Medicines Agency and Danish Plant Directorate (1996–2000). Data from 2001–2011 originate from VetStat

4.1.1 Data sources

Data on antimicrobial use at the product level have been collected in Denmark since 1996, including historical data back to 1990. In Denmark, all therapeutic medicine is prescription-only, and since 2001, data on all medicine prescribed for use in animals has been collected (at prescription level) in a national database (VetStat). Data on consumption of antimicrobial feed additives, including coccidiostatic agents (non-prescription) and antimicrobial growth promoters (no longer used), are also collected by VetStat.

Consumption data for 2012 - for use in DANMAP - were extracted from Vetstat by the Danish Veterinary and Food Administration (DVFA) on 22 March 2013. DTU National Food Institute has carried out no further validation of the received data. This is in contrast to previous years, where efforts were made to determine, for example, target species for antimicrobial agent sold to veterinary practitioners, but where target animals were not reported to VetStat. Therefore, the proportion of consumption in 'species unknown' is larger in this year's report than previous reports, for further details see Chapter 10, Materials and Methods.

4.1.2 Methods

Metrics of antimicrobial consumption are numerous, each with its own advantages and limitations. Therefore, the chosen measures must depend on the purpose and the available information.

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 and to enable international comparisons (Figure 4.1).

In DANMAP 2012, we introduce two new metrics to follow trends in antimicrobial consumption to ensure the robustness of the analyses over time and to facilitate comparisons between animal species, as well as comparisons between the veterinary and human sectors. The new metrics are defined below, and for more information on methodology, please refer to Chapter 10 and the web annex.

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).

In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which has been used since DANMAP 2003. For more details, see Chapter 10, Materials and Methods. The DADDs used in DANMAP 2012 are presented in the web annex.

DAPD (DADD per 1,000 animals per day) - estimated treatment proportion.

Trends in veterinary consumption, both within and across species, are presented in DAPD, allowing for comparison between sectors and adjusting for changes in live biomass. 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', and takes into account species differences in body-mass and life-span.

DAPD or estimated treatment proportion is a statistical measure, providing a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population (e.g. broiler population), on average, receives a certain treatment on a given day (Section 4.3 and Chapter 10, Materials and Methods). Furthermore, presenting the veterinary consumption in DAPD allows comparisons to the antimicrobial consumption in the human sector as expressed in defined daily dose per 1,000 inhabitants per day (DID), see Chapter 11, Terminology for a description of DID.

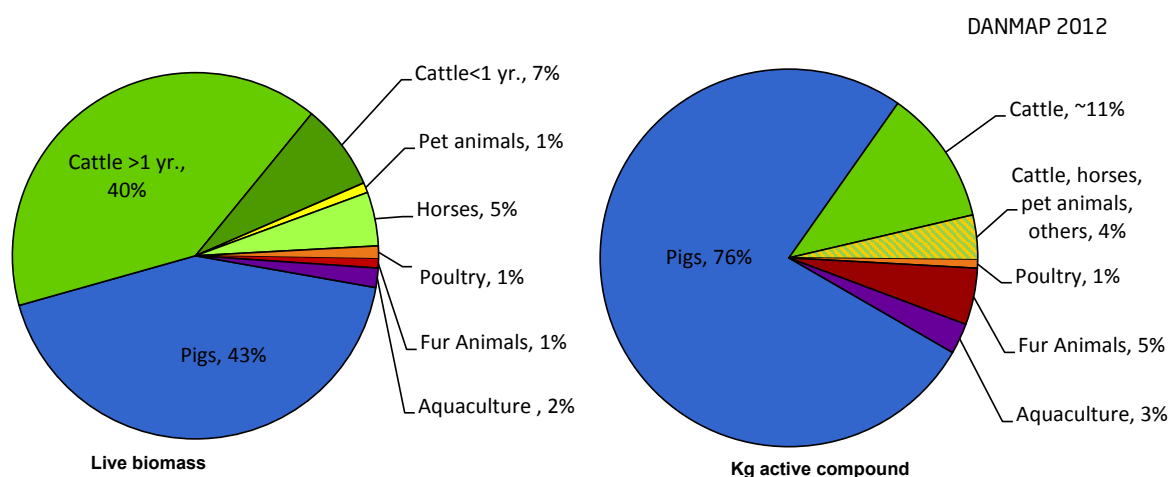
At European level, the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) project monitors veterinary usage in a number of countries. ESVAC monitoring is based on the quantity by weight of antimicrobials, using a "population correction unit" (PCU) as denominator to adjust for changes in size of the production animal population within the respective countries over time. A description of the methodology used by ESVAC is contained in the first report "Trends in the sales of veterinary antimicrobial agents in nine European countries 2005-2009" [www.ema.europa.eu].

In the context of DANMAP, we base our comparison on dosages in order to keep in focus the newer, potent antimicrobials such as fluoroquinolones and cephalosporins that are critically important in treatment of human infections. Further, the biomass of the live population is used as denominator to allow for comparisons of selection pressure between animal populations.

In the text that follows, unless otherwise stated, DAPD will be used to describe patterns in the veterinary antimicrobial consumption.

4.2 Total antimicrobial consumption

In 2012, the total veterinary consumption of antimicrobial agents, including agents used for companion animals, amounted to 112.3 tonnes active compound (Table 4.1), representing a 4% increase compared with 2011. The increase was mainly attributed to a 5% increase in amounts (tonnes) used in pigs. The two major species, cattle and pigs, comprise equal proportions of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have a very low consumption of antimicrobial agents compared with growing animals. In 2012, the antimicrobial consumption in pigs, cattle and poultry comprised 76%, ~11%, and ~1% of the total veterinary consumption, respectively (Figure 4.2).

Figur 4.2. Live biomass (mill kg) and antimicrobial consumption (kg) in main animal species, Denmark

Note: The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals, aquaculture). The estimation procedures are described in Chapter 10. For poultry: the figures comprise only the biomass and antimicrobial consumption for the main production types (turkey and broiler production)

Historically, the overall consumption – measured as kg active compound – was 46% lower in 2012 compared with 1994 – while the total meat production increased by 12% during this period (Table 3.1 and Figure 4.1). A major part of the decrease in consumption can be explained by the discontinued use of growth promoters (1994-1999).

However, from 2001 to 2009, the antimicrobial consumption in production animals increased by 36% (Figure 4.1). This increase was driven mainly by an increase in consumption in pigs and should be seen in the context that the number of pigs produced increased by 19% (Table 3.1). At the same time the proportion of live pigs (approx. 30 kg) being exported has increased and thus resulted in a 2% decrease in the biomass of the pig population.

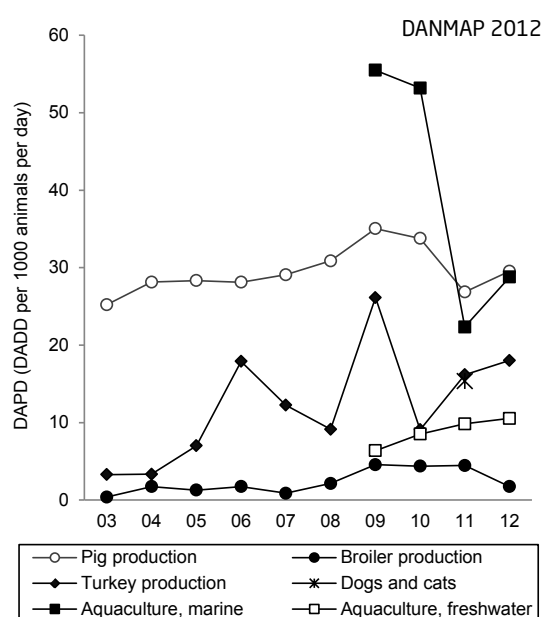
4.3 Antimicrobial consumption by animal species

For comparison between species, kilograms of meat produced has been used in many international comparisons; however, this measure overestimates the selection pressure in species with long lives (e.g. cattle), while underestimating the selection pressure in species slaughtered at an early age (e.g. poultry).

A comparison of trends in the consumption of antimicrobial agent, by species, is shown in Figure 4.3. Previous comparisons between species were based on gram active compound per kilogram of meat produced, and the consumption in aquaculture was very high compared with all other species [DANMAP 2011]. However, comparisons based on the DAPD provide a very different picture, with similar levels for pigs and marine aquaculture, and similar levels for freshwater aquaculture and poultry. There are two reasons for this: firstly, the typical drug of choice in aquaculture needs high dosages (in all species), and secondly, the lifespan of fish is very long compared to both pigs and poultry. Similarly, the difference between pig and poultry production also becomes less pronounced when using DAPD (Figure 4.3).

The consumption in dairy cattle is very low, measured in DAPD, compared with the antimicrobial consumption in calves (half of which are for slaughter), pig production and turkey production.

In 2011, antimicrobial sales for use in pet animals amounted to more than 15 DAPD (~10% underestimation, see DANMAP 2011). Thus, the consumption in pet animals was higher than for cattle and poultry, but lower than for pigs. The trends in consumption of medicines for oral use in pets indicate a similar level (2% decrease) in antimicrobial use for pets in 2012.

Figure 4.3. Antimicrobial consumption in pigs, broilers, turkey, aquaculture and pet animals, Denmark

Note: The DAPD is calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group at the total population (in tonnes)

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

DANMAP 2012

ATCvet groups	QJ01B	QJ01G	QJ01DA	QJ01MA	QJ01MB	QJ01FF	QJ01EA	QJ01XX	QJ01CE	QJ01CA	QJ01E	QJ01AA	QJ01X	
Therapeutic group	Amphenicols	Aminoglycosides	Cephalosporins	Fluoroquinolones	Other quinolones	Lincosamides	Macrolides	Pleuromutilins	Penicillin's, b-lactamase sensitive	Penicillin's, others ^(a)	Sulfonamides and trimethoprim ^(b)	Tetracyclines	Others	Total
<i>Pigs, total</i>	164	4716	1	7	-	2284	11203	7505	15896	7408	7622	28652	412	85870
- Sows and piglets	139	1970	0	7	-	455	540	637	8418	3645	6029	2068	57	23964
- Weaners	21	2437	1	0	-	806	6925	2869	1566	2573	1348	16602	352	35500
- Finishers	4	308	0	0	-	1022	3736	3999	5911	1190	244	9981	3	26398
- Age not given	0	1	0	0	-	0	3	0	2	0	1	1	0	8
<i>Cattle, total^(c)</i>	509	648	120	-	-	7	201	-	7339	990	1382	1675	9	12881
- Intramammaries	-	19	73	-	-	2	-	-	195	163	5	-	2	460
- Cows and bulls	15	308	43	-	-	2	127	-	6634	630	1048	1240	1	10047
- Calves < 12 months	476	276	2	-	-	2	65	-	360	181	294	384	7	2046
- Heifers, Steers	18	45	2	-	-	1	10	-	150	16	35	51	0	328
<i>Poultry, total^(d, e)</i>	5	28	-	-	1	14	278	6	29	157	126	163	2	809
- Turkeys	5	26	-	-	-	13	247	0	16	27	1	127	2	463
- Broiler production	0	0	-	-	-	0	15	0	10	111	105	12	-	253
- Species unknown	0	3	-	-	1	1	16	6	3	18	21	23	0	93
<i>Other production animal species</i>	166	411	0	3	532	204	679	0	1	2179	3434	749	1	8358
- Fur animals	5	411	0	3	0	204	679	0	1	2172	1240	733	1	5448
- Aquaculture	161	0	0	0	532	0	0	0	0	7	2194	16	0	2910
<i>Companion animals (pharmacy)</i>	-	5	272	12	-	67	7	-	64	645	408	52	43	165
- Pet animals - oral use ^(f)	-	1	270	12	-	67	7	-	27	643	293	50	42	1411
- Pet animals - other ^(g)	-	3	3	0	-	0	0	-	23	2	5	1	2	38
- Horses	-	1	0	-	-	0	0	-	14	0	111	1	0	127
<i>Target species not reported^(h)</i>	1	261	5	5	3	11	18	14	924	156	947	179	8	2843
Total	844	6069	398	27	536	2587	12386	7526	24254	11536	14128	31469	476	112338

Note: Only the ATCvet group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds

a) Penicillins with extended spectrum, cloxacillin and amoxicillin/clavulanic acid

b) Sulfaclozin (a prescription coccidiostat) is included in the sulfonamide/trimethoprim group

c) Fluoroquinolones reported for use in pigs were used in a clinical trial approved by the Danish Medicine Agency [Source: DVFA]

d) Approximately half of the prescribed antimicrobials for cattle was purchased of the animal owner at pharmacies; half was either administered or handed out by veterinary practitioners. In previous reports, reporting from large animal practice on medicines for cattle was validated against data on medicines sold from pharmacies to cattle practice (not mixed practice), but this was not performed for this report. Thus, the part of antimicrobial used for cattle not reported cannot be estimated

e) An important part of antimicrobial agents prescribed for poultry are sold in practice. Potential underreporting from practice has not been validated for 2012 and the magnitude can therefore not be estimated

f) In 2012, 103 kg sulfamethoxazole was used in poultry on special license, due to problems with resistance in E.coli infections. Sulfamethoxazole for use in poultry was not included in VetStat until January 2013, and thus target poultry species prior to this date is unknown. According to the practitioners it was mainly in young broilers, and therefore included under the broiler category in this table used, but not reported to the Vetstat database. According to the practitioners it was mainly in young broilers, and included under the broiler category in the table

g) Includes all medicines approved for oral treatment of dogs and cats, plus all oral medicines handed out to pet owners from the pharmacy

h) Includes parenteral and topical medicines sold from the pharmacies directly to the animal owner. Parenteral and topical medicines sold for use in practice are not included

i) Part of the medicines sold from pharmacy to practice, not reported to by veterinarian. Contains medicines mainly to companion animals and ruminants, see also d) and e)

4.3.1 Antimicrobial consumption in pigs

In 2012, the total antimicrobial consumption in pigs was 85.9 tonnes active compound (Table 4.1), representing an increase of 4.4 tonnes (5%) compared with 2011. Measured in DADD, the consumption increased by 6%.

The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.4 and in the web annex (Table A4.2).

On average, of the antimicrobial agents used for the production of a pig until slaughter, 22% is used in the sow section, 36% during weaning and 42% in the finisher section. The treatment proportion (DAPD) is much higher in the weaning pigs, compared with finishers and sows (Figure 4.4). However, the biomass of the weaning pigs is very small (7.2-30 kg, 4 weeks), compared with the finishers (31-107 kg, 12 weeks) and the sows.

The DAPD of the total population should reflect the trends in selection pressure in the population. Due to the differences in treatment proportion between age groups, the DAPD of the total population is affected by changes in population structure, e.g. increased export of live pigs at 30 kg. The increase in the export would in itself cause an increase in DAPD for the remaining population, because the DAPD for slaughter pigs is relatively low. Thus, a true impression of the antimicrobial consumption pattern and selection pressure in the pig production requires that changes in export and productivity are taken into account.

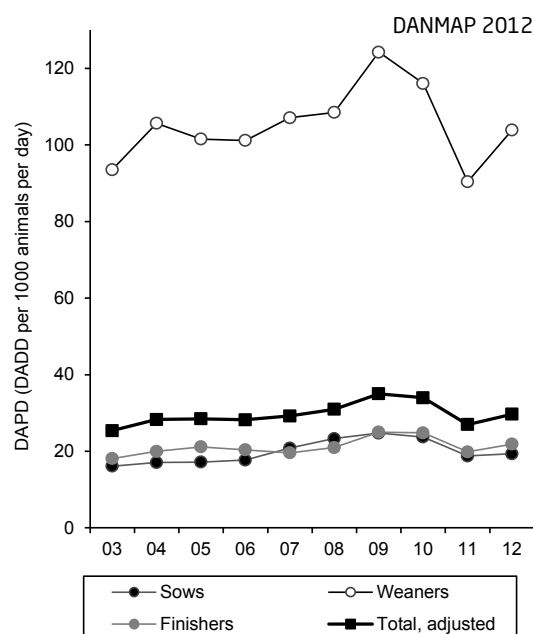
The antimicrobial consumption in pigs increased by 10% from 26 DAPD in 2011 to 30 DAPD in 2012 (Figure 4.4) when adjusted for changes in export, i.e. representing the change in treatment incidence of a pig from birth to slaughter. This 10% increase in DAPD was related to an increased consumption of almost 6%. The number of pigs produced decreased by 1.2% (Table 3.1). An additional decrease in biomass related to increased productivity and underestimation of weight of sows accounts for a minor part (~1%) of the increase in DAPD. Overall, the best estimate for the increase in antimicrobial use for the production of a slaughter pig would be an 8%-9% increase in 2012.

The DAPD increased the most in weaners (15%) and finishers (10%) and less in sow herds (2.9%), and this was almost entirely (97%) associated with an increasing use of primarily tetracyclines and macrolides in all age groups (Figure 4.5). Tetracyclines, macrolides and pleuromutilins have been the most commonly used antimicrobial agents in the Danish pig production for a decade (Figure 4.5). They are almost entirely administered orally, and particularly used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall treatment proportion (DAPD) of tetracycline increased by 15%, while the use of macrolides increased by 19%.

For the critically important antimicrobial agents, the use of fluoroquinolones increased, however remains at a very low level, constituting only 0.7% of the total consumption in pigs. A clinical trial aiming at eliminating infections with *Actinobacillus pleuropneumoniae* by fluoroquinolone treatment was carried out in 2012, and may explain the observed increase in fluoroquinolone consumption for pigs [DVFA]. The use of cephalosporins in pigs was close to none (1 kg) corresponding to 0.2% of the total consumption in this species (in DAPD).

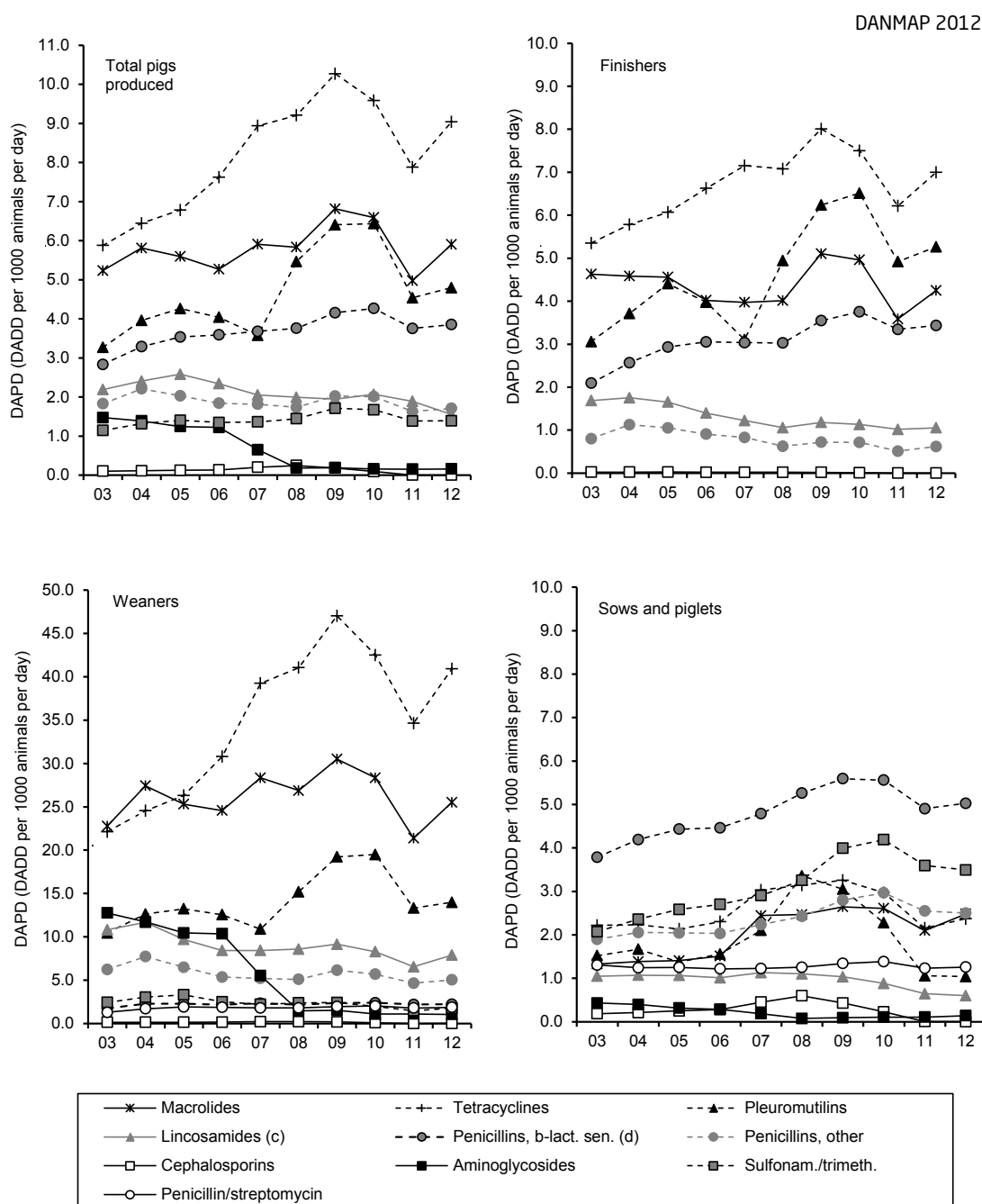
Over the last decade, the treatment proportion (DAPD) increased by 49% from 2003 to 2009. However, in 2010 and 2011, a decrease in DAPD by 23% compared with 2009 was observed, probably as a response to the DFVA's implementation of the "yellow card initiative" – a special provision for reduction of antimicrobial consumption in pig production (See DANMAP 2010 for further details). The reductions in antimicrobial use were associated with increasing use of vaccines and a slight decrease in productivity in some herds, but disease outbreaks did not increase [Danish Veterinary Bulletin no. 6, 2012]. With the increase in antimicrobial consumption in pigs in 2012, the consumption is still 16% lower than in 2009, and similar to the 2007 level.

Figure 4.4. Antimicrobial consumption ^(a) in the pig production, and the distribution on age groups, Denmark



Note: The "adjusted total" is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in piglets pre-weaning

a) The DAPD is 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)

Figure 4.5. Antimicrobial consumption^(a) in the total pig production^(b), and in finishers, weaners, sows and piglets, Denmark

Note: Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure. Data from veterinary practice are not included (<1% of the consumption in pigs)

a) The DAPD is 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)

b) Total pigs produced includes pigs exported at 30 kg, which has increased in numbers from 1.7 million in 2004 to 8.8 million in 2012, comprising an 26%, although consumption in these pigs is included only from birth to 30 kg body weight. See discussion in the DANMAP 2011

c) Lincosamide/spectinomycin combinations comprise 65% of this group

d) Beta-lactamase sensitive penicillins

4.3.2 Antimicrobial consumption in poultry

In Denmark, poultry production comprises mainly the broiler production (*Gallus gallus*), followed by egg layers (*Gallus gallus*) and turkey production. In addition, there is a small production of ducks, geese, and game birds. Consumption of antimicrobial agents for systemic use in poultry (2003–2012), given as defined animal daily dose (DADD) to the different species, are presented in the web annex (Table A4.3).

In 2012, the total antimicrobial consumption in poultry was 809 kg active compound, including 103 kg sulfamethoxazole used on special license, representing a 5% decrease compared with 2011. Sulfamethoxazole for use in poultry was not included in VetStat until January 2013, and thus target poultry species prior to this date is unknown (Table 4.1). Sulfamethoxazole is primarily prescribed to treat *E. coli* or Staphylococci (joint) infections in young broilers [Personal communication, S. Kabell, Danish Agriculture and Food Council].

In the period 2002–2008, the annual consumption fluctuated between 400–600 kg. However, disease problems caused a steep increase in antimicrobial consumption for poultry in 2009 (Figure 4.6, see also DANMAP 2009). For the layers, broiler rearing, and the turkey production, the decrease in antimicrobial consumption seems to indicate that these disease problems were under control in 2010 and 2011. In 2012, a steep decrease in reported treatment proportion (DAPD) in broilers was observed. However, it is not known whether this is altogether a true decrease in antimicrobial consumption in broilers, or partly due to incomplete reporting, because reporting has not been validated for 2012. However, during 2012, ESBL-producing bacteria became a much discussed issue, thus the poultry industry made a serious effort to minimize the use of antibiotics.

In Denmark, the antimicrobial consumption in the broiler production is generally low compared with other species (Figure 4.3). Therefore, a few disease outbreaks in some farms will seriously affect the national consumption, causing considerable fluctuations in annual consumption data. In 2012, the consumption decreased to 2.3 DAPD in broilers, indicating that the disease problems observed in 2009–2011 are almost solved by other means. In the broiler production in total (including rearing flocks), the consumption fell to 1.8 DAPD, corresponding to a 61% reduction. However, for broilers this is 2.5 times higher than the level in 2003–2008 (Figure 4.6).

In 2012, an estimated 9% of the antimicrobial agents prescribed for the broiler production were used for parent and grandparent flocks.

For broilers, amoxicillin has been the most commonly used antimicrobial agent for at least a decade. However, during the period with higher consumption (2009–2011), tetracyclines (mainly doxytetracycline) were also important (Figure 4.6). Fluoroquinolones were the second most commonly used antimicrobial agent until 2007 (when new medicines were approved for poultry), but has not been used in broilers in 2010–2012.

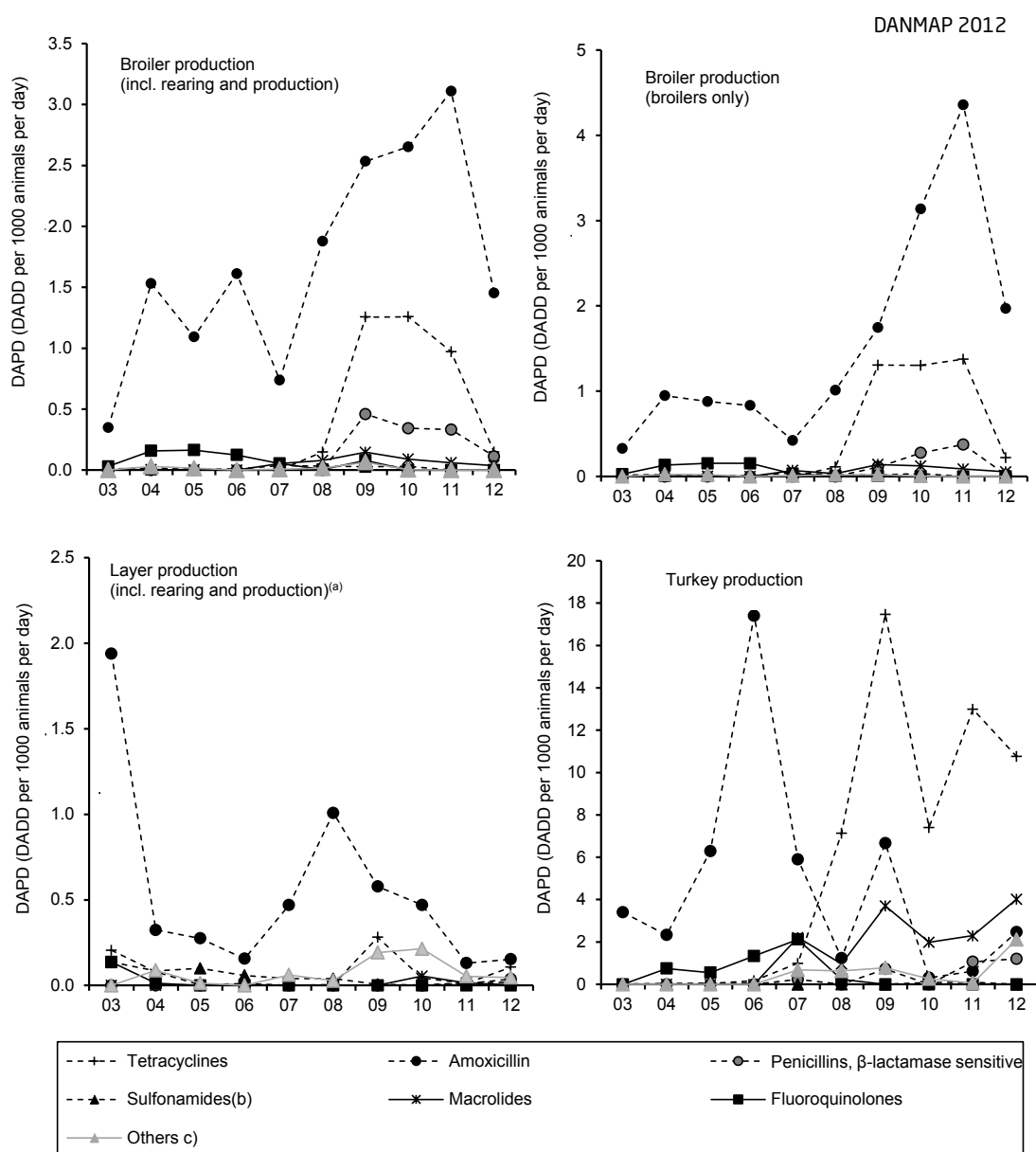
The antimicrobial consumption in the layer production is low with large annual fluctuations. The treatment proportion in 2012 (0.4 DAPD) was about 50% higher than in 2011, and twice the average consumption during the past decade. Amoxicillin and tetracycline have comprised the vast majority of antimicrobial agents used in the layer production, but during 2010–2012, pleuromutilins comprised 12% of the consumption (web annex, Table A4.3).

In turkeys, both the annual consumption and the production are highly variable (Figure 4.6). In 2012, the treatment proportion was 21 DAPD, corresponding to a 20% increase compared with 2011. Reviewing the past decade, the consumption in 2012 was twice the average annual consumption than the previous nine years (Figure 4.3). The peak in 2009 was mainly due to *Pasteurella multocida* infections (according to the poultry practitioners), and a vaccination campaign was conducted to control the disease in April–October 2009. Also, vaccination against haemorrhagic enteritis (viral) in turkeys was initiated in April 2010.

Please note that the estimation of biomass is very difficult for the turkey production due to variability in slaughter age and weight, and the trends must be interpreted with caution. Measured in standard doses (DADDs), without relating to population size, the consumption increased by 51% in 2012 (web annex Table 4.3).

Tetracyclines comprised a significant part (50%–70%) of antimicrobial use in turkeys since 2008, but the overall increase in 2012 was mainly related to increasing use of macrolides. Prior to 2007, amoxicillin constituted 73%–99% of the antimicrobial consumption in turkeys (Figure 4.6). The changes in prescription practice occurred after the marketing of tetracyclines and other agents for use in poultry during 2007–2008. As for broilers, fluoroquinolones were commonly used previously but have not been used for turkeys since 2008. Cephalosporins have not been used for at least 12 years (if ever).

Figure 4.6. Consumption of antimicrobial agents in the poultry production, Denmark



Note: Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure. Data from veterinary practice are not included

a) The DAPD is 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)

b) Includes mainly sulfaclozin registered as a coccidiostat

c) Lincosamide/spectinomycin combinations comprise 65% of this group

d) Beta-lactamase sensitive penicillins

4.3.2 Antimicrobial consumption in cattle

Data on antimicrobial consumption in cattle are not as accurate as data in the other major species because a large proportion of the antimicrobial agents are sold via veterinary practice, and reporting from practices is incomplete. In contrast to previous years, we have not attempted to estimate the proportion of medicines from veterinary practices that were used for cattle in 2012.

Pharmacy data, including sales to veterinary cattle practices, indicate that the overall consumption in cattle has been stable, around 14 tonnes per year from 2005–2012. During this period, the veal and beef production has fluctuated around a consistent level, and the milk production has increased slightly. In 2012, the beef production was 2% lower, and the milk production 6% higher compared with the average from 2005–2011 (Table 3.1).

In general, the majority of the parenteral use in cattle is for cows (Table 4.1), and is mainly prescribed for mastitis. The systemic use of fluoroquinolones in cattle was only 0.04 kg active compound in 2012, and has been at a low level since 2003. In 2012, systemic use of 3rd and 4th generation cephalosporins in cattle increased by 5% to 47 kg and intramammary use decreased by 13% to 7 kg compared with 2011. To note, the use of 3rd and 4th generation cephalosporins for systemic use peaked in 2008, and for intramammary use in 2007 (Figure 4.7).

Data on intramammary use, based on pharmacy data show an unchanged overall level of intramammary treatment from 2001 to 2012. However, drying-off treatment increased by 20% while therapeutic treatment decreased by 9% in 2012 (Tables 4.2). From 2005 to 2012, the overall intramammary treatments per cow per year decreased by 12%, while the proportion of intramammary medicines containing only penicillins increased from 35% to 63% (Table 4.3). The use of antimicrobial agents for treatment of mastitis in cattle has been regulated since 2006 [Order (DK) 1045/2006 and 785/2010], and simple penicillins should be used, unless resistance testing indicates otherwise. However, these trends are probably also a result of a “milk quality campaign” run by the cattle association (Agriculture and Food Council) since 2010. The goals of the campaign are to reduce treatment of clinical mastitis by 50%, mainly through a reduction of treatment of subclinical mastitis, but also by increasing the frequency of cell counts to determine the need for treatment.

The Agriculture and Food Council does accept some degree of increase in drying-off treatment in cases where it is a consequence of refraining to treat during lactation. Furthermore, Order (DK) 785/2010 provides legal regulations of use of antimicrobial agents for mastitis in cattle (recommending using simple penicillins) and the industry has also emphasized that farmers should use narrow spectrum penicillins to treat mastitis caused by Gram-positive bacteria, unless sensitivity testing expresses resistance towards these antimicrobials.

Table 4.2. Number of treatments with antimicrobial agents for intramammary application in cattle, Denmark DANMAP 2012

	2005	2006	2007	2008	2009	2010	2011	2012
Total doses per indication	DADDs (1000's)							
Drying off treatment (4 teats)	73	75	71	76	82	99	97	117
Therapeutic treatment (2 teats)	420	408	388	377	378	350	307	279

Note: For intramammary therapeutic treatment, 1 DADD is defined as the dose to treat two teats for 24 hours. For drying off treatment, 1 DADD is defined as the dose to treat 4 teats. One product used for both indications is included as half drying off and half therapeutic treatments

Table 4.3. Use of antimicrobial agents for intramammary application in cattle, Denmark DANMAP 2012

	2005	2006	2007	2008	2009	2010	2011	2012
Doses per antimicrobial class	DADDs (1000's)							
Penicillins ^(a)	201	211	211	236	282	314	318	324
Aminoglycoside-benzylpenicillin combinations ^(b)	130	104	101	101	110	93	48	47
Cephalosporins, 1st generation	103	98	89	85	89	89	99	105
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34	30
Others ^(c)	21	20	16	15	14	12	9	8
Total	566	558	544	549	570	559	508	514
Total DADD per cow per year	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9

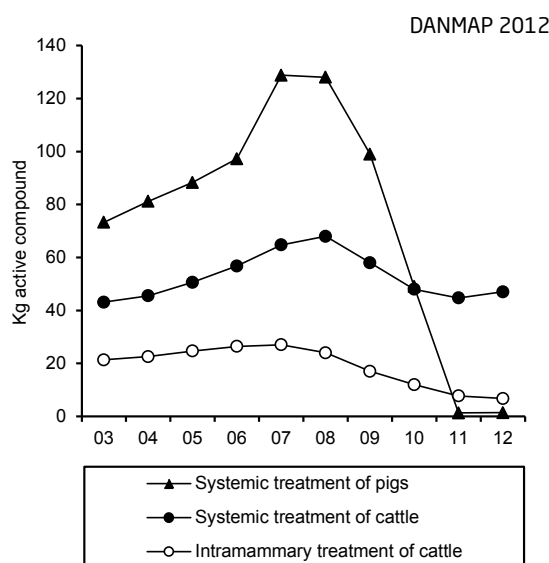
Note: For intramammary application, 1 DADD is defined as the dose to treat two teats for 24 hours

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

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

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

Figure 4.7. Consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark



4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The antimicrobial consumption in **aquaculture** increased by 8% to 2,900 kg in 2012 compared with 2011 (Table 4.1). Measured in standard doses (DADD), the major class of antimicrobial used was sulfonamide/trimethoprim, comprising 53% of the consumption in aquaculture in 2012. The consumption of quinolones (oxolinic acid) comprised 39% and florfenicol 8%.

Assuming an unchanged production volume, the treatment proportion in marine aquaculture was 29 DAPD in 2012, representing a 28% increase compared with 2011 (Figure 4.3). This increase is partly related to an increase in production. However, the antimicrobial consumption in both 2012 and 2011 was very low compared to previous years, probably due to cold summers in 2011–2012. Fish immunology and infection is very sensitive to water temperatures. Improved vaccination against furunculosis between 2006–2010 may also have contributed to the observed decrease in antimicrobial consumption. [personal communication: N.H. Henriksen, Danish Aquaculture].

In freshwater fish, the antimicrobial consumption fluctuates less than in the marine aquaculture. In 2012, the treatment proportion in freshwater aquaculture increased by 7%, to 11 DAPD, assuming the same production volume as in 2011. There has been an increasing trend in antimicrobial consumption since 2009. Some structural changes in the production could be a partial explanation but at present, the cause is unknown [personal communication: N.H. Henriksen, Danish Aquaculture].

In 2012, the production of **mink** increased to 17 million mink from 15 million in 2011 (Source: Copenhagen Fur). Antimicrobial consumption in fur animals increased to 5.4 tonnes, representing a 13% increase in kg active compound compared with 2011 (Table 4.1). Aminopenicillins remained the most commonly used antimicrobial class in fur animals, comprising 40%. The use of fluoroquinolones increased from 0.4 kg in 2010 to 3 kg in 2012, when it was prescribed for enteritis in May–June 2012, presumably for the kits. During the past five-year period, the antimicrobial use has gradually increased by 60%, while the production of mink has increased

by 21%, from 14 million since 2008. This increase may partly be explained by an increased focus on animal welfare in the mink production by both the Industry (Kopenhagen Fur) and the Authorities (DVFA).

The information available on antimicrobial consumption in **pet animals** is not as detailed as for production animals. Therefore, in 2012, we only estimated the consumption of antimicrobial agents used for oral treatment in pet animals (mainly cats and dogs).

In 2011, antimicrobial sales for use in pet animals amounted to more than 15 DAPD; this estimate does not include parenteral and topical antimicrobial use in mixed practice, causing an underestimation of approximately 10%–15%. Consumption of antimicrobial agents for oral use amounted to 12 DAPD in 2012, corresponding to a 2% decrease in treatment proportion compared with 2011 (Figure 4.8).

The use of the broad-spectrum agent amoxicillin/clavulanic acid increased by 2%. This is part of a continuous increase of 91% since 2005. The use of fluoroquinolones for oral use increased by 7% in 2012, compared with 2011, whereas the use of 1st generation cephalosporin decreased by 12%. In pet animals (mainly cats and dogs), the prescription for oral treatment measured in DAPD has increased by 30% since 2005.

The treatment proportion of 3rd and 4th generation cephalosporins was estimated at 0.7 DAPD in 2012, similar to 2011. The use of fluoroquinolones for oral use was 0.6 DAPD in 2012. For comparison, the use of cephalosporins for systemic use was 0.2 DAPD in cattle in 2011, while the use in pigs is now close to none, and it has not been used in poultry for at least 12 years.

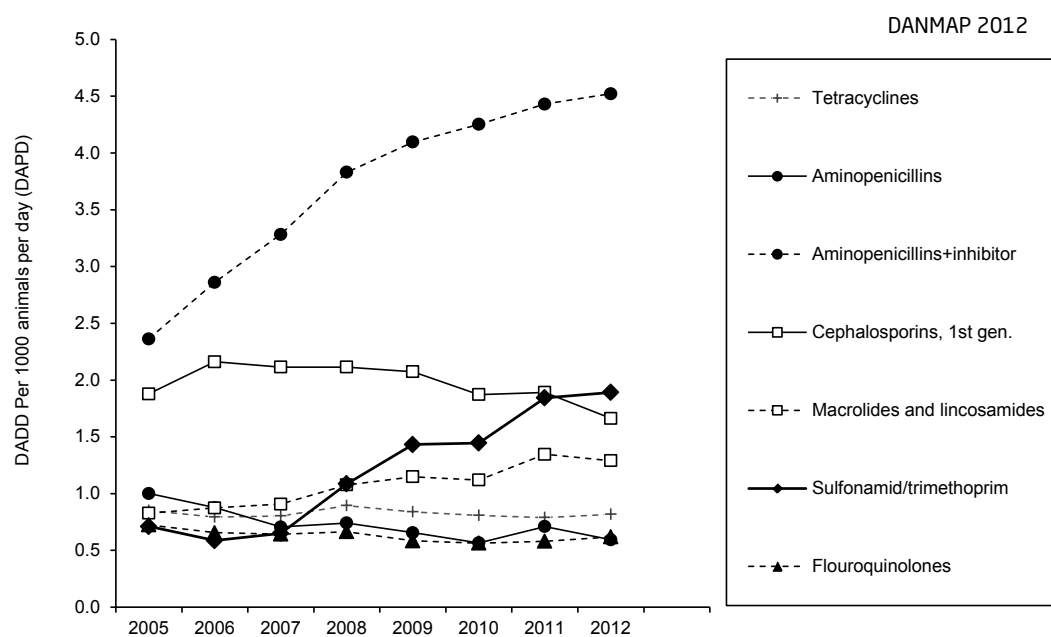
The use of fluoroquinolones for oral use in pet animals was 12 kg in 2012. This means that the total use of fluoroquinolones in pet animals is about half of the total veterinary use (kg) of fluoroquinolones. Regarding amoxicillin/clavulanic acid, 91% of the veterinary consumption (kg) was used in pet animals; this combination has a very broad spectrum and should be reserved for infections caused by bacteria that are resistant to more narrow spectrum agents, or as a potential empirical choice for severe infections where instant effect is essential.

In conclusion, the treatment proportion for critically important antimicrobial agents is much higher for pet animals compared with food animals.

Considering the close contact between pet animals and humans, and the increasing evidence for transfer of resistance between the pet reservoir and humans, the high consumption of broad spectrum antimicrobial agents in pets is a matter of concern. The new national guideline from the Danish Health and Medicines Authority aim to ensure that the critically important antibiotics are reserved for severely ill people or are only used when there are no alternatives, including especially targeting the use of carbapenems, and cephalosporins (Textbox 3).

A large proportion of antimicrobials used for pet animals are prescribed for treatment chronic or recurrent disease, mainly dermatitis. Particularly the consumption of critically important antimicrobial agents in pet animals could pose an important risk to owners of diseased dogs that are frequently treated. Presently, there is no information available concerning the prevalence of antimicrobial resistance in pet animals.

Vibeke Frøkjær Jensen and Birgitte Borck Høg

Figure 4.8 Antimicrobial consumption (oral treatments) in pets^(a), Denmark

Note: The DAPD is 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)

a) The live biomass for pets only include dogs (average weight: 20 kg), and cats (average weight: 4 kg). Census data are available only for 2000 (Statistics Denmark), and the population size was assumed to be constant (in accordance with data in the Danish Dog register; personal communication), while the cat population may have increased. The vast majority of antimicrobials for pets are used in dogs



5. Antimicrobial consumptions in humans

5.1 Introduction

In Denmark, systemic antimicrobial agents for humans are available only on prescription. In November 2012 a new set of national guidelines for use of antimicrobial agents especially concerning the use of the “critical important antibiotics”: fluoroquinolones, cephalosporins and carbapenems was published (Textbox 3). Some of the Danish Regions already had made new guidelines which were in agreement with the recommendation before it was published, in order to reduce the number of multi-resistant bacteria (e.g. ESBL-producing bacteria, MRSA and *Clostridium difficile*).

Throughout this section, the antimicrobial consumption in 2012 is compared to that of 2011 and of the last decade (2003). Combinations of penicillins, including beta-lactamase inhibitors (J01CR), are referred to as ‘combination penicillins’.

In this section, the term ‘antimicrobial agents’ covers only antibacterial agents for systemic use in humans, agents used for treatment of infections caused by virus or fungi are not included. Currently available antimicrobial agents for systemic treatment in humans (and in animals) are listed in Table 3.3.

Antimicrobial agents have been classified as either narrow-spectrum or broad-spectrum agents according to the spectrum of the activity (Table 5.1).

5.2 Total consumption in both primary health care and hospital care

Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, and can be used for comparison between sectors and for illustration of the consumption in hospital care without taking account of hospital activity (discharges) (Figure 5.1).

In 2012, the total consumption of antimicrobial agents for systemic use (primary health care and hospital care) decreased with 2% (18.48 DID in 2012 compared to 18.90 DID in 2011, Figure 5.1). The total consumption of broad-spectrum agents increased in 2012 (8.21 DID in 2012 compared to 7.80 DID in 2011, Figure 5.2). Primary health care represented 90% of all prescribed DDDs in Denmark in 2012 (Figure 5.1).

The distribution of DIDs between primary health care and hospital care differed between antimicrobial agents (Figure 5.3). For most of the antimicrobial agents, the consumption was higher in primary health care, with exception of cephalosporins and related substances, aminoglycosides and imidazole derivatives.

Since 2003, the overall consumption of antimicrobial agents has increased by 23%, or 3.44 DID (Figure 5.1). During the same period, broad-spectrum agents have increased by 3.5 DID (74%), comprising 31% of the overall consumption in 2003 and 44% in 2012. The proportion of DDDs prescribed in primary health care remained relatively constant during the last decade, between 89%–90%.

The detailed distribution of DIDs among antimicrobial groups in primary health care and hospital care is presented in Table 5.2 and Table A5.1 in the web annex.

In 2012, 48.7 tonnes of antimicrobial agents for systemic use were used in humans in Denmark. This level is approximately 2.5 tonnes less than reported in 2011 but still represents an increase of 4.8 tonnes (10%) compared to 2003 (Table A5.2 in web annex).

Table 5.1. Classification of antimicrobial agents for systemic use in humans into narrow-spectrum and broad-spectrum agents, Denmark

DANMAP 2012

ATC group ^(a)	Therapeutic group
Narrow-spectrum	
J01CE	Beta-lactamase sensitive penicillins
J01CF	Beta-lactamase resistant penicillins
J01DB	First-generation cephalosporins (included in data from primary health care as a broad-spectrum agent in the group J01D)
J01DF	Monobactams
J01EA	Trimethoprim and derivatives
J01EB	Short-acting sulfonamides
J01FA	Macrolides
J01FF	Lincosamides
J01XA	Glycopeptides
J01XC	Steroid antibacterials (fusidic acid)
J01XD	Imidazol derivatives
J01XE	Nitrofurans derivatives (nitrofurantoin)
J01XX	Other antibacterials
Broad-spectrum	
J01AA	Tetracyclines
J01CA	Penicillins with extended spectrum
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors
J01D	Cephalosporins and related substances (primary health care only)
J01DC	Second-generation cephalosporins
J01DD	Third-generation cephalosporins
J01DH	Carbapenems
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives
J01GB	Aminoglycosides
J01MA	Fluoroquinolones
J01XB	Polymyxins

a) From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.1. Total consumption of antimicrobial agents (J01) in humans by sector, Denmark

DANMAP 2012

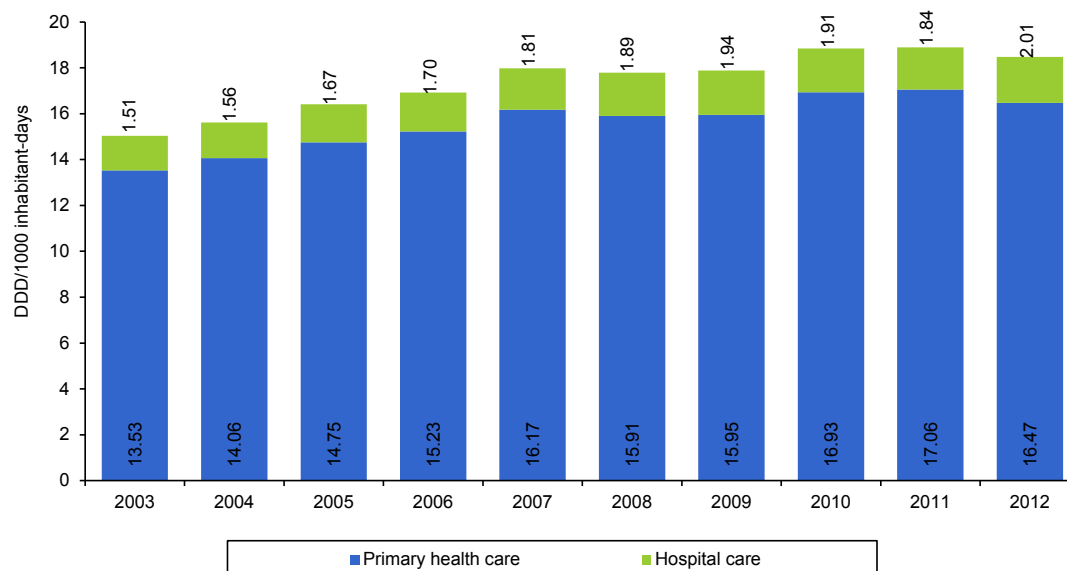
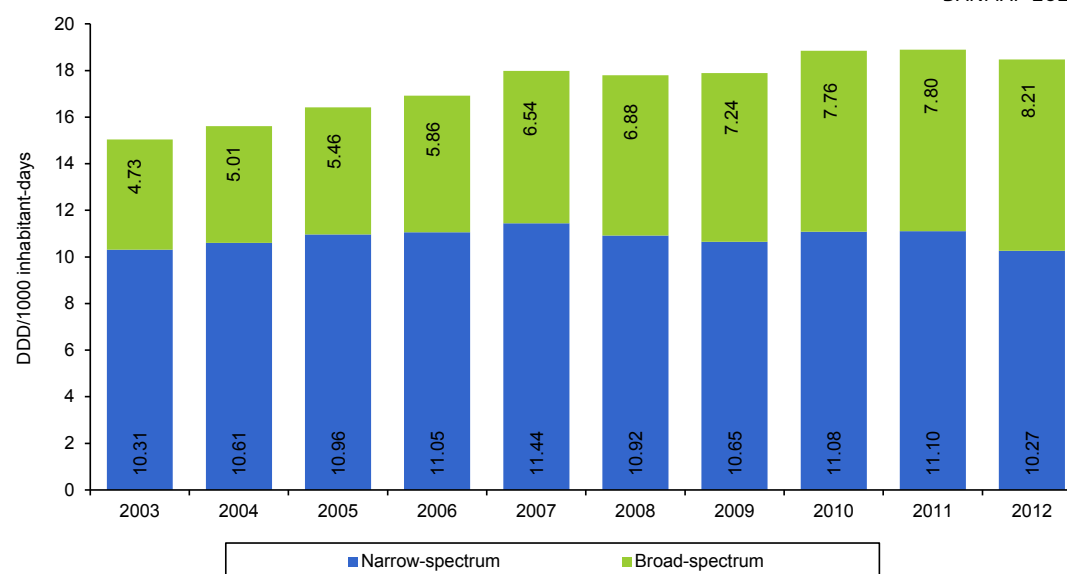


Figure 5.2. Total consumption of antimicrobial agents (J01) in humans by narrow-spectrum and broad-spectrum agents, Denmark

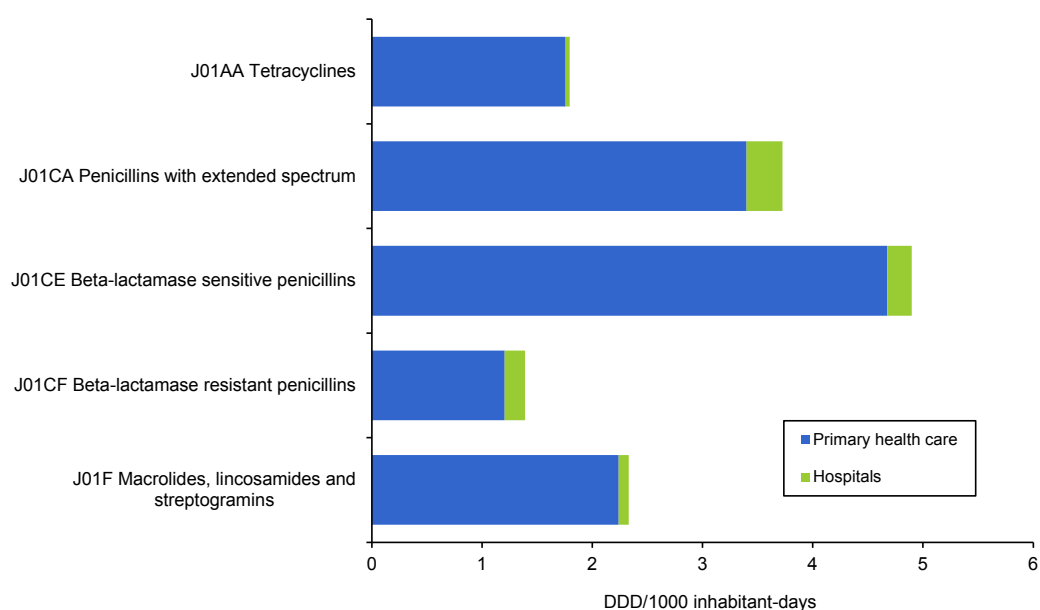
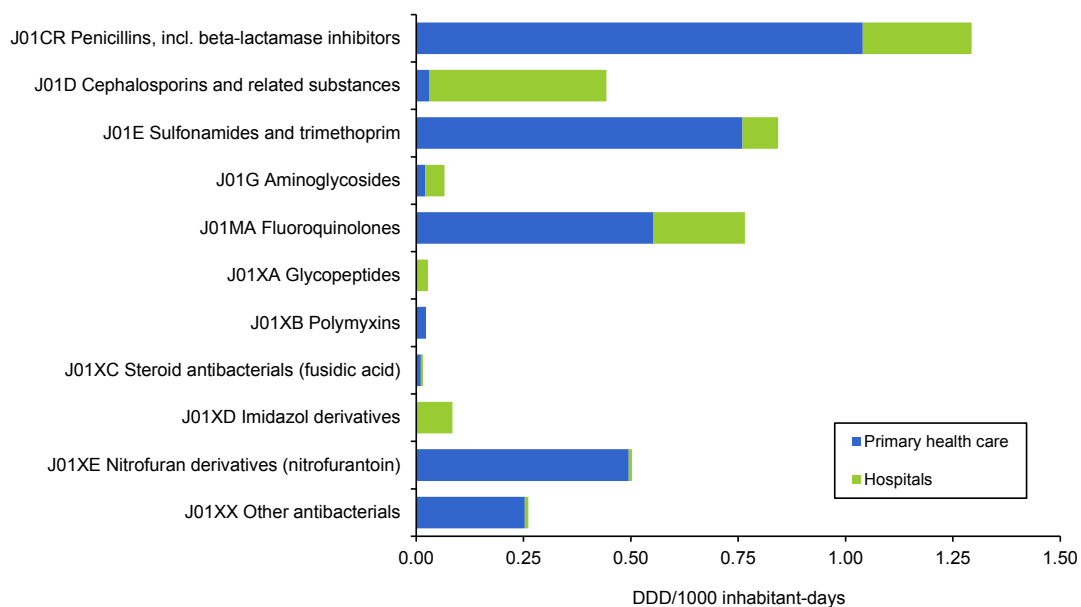
DANMAP 2012



Note: "Narrow-spectrum" includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofurantoin derivatives, and 'other antibiotics'
 "Broad-spectrum" includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, cephalosporins and related substances, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones, and polymyxins

Figure 5.3. Distribution of DIDs between primary health care and hospital care, Denmark

DANMAP 2012



Consumption of antimicrobial agents and incidence of multi-resistant bacteria in Greenland

Background: Greenland has a population of 56,749 inhabitants (January 2012) and Nuuk is the capital with around 16,000 inhabitants. Greenland has its own Ministry of Health and the country is divided into five health regions: Qeqqa (the former health districts Sisimiut and Maniitsoq), Disko (the former health districts Aasiaat, Kangaatsiaq, Qeqertarsuaq and Qasigiannnguit), Avannaa (the former health districts Uummannaq, Upernavik and Qaanaaq), Sermersooq (the former health districts Nuuk, Paamiut/Ivittuut, Tasiilaq and Illoqqortoormiut), and Kujataa (the former health districts Qaqortoq, Nanortalik and Narsaq). The largest hospital, Dronning Ingrid's Hospital, is situated in Nuuk (185 beds). There are several smaller hospitals and health care centers in the five health regions. Around 15-16,000 persons are admitted to hospital once or several times a year. The primary health care is organized different from that in Denmark; there are no general practitioners with private practice, and the hospital clinics are used for patients from the primary health care. In Nuuk, a large health care center has combined function as medical clinic, emergency room and primary health care with doctors and nurses. The settlements have nursing stations (supervised by doctors via phone or telemedicine and doctors visiting three to four times a year). Medication on prescription is free of charge. Patients with specific/serious diseases are transferred to Denmark or Iceland for further treatment (e.g. hemodialysis, cancer treatment, brain surgery etc.).

Resistant bacteria: From 2004 to 2012, eight patients have been diagnosed with MRSA, 23 patients with ESBL-producing *Enterobacteriaceae*, and 24 patients with *Clostridium difficile* 027. Most of these resistant bacteria were imported from Denmark or abroad, but in some cases, especially in patients with an ESBL-producing *Enterobacteriaceae*, treatment with broad-spectrum antimicrobial agents in Greenland has probably selected for these bacteria.

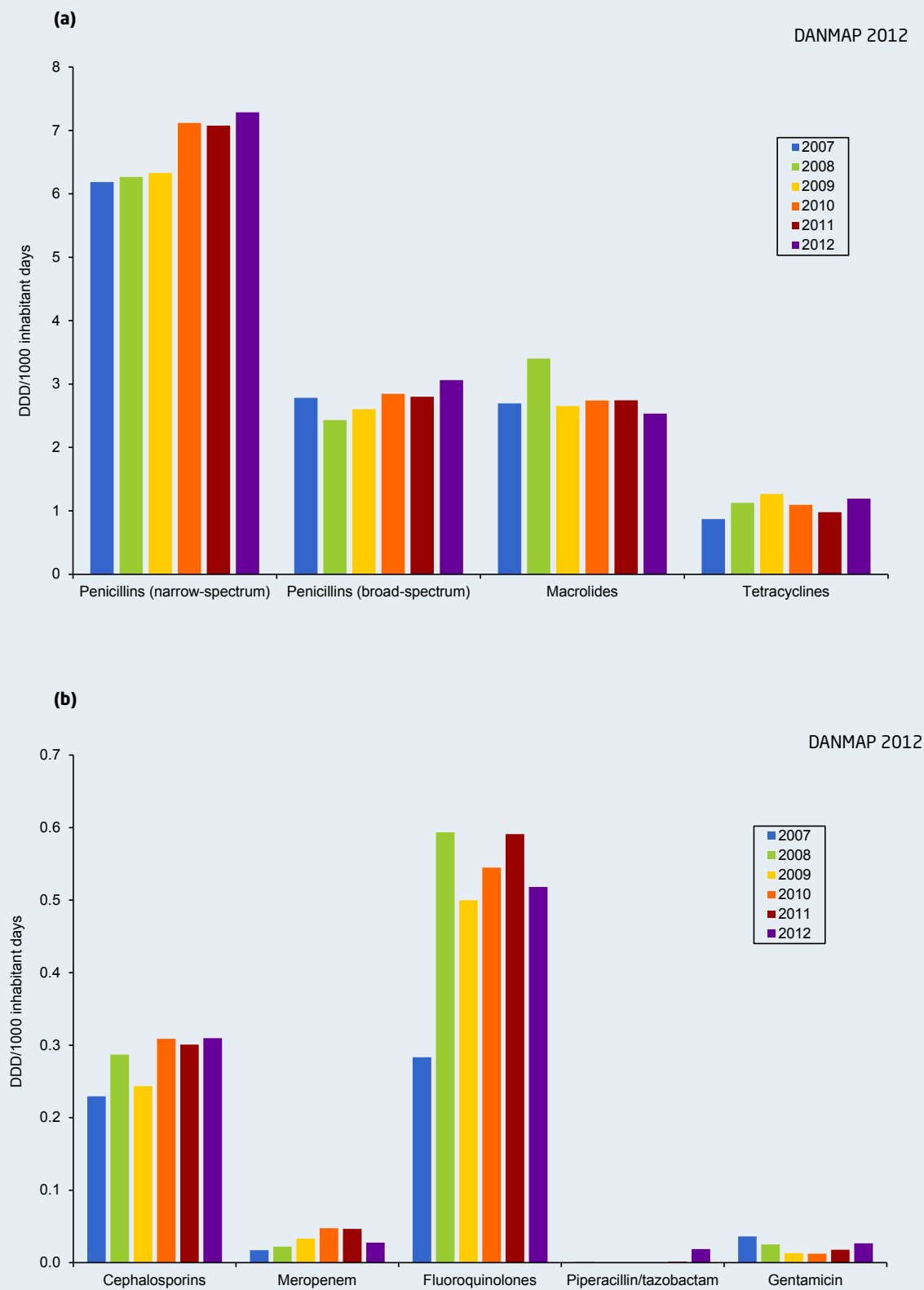
Consumption of antimicrobial agents: There are no exact figures for consumption of antimicrobial agents in Greenland, only for purchased agents. Figure 1 shows the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2007 to 2012. From 2007–2012, an increase in purchase of narrow-spectrum (18%) and broad-spectrum penicillins (11%) has been seen. From 2011 to 2012, an increase in the sale of piperacillin-tazobactam (18%) and a decrease in broad-spectrum antimicrobial agents such as macrolides (7%), fluoroquinolones (12%), and meropenem (40%) have been seen. The sale of cephalosporins (mainly ceftriaxone) has been at the same level from 2011 to 2012.

Conclusion: Continued focus on resistant microorganisms and use of broad-spectrum antimicrobial agents in Greenland is important in the future.

Anne Kjerulf, Jette Holt, Anne Birgitte Jensen, Turid B. Skifte, Peter Poulsen, Inge Mortensen

For further information: Anne Kjerulf (alf@ssi.dk)

Figure 1. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1,000 inhabitants/day) 2007-2012: (a) consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines; and (b) consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin. (Note: Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin and dicloxacillin, and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor)



Increased focus on the use of antimicrobial agents in the Faroe Islands

Background: The Faroe Islands (FI) consists of 18 islands, inhabited by approx. 48,000 inhabitants, 19,000 of whom live in the capital Tórshavn. The main hospital (Landssjúkrahúsið, LS, with 170 beds) is located in Tórshavn, and there are two smaller hospitals in Klaksvík (36 beds) and Súðuroy (26 beds). The healthcare system is comparable to the Danish healthcare system with general practitioners responsible for primary care and secondary care provided by the hospital. LS has a local as well as a centralized function. In the case of specified diseases, patients are referred to hospitals in Denmark or other foreign hospitals. The healthcare staff is well educated and constitutes a stable workforce. However, consultants, mainly from Denmark, perform specialized treatment where the number of patients is too small to support full-time specialist employment.

Food is either locally produced or similar to what can be bought in Danish supermarkets (same origin), although with a greater share of products from Iceland and Norway.

Data and data sources: Data for antimicrobial consumption for FI and for LS were supplied by the Chief Pharmaceutical Office. Bed days and data on MRSA and ESBL-producing bacteria were obtained from LS.

Results: Since the first case of MRSA in 2004, a total of 32 cases of MRSA have been identified (21 with infection and 11 carriers). From 2006–2012, 13 ESBL-producing *Escherichia coli* and 4 ESBL-producing *Klebsiella pneumoniae* have been detected, furthermore one patient had both an ESBL-producing *E. coli* and an ESBL-producing *K. pneumoniae*. The total consumption of antibacterial agents in primary health care (defined by individual prescriptions) was 14.17 DID in 2012. An almost total stop in the use of pivampicillin and pivmecillinam and a rise in the use of amoxicillin with enzyme inhibitor (251%) as well as ciprofloxacin (47%) have been detected from 2007 to 2012 (Figure 1). This change reflects a ban of the use of pivampicillin and pivmecillinam due to identification of a potentially fatal carnitine transporter gene defect in 1/3.600 inhabitants of the Faroe Islands [Joensen F *et al.* 2006. Ugeskr Laeg 168: 667-670]. Screening is possible and it is considered to lift the ban in order not to stimulate quinolone use further.

For many years, LS has had a policy of rational use of antimicrobial agents. However, a rise in the use of broad-spectrum antimicrobial agents from 2007–2012 has been detected, exemplified by cefuroxime (41%), ciprofloxacin (413%), and meropenem (102%) (Figure 2). LS has therefore increased the focus on reduction of the use of broad-spectrum antimicrobial agents. This will be done by performing regular audits in all clinical wards, and securing continued compliance with infection control guidelines in order to prevent transmission of possible resistant bacteria.

Conclusion: Increased focus on the use of broad-spectrum antimicrobial agents and resistant bacteria is the current strategy of the Faroe Islands.

Elsebeth Tvenstrup Jensen, Anne Kjerulf, William Smith, Niels Joensen and Hjørdis Reinert

For further information: Elsebeth Tvenstrup Jensen (etj@ssi.dk)

Figure 1. All primary health care prescriptions for pivampicillin, pivmecillinam, amoxicillin and enzyme inhibitor, and ciprofloxacin (DDD/1,000 inhabitants/day)

DANMAP 2012

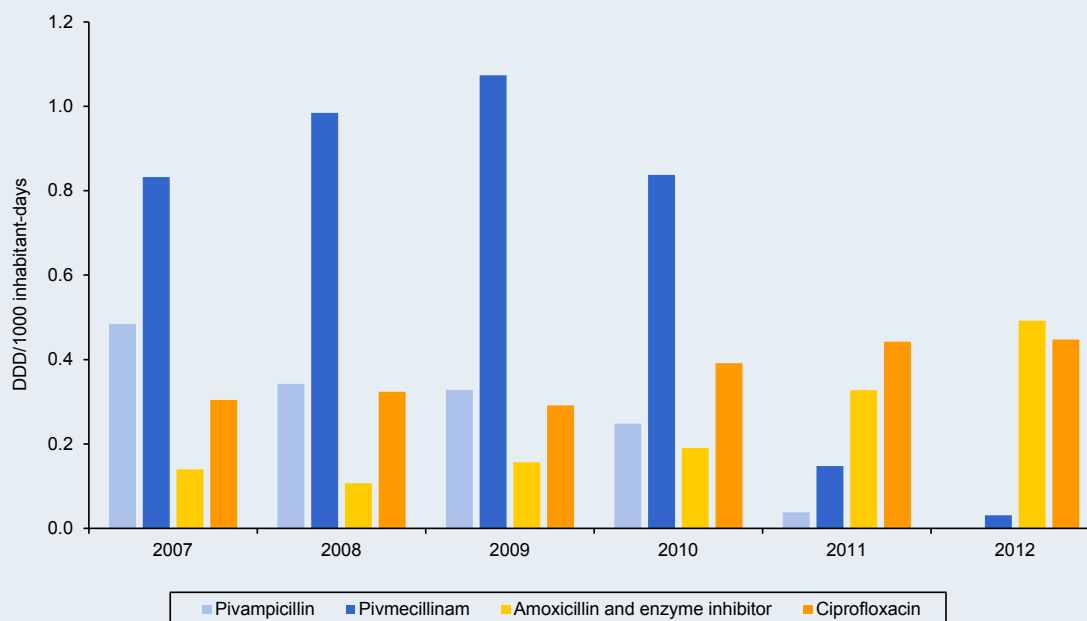
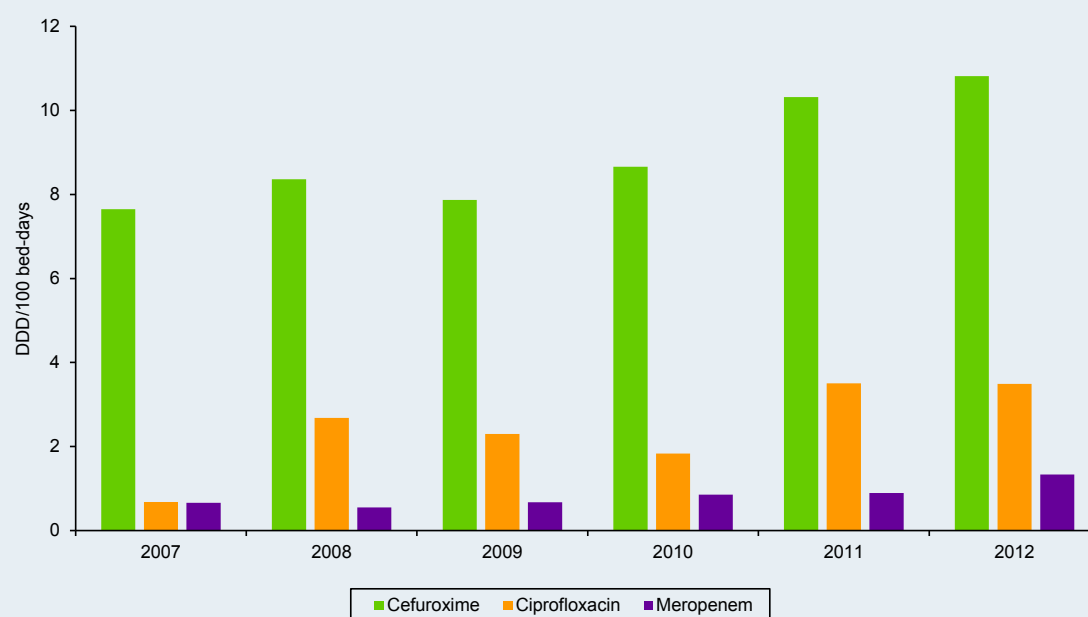


Figure 2. Use of cefuroxime, ciprofloxacin, and meropenem at the LS hospital (DDD/100 bed-days)

DANMAP 2012



New guidelines on prescribing antibiotics in primary health care and hospitals

Background: In November 2012, The Danish Health and Medicines Authority issued a new set of guidelines that outline the standards required for physicians in prescribing antibiotics. Below is a short description of the guidelines, the full length version can be found at: <http://www.sst.dk/~media/English/Guidelines%20on%20Antibiotics.ashx>

The guidelines aim to change the pattern of prescription of antibiotics to become more rational - including ensuring that unnecessary use is reduced - with the aim of preventing the development of antibiotic resistance; and to ensure that the critically important antibiotics are reserved for severely ill people or are only used when there are no alternatives, including especially targeting the use of carbapenems, fluoroquinolones and cephalosporins.

The basic rules that apply to physicians' prescription of antibiotics for systemic use are as follows:

- Antibiotic treatment must be expected to prevent severe or life-threatening events or to reduce the period of illness considerably.
- Clinical and diagnostic testing must be carried out such that it at least determines that bacteria are the likely cause of illness.
- The antibiotic selected must be as narrow-spectrum as possible and influence the normal bacterial flora as little as possible in accordance with the general and local guidelines for the use of antibiotics.
- If the initial treatment is not successful, the choice of antibiotic must be reassessed and perhaps changed based on microbiological testing.
- The treatment must be as brief as possible and be in accordance with the evidence available in the field.
- The diagnosis that results in the prescription must be specifically outlined in the prescription system, including on the prescription and in the medical records.

General rules on prescribing antibiotics at hospitals:

- Each department or hospital must have instructions for prescribing and using antibiotics.
- If there is a reason to deviate from these instructions, the reason for this must be entered into the medical records in connection with prescription.
- The department or hospital should always take samples for microbiological testing before initiating antibiotic treatment. Occasional exceptions might include cholecystitis and erysipelas.
- For all treatment with antibiotics, the indication, dose and expected duration of treatment must be entered in the person's medical record.
- A physician must reassess the indication, choice of medicine, dose and duration of treatment within 48 hours and should assess this at least every 3 days thereafter.
- Critically important antibiotics should be primarily used when the person has or may be expected to have life-threatening illness or relevant microbiological test results are available.

Specific rules for carbapenems, fluoroquinolones and cephalosporins:

The recommendation outlines specific rules for the use of carbapenems, fluoroquinolones and cephalosporins.

For the primary sector applies:

Regarding use of antimicrobial agents by general practitioners and other physicians in the primary sector, the use of carbapenems is not allowed at all, and the use of fluoroquinolones and cephalosporins should be minimized and only used when microbiological diagnostics indicate that other antibiotics cannot be used.

Treatment with fluoroquinolones before the microbiological test results are available may only be initiated among:

- people allergic to penicillin who have acute exacerbation of chronic obstructive pulmonary disease, are clinically affected and fulfil the following criteria: increased dyspnoea, increased expectoration and increasing purulent expectorate;
- people allergic to penicillin who have pyelonephritis;
- people with severe gastroenteritis who have a higher risk of complications (such as those older than 60 years, with arteriosclerosis or with immune suppression) and among whom *Salmonella* infection is suspected; and
- men older than 35 years with epididymitis.

Cephalosporins can be prescribed for patients with one of two indications:

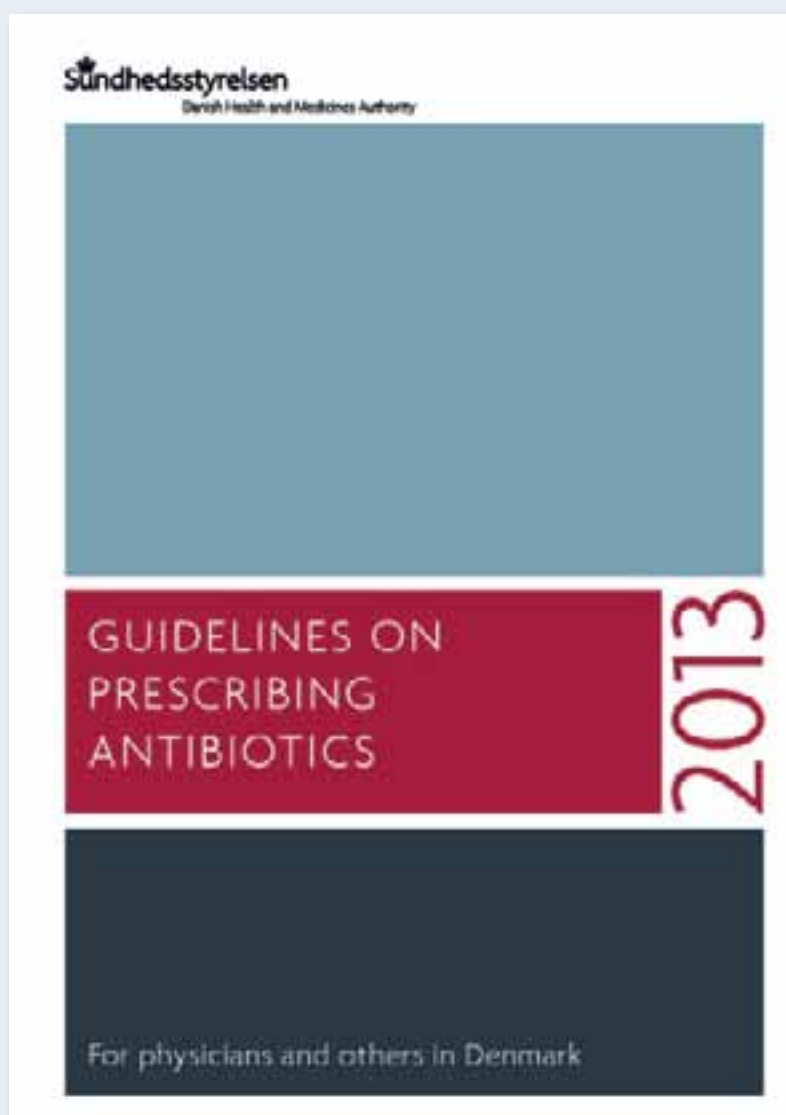
- Pregnant women with penicillin allergy who require treatment for a proven infection (fluoroquinolones and macrolides are not recommended for pregnant women).
- Patients with penicillin allergy and suspected meningococcal disease, see Health Protection Agency guidelines treatment of meningococcal disease No. 9235 of 23 May 2012.

For hospitals applies:

- Carbapenems as first-line treatment should only be used empirically when septic shock or severe sepsis, synergistic gangrene or a similar life-threatening acute infection is suspected.
- Carbapenems may be used as second-line treatment if the symptoms progress during treatment with another less broad-spectrum antibiotic treatment for infections of unknown cause.
- For microbiologically verified infections, carbapenems should only be used if the paraclinical results indicate that less broad-spectrum treatment is inadequate.
- Fluoroquinolones should only be used in connection with microbiological testing demonstrating that other antibiotics cannot be used or if the pharmaceutical properties of fluoroquinolones are especially suitable and/or if the person is allergic to penicillin.
- Cephalosporins may be used empirically after samples have been obtained for microbiological testing from normally affected people suspected of having an infectious disease with unknown bacterial origin.
- Cephalosporins may be used for surgical prophylaxis in accordance with the local instructions on the use of antibiotics if they are validated as being the best choice: that is, more narrow-spectrum antibiotics or combinations thereof would not have the same effect.
- Cephalosporins should otherwise only be used in connection with microbiological testing demonstrating that penicillin products or other less broad-spectrum medicines cannot be used.

Jenny Dahl Knudsen and Niels Frimodt-Møller

For further information: Jenny Dahl Knudsen (inge.jenny.dahl.knudsen@regionh.dk)



5.3 Primary health care

5.3.1 Total consumption in primary health care

In 2012, the total consumption of antimicrobial agents for systemic use in primary health care decreased with 3% compared to 2011 (17.06 in 2011 to 16.47 DID in 2012) and thus deviating from the general upwards tendency observed since 1995. Several changes were observed from 2011 to 2012 (Table 5.2). Increases were observed for 'combination penicillins' (0.16 DID), tetracyclines (0.12 DID), beta-lactamase resistant penicillins (0.07 DID) and trimethoprim and derivatives (0.02 DID). Consumption decreased notably within two groups: beta-lactamase sensitive penicillins (0.63 DID) and macrolides (0.28 DID).

Compared to 2011, each patient treated in primary health care used a higher number of DDDs in 2012 (20.6 DDD vs. 19.4 DDD) (Table 5.3), as discussed in section 5.3.2.

As observed during previous years, the consumption of antimicrobial agents was markedly larger in the second half of the year, particularly for macrolides and beta-lactamase sensitive penicillins. This pattern is most likely caused by the generally increased burden of lower respiratory tract infections (LRTIs) in the winter months of 2012 as illustrated by the incidence of *Mycoplasma pneumoniae* infections (Figure 5.4).

According to national guidelines, LRTIs of suspected bacterial origin are treated with beta-lactamase sensitive penicillins and suspected or confirmed *M. pneumoniae* infections with macrolides.

As in previous years, beta-lactamase sensitive penicillins represented the largest therapeutic group of antimicrobial agents consumed in 2012 (29%), followed by penicillins with extended spectrum (21%) and macrolides (14%) (Figure 5.5). Penicillins accounted for 65% of the total consumption in 2012. Consumption of broad-spectrum agents increased by 0.3 DID (4.1%) compared to 2011 (Figure 5.6).

From 2003 to 2012, antimicrobial consumption increased by 21.7% from 13.53 to 16.47 DID (Table 5.2). Broad-spectrum agents represented 6.85 DID (42%) of the total consumption in 2012 compared to 3.98 DID (29%) in 2003; representing an increase of 72% (Figure 5.6 and 5.7).

5.3.2 Measures at treated patient level

The total number of DDDs per treated patient was 20.6 compared to 19.4 in 2011. This can be explained by the fact that, although fewer patients were treated, more DDDs were prescribed for each package than in 2011 (Table A5.3 and Table A5.4 in web annex).

5.2. Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

DANMAP 2012

ATC group ^(a)	Therapeutic group	Year									
		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01AA	Tetracyclines	1.07	1.17	1.28	1.38	1.48	1.54	1.61	1.69	1.64	1.76
J01CA	Penicillins with extended spectrum	2.52	2.63	2.79	2.95	3.25	3.26	3.29	3.47	3.41	3.40
J01CE	Beta-lactamase sensitive penicillins	5.07	5.20	5.28	5.40	5.67	5.30	5.12	5.25	5.31	4.68
J01CF	Beta-lactamase resistant penicillins	0.85	0.92	0.97	1.05	1.09	1.12	1.13	1.17	1.14	1.21
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.05	0.06	0.08	0.12	0.19	0.27	0.45	0.68	0.89	1.05
J01D	Cephalosporins and related substances	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.38	0.41	0.44	0.47	0.49	0.49	0.48	0.51	0.50	0.52
J01EB	Short-acting sulfonamides	0.36	0.36	0.35	0.35	0.31	0.28	0.27	0.26	0.24	0.22
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
J01FA	Macrolides	2.13	2.23	2.41	2.31	2.42	2.28	2.21	2.44	2.47	2.19
J01FF	Lincosamides	0.01	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.04
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
J01MA	Fluoroquinolones	0.25	0.28	0.33	0.37	0.44	0.51	0.52	0.57	0.57	0.55
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01
J01XD	Imidazole derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.42	0.43	0.45	0.46	0.47	0.47	0.49	0.51	0.50	0.49
J01XX	Other antibacterials (methenamine >99%)	0.32	0.30	0.28	0.27	0.26	0.27	0.26	0.27	0.26	0.25
J01	Antibacterial agents for systemic use (total)	13.53	14.06	14.75	15.23	16.17	15.91	15.95	16.93	17.06	16.47

a) From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

For the leading groups of antimicrobial agents each treated patient received 11.7–22.3 DDDs in 1.5–1.7 packages with the exception of tetracyclines (47.6 DDDs in 2.1 packages) (Table 5.3). The large number of DDDs prescribed for tetracyclines are primarily due to acne treatment which requires higher dosages for longer periods (Textbox 5).

Three different indicators of antimicrobial consumption at treated patient level in primary health care are available (Figure 5.8). From 2003 to 2012, the largest decreases were observed in DDDs per treated patient (29%) and DDDs per prescribed package (24%) (Table 5.3).

The reasons for the changes at treated patient level are still unclear. However, following the removal of broad codes of indication on prescriptions for antimicrobial agents in early 2012, it is expected that future editions of DANMAP will be able to shed light on the disease-or pathogen-specific reasons for upward or downward trends in consumption of certain antimicrobial agents.

5.3.3 Tetracyclines

In 2012, the overall consumption of tetracyclines increased by 0.12 DID (7.2%) compared to 2011 (Table 5.2). The most commonly used substance was tetracycline (0.78 DID, 44%) followed by doxycycline (0.60 DID, 34%) and lymecycline

(0.38 DID, 22%) (Figure 5.9). Compared to 2011, consumption of doxycycline increased while consumption of tetracycline, lymecycline and oxytetracycline remained relatively constant. Since the early 2000s, a considerable increase in the consumption of tetracyclines has been observed (Table 5.2). This topic is further highlighted in Textbox 5.

5.3.4 Penicillins

The overall consumption of penicillins in 2012 showed a small decrease of 0.41 DID (3.8%) compared to 2011 (Table 5.2). Increases in consumption were observed for 'combination penicillins' (18%) and beta-lactamase resistant penicillins (6%). For the individual substances, the consumption of flucloxacillin, amoxicillin and enzyme inhibitor, and pivmecillinam increased. The consumption of phenoxymethylpenicillin decreased by 29% (Figure 5.10). The use of 'combination penicillins' (primarily amoxicillin/clavulanic acid) is currently advocated for broad treatment of respiratory infections, particularly in patients with exacerbation of chronic obstructive pulmonary disease.

During the past decade (2003–2012), the consumption of penicillins increased by 1.85 DID (22%). This increase was apparent for penicillins with extended spectrum, 'combination penicillins' and beta-lactamase resistant penicillins (Table 5.2). Phenoxymethylpenicillin continues to be the most commonly consumed penicillin; however the order has changed among the other substances during the last decade (Figure 5.10).

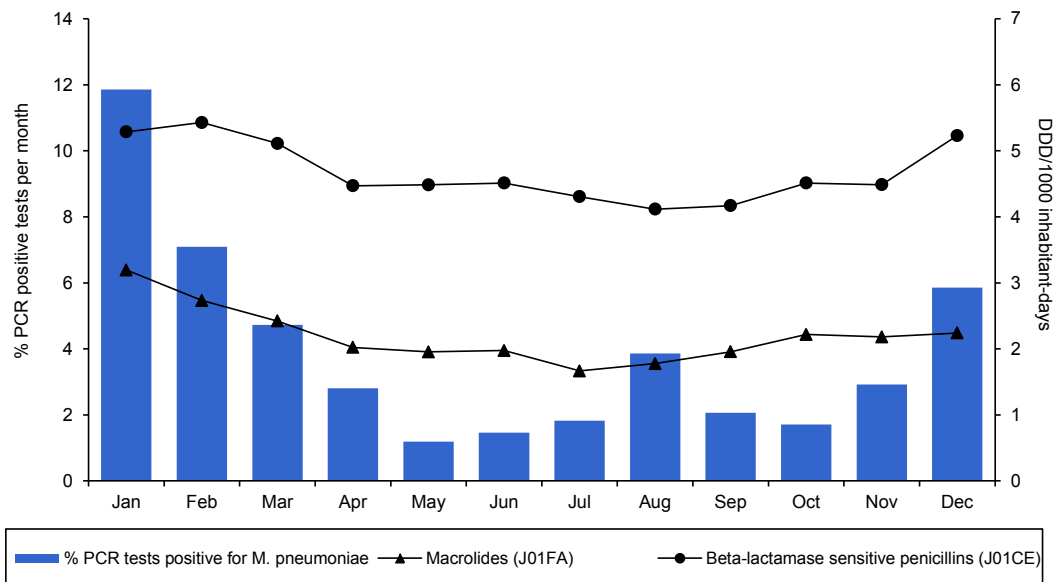
Table 5.3. Number of DDDs and packages per treated patient among leading groups of antimicrobial agents in primary health care, Denmark

ATC group ^{a)}	Therapeutic group	Indicator	Year									
			2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01AA	Tetracyclines	DDD / patient	34.4	36.9	39.0	40.9	43.0	44.4	45.2	45.9	44.0	47.6
		Packages / patient	1.9	1.9	2.0	1.9	2.0	2.0	2.0	2.0	1.9	2.1
		DDD / package	18.1	19.0	19.6	21.0	22.0	22.7	22.7	22.7	22.6	23.1
J01CA	Penicillins with extended spectrum	DDD / patient	13.4	13.6	13.9	14.2	14.4	14.7	14.8	14.9	14.8	16.1
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7
		DDD / package	8.2	8.4	8.5	8.9	9.0	9.2	9.2	9.0	9.2	9.7
J01CE	Beta-lactamase sensitive penicillins	DDD / patient	10.7	11.1	11.3	11.5	11.7	11.8	11.8	11.8	11.8	11.8
		Packages / patient	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4
		DDD / package	7.3	7.5	7.7	8.0	8.2	8.2	8.4	8.4	8.4	8.4
J01CF	Beta-lactamase resistant penicillins	DDD / patient	11.8	12.4	12.7	13.0	13.4	13.7	13.9	14.2	13.8	15.5
		Packages / patient	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.4	1.6
		DDD / package	7.4	7.8	8.0	8.6	8.7	9.0	9.1	9.3	9.6	9.7
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDD / patient	16.6	17.2	16.8	19.3	19.1	19.9	20.4	21.1	21.9	22.3
		Packages / patient	1.8	2.0	2.0	1.8	1.6	1.6	1.5	1.5	1.6	1.6
		DDD / package	9.1	9.1	9.3	10.7	11.7	12.4	13.3	13.7	14.1	14.3
J01FA	Macrolides	DDD / patient	12.1	12.4	12.4	12.6	12.4	12.5	12.5	12.2	11.5	12.4
		Packages / patient	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDD / package	7.8	7.9	8.0	8.3	8.1	8.1	8.1	8.1	7.9	8.0
J01MA	Fluoroquinolones	DDD / patient	10.3	9.5	9.6	10.3	10.6	11.0	11.2	11.2	11.5	11.7
		Packages / patient	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDD / package	6.6	6.4	6.5	6.9	7.0	7.5	7.6	7.6	7.7	7.8
J01	Antibacterial agents for systemic use (total)	DDD / patient	16.4	17.0	17.5	17.9	17.3	18.9	19.2	19.6	19.4	20.6
		Packages / patient	2.1	2.1	2.1	2.0	1.9	2.1	2.1	2.1	2.1	2.1
		DDD / package	7.9	8.1	8.3	8.7	8.9	9.1	9.3	9.3	9.3	9.7

a) From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4. Monthly consumption in 2012 of macrolides and beta-lactamase sensitive penicillins and PCR positive *Mycoplasma pneumoniae* tests in primary health care, Denmark

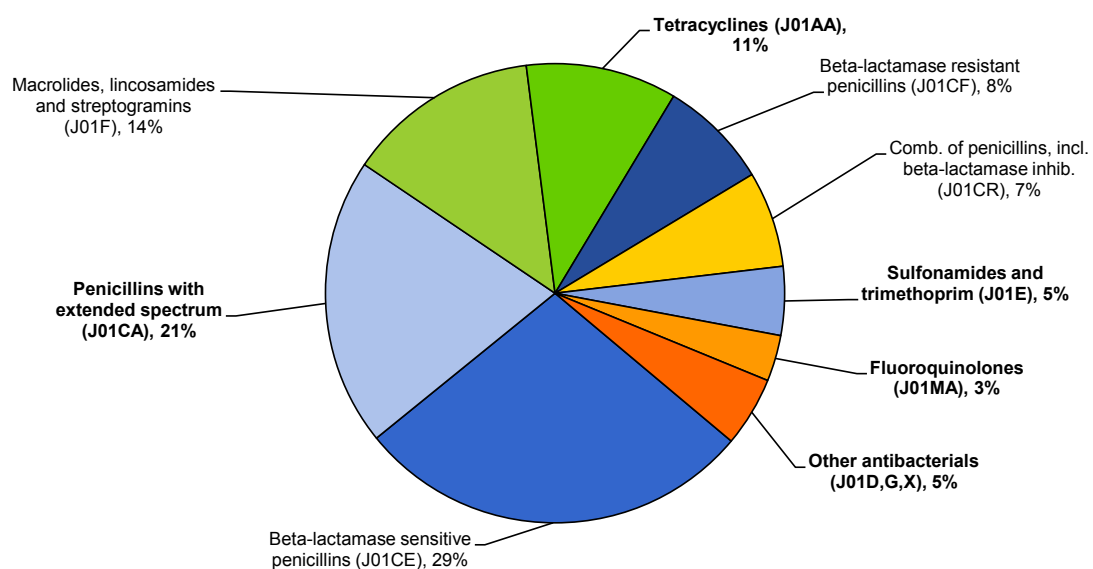
DANMAP 2012



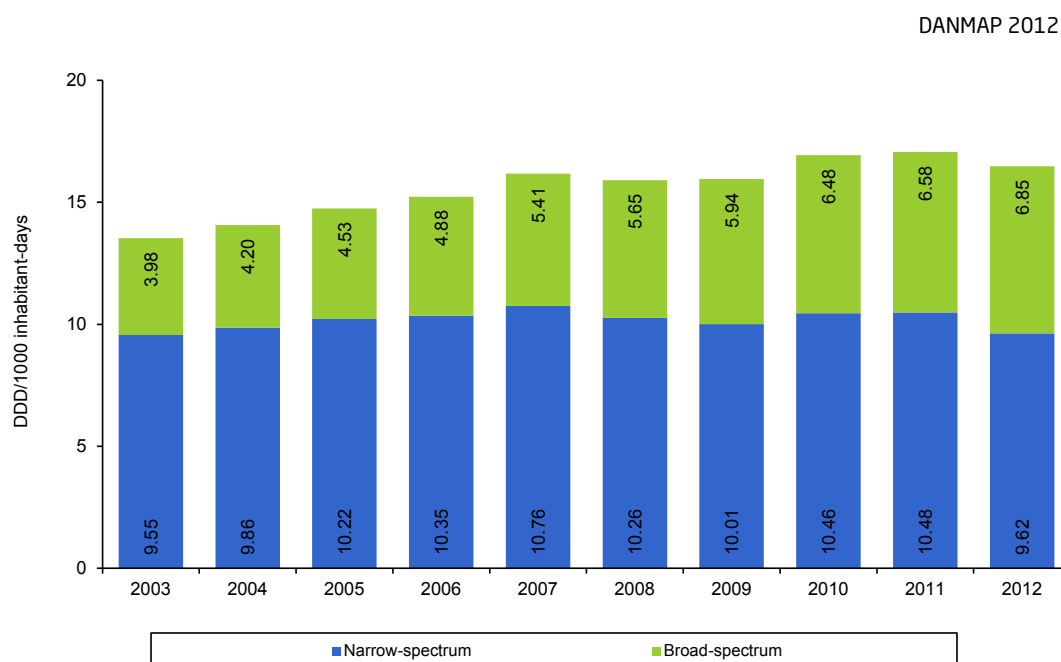
Note: Derived from weekly data representing only those tests sent for analysis at Statens Serum Institut, not national totals

Figure 5.5. Distribution of the total consumption of antimicrobial agents in primary health care, Denmark

DANMAP 2012



Note: Bold highlights indicate broad-spectrum antimicrobial agents

Figure 5.6. Consumption of antimicrobial agents (J01) in primary health care by narrow-spectrum and broad-spectrum agents, Denmark

Note: "Narrow-spectrum" includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofurantoin derivatives, and 'other antibiotics'
 "Broad-spectrum" includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, cephalosporins and related substances, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones, and polymyxins

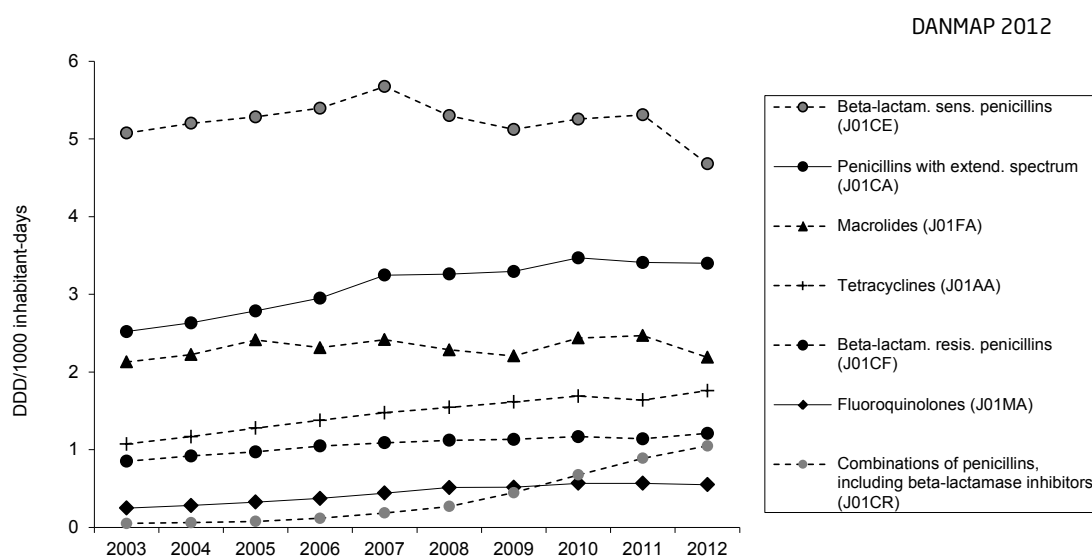
Figure 5.7. Consumption of leading antimicrobial groups for systemic use in primary health care, Denmark

Figure 5.8. Indicators of antimicrobial consumption (J01) in primary health care, Denmark

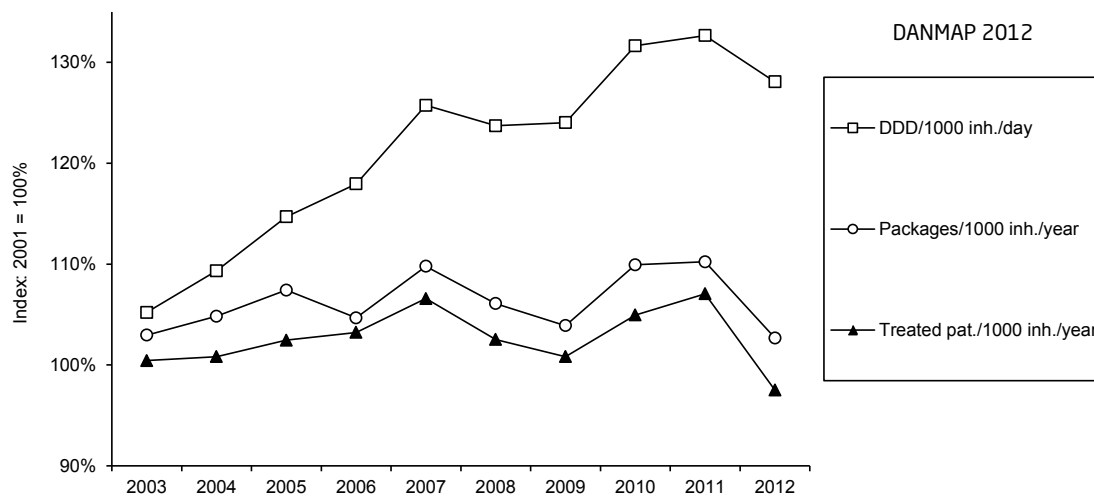


Figure 5.9. Consumption of tetracyclines in primary health care, Denmark

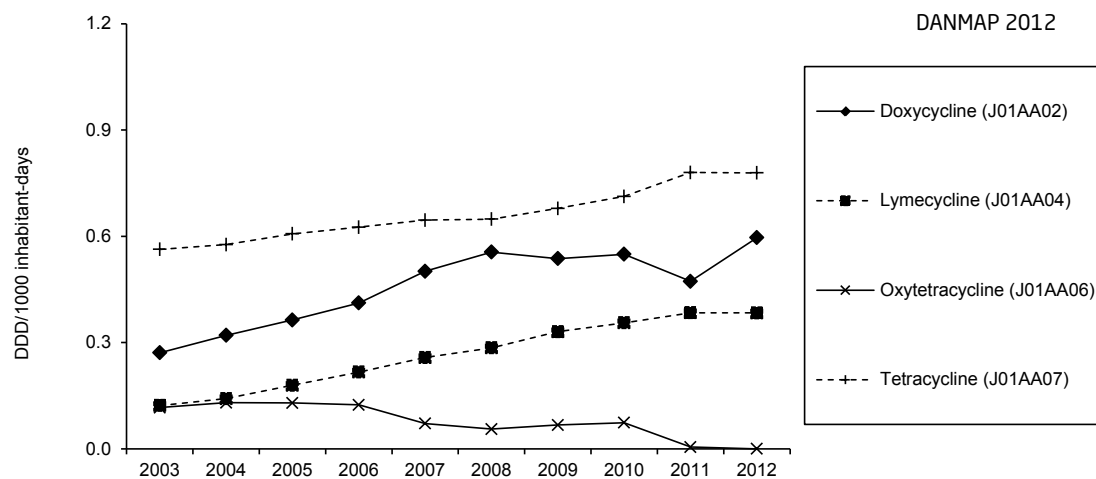
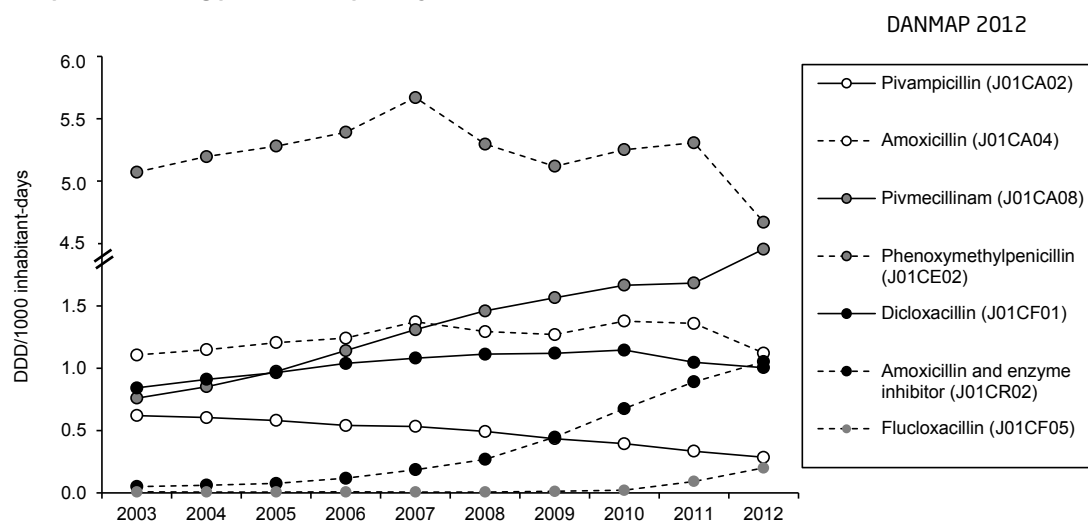


Figure 5.10. Consumption of leading penicillins in primary health care, Denmark



5.3.5 Macrolides

From 2011–2012, the consumption of macrolides decreased by 0.28 DID (11.4%) (Table 5.2). A decreased consumption was observed for all substances apart from clarithromycin (Figure 5.11). The consumption of roxithromycin decreased for the first time since 2008. The high consumption of macrolides in 2010 and 2011 may be explained by two epidemic waves of *M. pneumoniae* infection (DANMAP 2010 & 2011) and the return to a generally lower consumption in 2012 may be explained by a more normal incidence of *M. pneumoniae* infection in 2012 (Figure 5.4).

From 2003 to 2012, the consumption of roxithromycin increased by 0.47 DID, with smaller increases also observed for clarithromycin (0.13 DID) and azithromycin (0.09 DID) (Figure 5.11). The consumption of erythromycin decreased substantially (0.62 DID), most likely in response to changes in national guidelines which in 2004 substituted the first-choice macrolide in primary care from erythromycin to roxithromycin and subsequently also to clarithromycin in 2007.

These two substances are now the recommended first choices for treatment of respiratory infections in people with penicillin allergy or suspected *M. pneumoniae* infection. During the whole period, azithromycin has been the recommended treatment for urethritis/cervicitis and epididymitis.

5.3.6 Fluoroquinolones

The consumption of fluoroquinolones in 2012 (0.55 DID) remained at almost the same level as observed in 2011 (0.57 DID) (Table 5.2). Ciprofloxacin accounted for the majority of the fluoroquinolone consumption (94%), followed by moxifloxacin (4.1%) and ofloxacin (1.6%) (Figure 5.12).

During the past decade, the consumption of fluoroquinolones has increased by 0.30 DID (120%), most likely driven by the introduction of generic versions in Denmark in late 2001 [Jensen *et al.* 2010. J Antimicrob Chemother. 65: 1286–91]. As ciprofloxacin is strongly associated with resistance, this increase is particular grounds for concern.

Figure 5.11. Consumption of macrolides in primary health care, Denmark

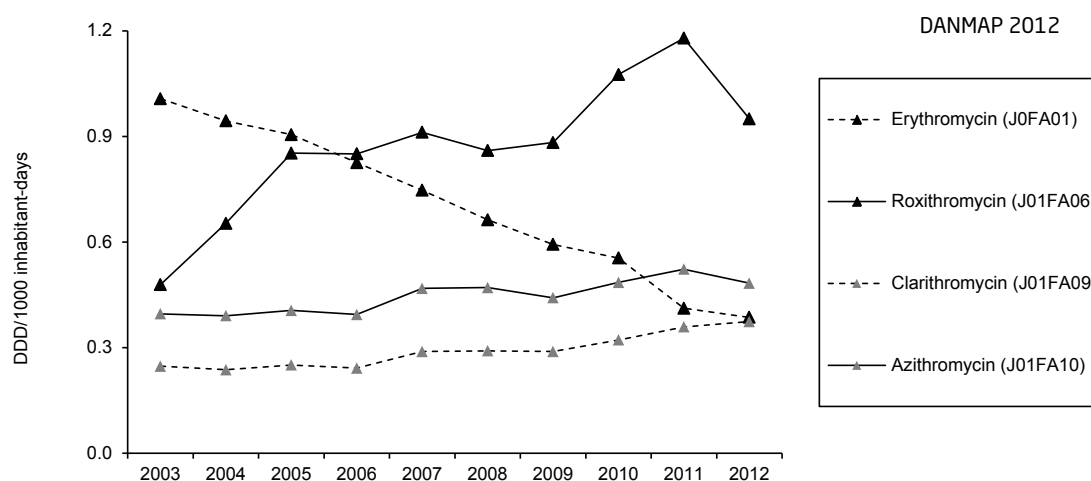
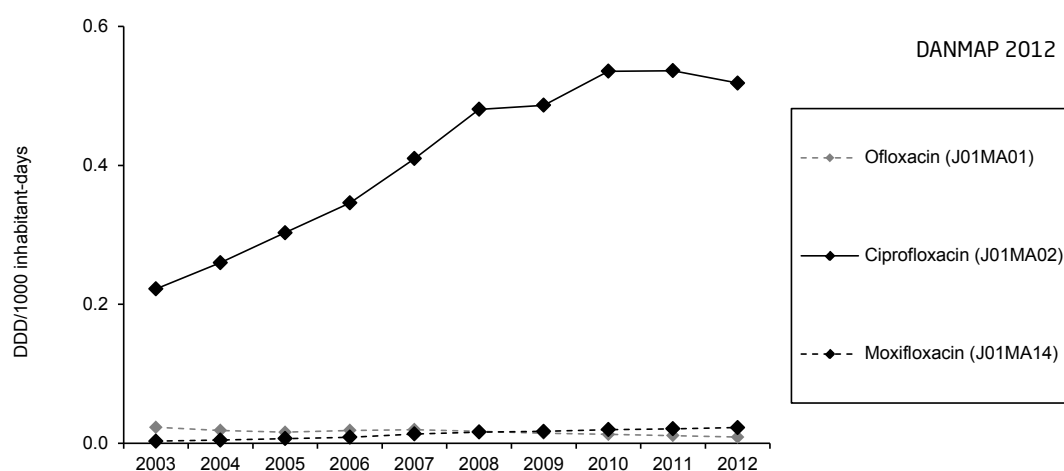


Figure 5.12. Consumption of leading fluoroquinolones in primary health care, Denmark



5.4 Hospital care

5.4.1 Introduction

The consumption of antimicrobial agents in the hospital sector is presented as DDD per 100 occupied bed-days (DBD). Furthermore, data are also presented as DDD per 100 admissions (DAD) to account for hospital activity and as DID to enable comparison with primary health care and to document the consumption across the entire hospital sector, irrespective of hospital activity.

The hospital sector encompasses all hospitals in Denmark, i.e. rehabilitation centres, hospices, private-, psychiatric-, specialized-, and somatic hospitals. Somatic hospitals account for the majority (97%) of the antimicrobial consumption in the hospital sector. Antimicrobial consumption is therefore correlated only to bed-days and admissions in somatic hospitals and not to bed-days and admissions in other hospital types since psychiatric hospitals contribute a large proportion of bed-days and admissions but only a small proportion of the antimicrobial consumption.

The hospitalization pattern in Denmark has changed significantly during the past decade: more people are admitted to somatic hospitals but average length of stay has been shortened (Figure 5.13, Table A5.5 in web annex) and outpatient treatment has increased considerably. Therefore, the hospital activity and subsequent selection pressure for the emergence of resistance were higher in 2012 than in 2003.

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

Consumption (DBD) in somatic hospitals compared to 2011

The consumption of antimicrobial agents in somatic hospitals increased with 2.28 DBD (3%) from 90.84 DBD in 2011 to 93.12 DBD in 2012 (Table 5.4). The distribution of the different antimicrobial agents is shown in Figure 5.14.

From 2011 to 2012, increased consumption was seen for 'combination penicillins' (3.49 DBD, 41%), beta lactamase resistant penicillins (1.07 DBD, 15%), and beta lactamase sensitive penicillins (0.81 DBD, 9%), while decreased consumption was observed for 2nd generation cephalosporins (1.99 DBD, 12%), 3rd generation cephalosporins (0.32 DBD, 23%), carbapenems (0.30 DBD, 7%), and fluoroquinolones (0.68 DBD, 6%) (Table 5.4). The changes were in agreement with the guidelines from the Danish Health and Medicines Authority described in Textbox 3.

Furthermore, tetracyclines increased with 0.40 DBD (34%). This could in part be explained by purchase of a large amount of a tetracycline for prophylactic treatments after *Brucella* exposures in the Capital Region, but in the end only a minor part of the tetracycline was used for treatment (Jenny Dahl Knudsen, personal communication).

Consumption (DBD) in somatic hospitals - the last decade

From 2003–2012, the total consumption of antimicrobial agents increased by 39.4 DBD (73%) (Table 5.4). This increase was due to a combination of increased DDDs and a decreased number of hospital bed-days. During the past decade, the consumption of broad-spectrum antimicrobial agents in the somatic hospitals has increased by 133%, from 27.11 DBD in 2003 to 63.14 DBD in 2012 (Table 5.4).

The changes in leading groups of antimicrobial agents used in hospitals during 2003–2012 are shown in Figure 5.15. The consumption of fluoroquinolones in somatic hospitals increased by 6.12 DBD (157%) and the consumption of carbapenems increased by 3.18 DBD (468%) during the past decade. Consumption of cephalosporins increased by 8.29 DBD (118%) from 2003–2012 (Table 5.4).

Figure 5.13. Number of bed-days and admissions in somatic hospitals, Denmark

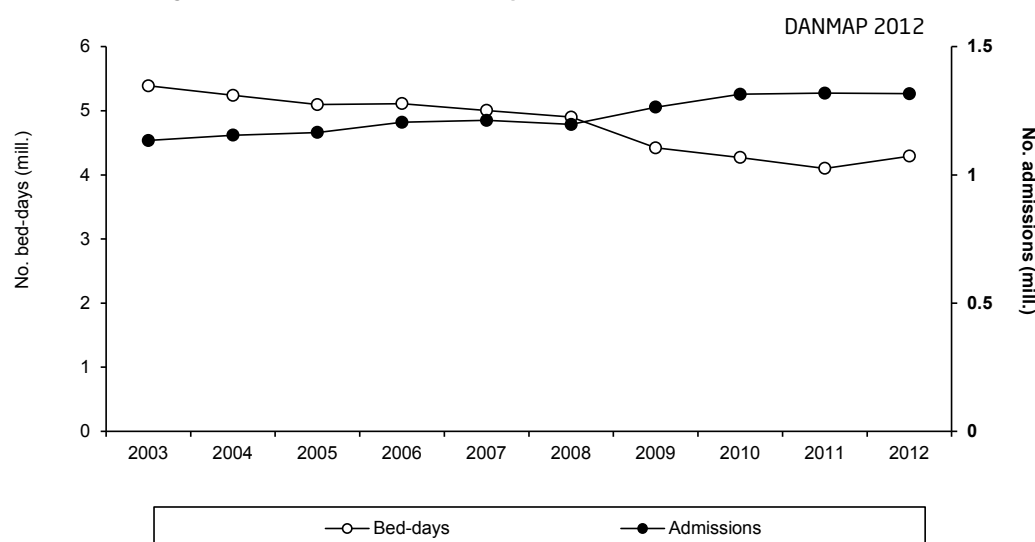
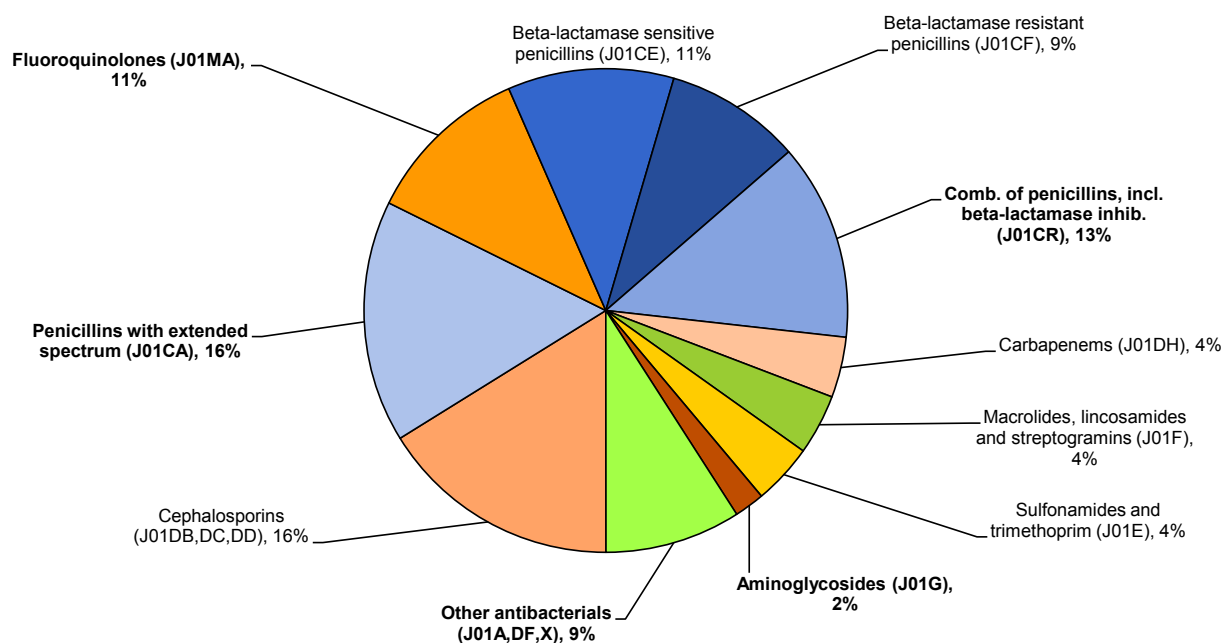


Figure 5.14. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark

DANMAP 2012



Note: Bold highlights indicate broad-spectrum antimicrobial agents

Table 5.4. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark
DANMAP 2012

ATC group ^(a)	Therapeutic group	Year									
		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
J01AA	Tetracyclines	0.30	0.32	0.33	0.39	0.63	0.78	1.04	1.09	1.18	1.58
J01CA	Penicillins with extended spectrum	11.55	11.51	12.90	13.00	13.42	13.96	15.37	14.61	14.41	14.90
J01CE	Beta-lactamase sensitive penicillins	11.85	12.02	12.17	10.67	10.79	9.98	9.90	9.49	9.32	10.13
J01CF	Beta-lactamase resistant penicillins	6.54	6.78	6.71	6.51	6.70	6.81	7.40	7.71	7.30	8.37
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.49	0.84	1.16	1.83	2.95	4.00	5.65	7.13	8.51	12.00
J01DB	First-generation cephalosporins	0.14	0.17	0.15	0.14	0.13	0.18	0.13	0.13	0.13	0.12
J01DC	Second-generation cephalosporins	6.24	6.91	8.39	9.38	12.31	13.32	15.76	16.21	16.14	14.15
J01DD	Third-generation cephalosporins	0.67	0.67	0.83	0.83	1.03	1.25	1.42	1.26	1.39	1.07
J01DF	Monobactams	0.01	0.00	0.00	0.00	0.04	0.07	0.06	0.09	0.19	0.15
J01DH	Carbapenems	0.68	0.85	1.16	1.38	2.13	2.70	3.15	4.02	4.16	3.86
J01EA	Trimethoprim and derivatives	0.43	0.41	0.41	0.42	0.44	0.44	0.44	0.36	0.36	0.38
J01EB	Short-acting sulfonamides	1.14	1.06	0.99	0.75	0.34	0.35	0.35	0.33	0.25	0.20
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	1.54	1.86	2.11	2.12	1.52	1.95	2.28	3.04	4.11	3.33
J01FA	Macrolides	2.95	2.85	2.89	2.83	3.08	3.06	3.42	3.52	3.69	3.56
J01FF	Lincosamides	0.22	0.23	0.24	0.31	0.35	0.41	0.50	0.47	0.53	0.62
J01GB	Aminoglycosides	1.71	2.00	1.95	1.81	1.79	1.64	1.56	1.71	1.91	2.14
J01MA	Fluoroquinolones	3.90	4.93	6.14	6.74	8.16	9.53	10.71	10.44	10.70	10.02
J01XA	Glycopeptides	0.42	0.47	0.52	0.56	0.63	0.68	0.99	1.07	1.24	1.29
J01XB	Polymyxins	0.03	0.06	0.12	0.12	0.05	0.05	0.07	0.10	0.09	0.09
J01XC	Steroid antibacterials (fusidic acid)	0.22	0.22	0.25	0.28	0.28	0.26	0.31	0.34	0.27	0.23
J01XD	Imidazole derivatives	2.32	2.43	2.62	2.78	2.62	3.27	3.84	3.93	4.19	4.16
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.30	0.28	0.29	0.29	0.28	0.29	0.36	0.31	0.33	0.34
J01XX05	Methenamine	0.08	0.10	0.08	0.11	0.09	0.10	0.09	0.08	0.10	0.09
J01XX08	Linezolid	0.04	0.07	0.15	0.20	0.16	0.21	0.22	0.22	0.32	0.32
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02
J01	Antibacterial agents for systemic use (total)	53.77	57.04	62.58	63.47	69.94	75.28	85.03	87.72	90.84	93.12

a) From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

5.4.3 Other measures of somatic hospital consumption

DDD per 100 admissions (DAD)

In Denmark, the hospitalization pattern has changed over the last decade. Today, more patients are admitted to the somatic hospitals but each length of stay has been shortened. It can therefore be relevant to measure the hospital consumption in relation to admissions.

When expressed as DAD (DDD per 100 admissions), the total consumption of antimicrobial agents in Danish somatic hospitals increased by 8% from 2011 to 2012 (Table 5.5). Increases in consumption were partly observed for the same therapeutic groups as when expressed in DBD: 'combination penicillins' (12.67 DAD, 48%), beta lactamase resistant penicillins (4.59 DAD, 20%), beta lactamase sensitive penicillins (4.06 DAD, 14%), penicillins with extended spectrum (3.83 DAD, 9%)

and tetracyclines (1.49 DAD, 41%). A decreased consumption was observed for 2nd generation cephalosporins (4.02 DAD, 8%), fluoroquinolones (0.63 DAD, 2%) and 3rd generation cephalosporins (0.83 DAD, 19%).

From 2003–2012, the consumption of antimicrobial agents increased by 19%, from 255.59 DAD in 2003 to 303.71 DAD in 2012. As observed in previous years, this increase was primarily driven by an increase in the number of DDDs but counterbalanced by an increase in the number of hospital admissions.

DDD per 1,000 inhabitants per day (DID)

The consumption of antimicrobial agents in somatic hospitals has increased by 0.50 DID (33%) from 2003 to 2012. Broad-spectrum agents have increased by 0.60 DID (79%), comprising 68% of the total consumption in 2012 compared to 50% in 2003 (Figure 5.16).

Katrin Gaardbo Kuhn, Maja Laursen, Stefan S. Olsen,
Line Skjot-Rasmussen and Anette M. Hammerum

Table 5.5. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

DANMAP 2012											
ATC group ^(a)	Therapeutic group	Year									
		2003	2004	2005	2006	2007	2008 ^(b)	2009	2010	2011	2012
J01AA	Tetracyclines	1.43	1.45	1.45	1.67	2.59	3.19	3.63	3.55	3.66	5.15
J01CA	Penicillins with extended spectrum	54.88	52.22	56.43	55.13	55.39	57.18	53.76	47.46	44.77	48.60
J01CE	Beta-lactamase sensitive penicillins	56.33	54.53	53.20	45.26	44.55	40.90	34.61	30.83	28.98	33.04
J01CF	Beta-lactamase resistant penicillins	31.11	30.77	29.33	27.60	27.64	27.89	25.86	25.04	22.71	27.30
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	2.35	3.82	5.09	7.77	12.17	16.37	19.74	23.15	26.47	39.14
J01DB	First-generation cephalosporins	0.67	0.76	0.67	0.60	0.55	0.72	0.46	0.43	0.41	0.40
J01DC	Second-generation cephalosporins	29.66	31.36	36.70	39.76	50.81	54.55	55.12	52.65	50.19	46.17
J01DD	Third-generation cephalosporins	3.17	3.06	3.62	3.53	4.24	5.10	4.98	4.10	4.33	3.50
J01DF	Monobactams	0.02	0.02	0.02	0.00	0.18	0.27	0.21	0.29	0.60	0.48
J01DH	Carbapenems	3.24	3.85	5.05	5.86	8.78	11.08	11.01	13.07	12.55	12.60
J01EA	Trimethoprim and derivatives	2.05	1.86	1.78	1.78	1.81	1.80	1.56	1.17	1.11	1.23
J01EB	Short-acting sulfonamides	5.44	4.82	4.32	3.18	1.41	1.43	1.21	1.09	0.78	0.63
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	7.32	8.44	9.21	8.98	6.28	7.98	7.96	9.88	12.79	10.87
J01FA	Macrolides	14.03	12.92	12.64	12.01	12.70	12.53	11.97	11.45	11.47	11.61
J01FF	Lincosamides	1.05	1.04	1.05	1.31	1.46	1.69	1.74	1.52	1.63	2.01
J01GB	Aminoglycosides	8.14	9.07	8.55	7.68	7.39	6.71	5.45	5.56	5.95	6.99
J01MA	Fluoroquinolones	18.53	22.38	26.87	28.58	33.66	39.04	37.45	33.92	33.30	32.67
J01XA	Glycopeptides	1.97	2.12	2.28	2.38	2.61	2.77	3.48	3.47	3.87	4.20
J01XB	Polymyxins	0.14	0.27	0.54	0.53	0.22	0.21	0.24	0.32	0.28	0.30
J01XC	Steroid antibacterials (fusidic acid)	1.04	1.01	1.11	1.19	1.17	1.05	1.09	1.12	0.85	0.76
J01XD	Imidazole derivatives	11.03	11.02	11.47	11.81	10.83	13.39	13.43	12.76	13.03	13.55
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	1.41	1.26	1.28	1.24	1.17	1.19	1.27	1.01	1.02	1.12
J01XX05	Methenamine	0.37	0.45	0.36	0.46	0.38	0.43	0.31	0.27	0.32	0.28
J01XX08	Linezolid	0.21	0.34	0.64	0.86	0.68	0.84	0.76	0.72	0.99	1.04
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.03	0.06	0.06	0.07	0.05	0.06
J01	Antibacterial agents for systemic use (total)	255.59	258.81	273.67	269.18	288.70	308.39	297.36	284.89	282.53	303.71

a) From the 2012 edition of the Anatomical Therapeutic Chemical (ATC) classification system

b) The number of admissions was affectedly low in 2008 due to a major hospital strike

Figure 5.15. Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), Denmark

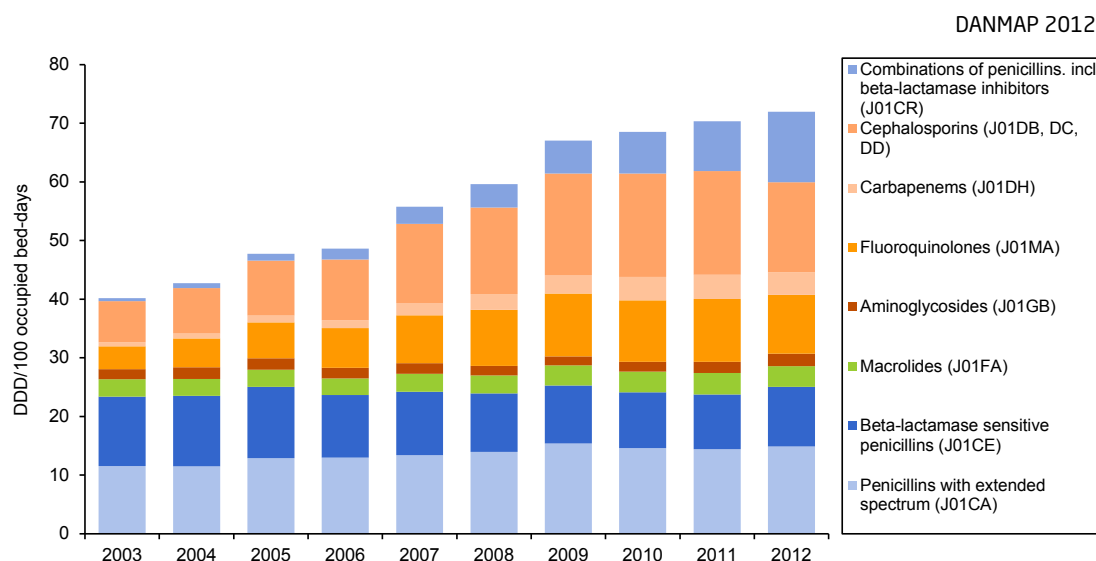
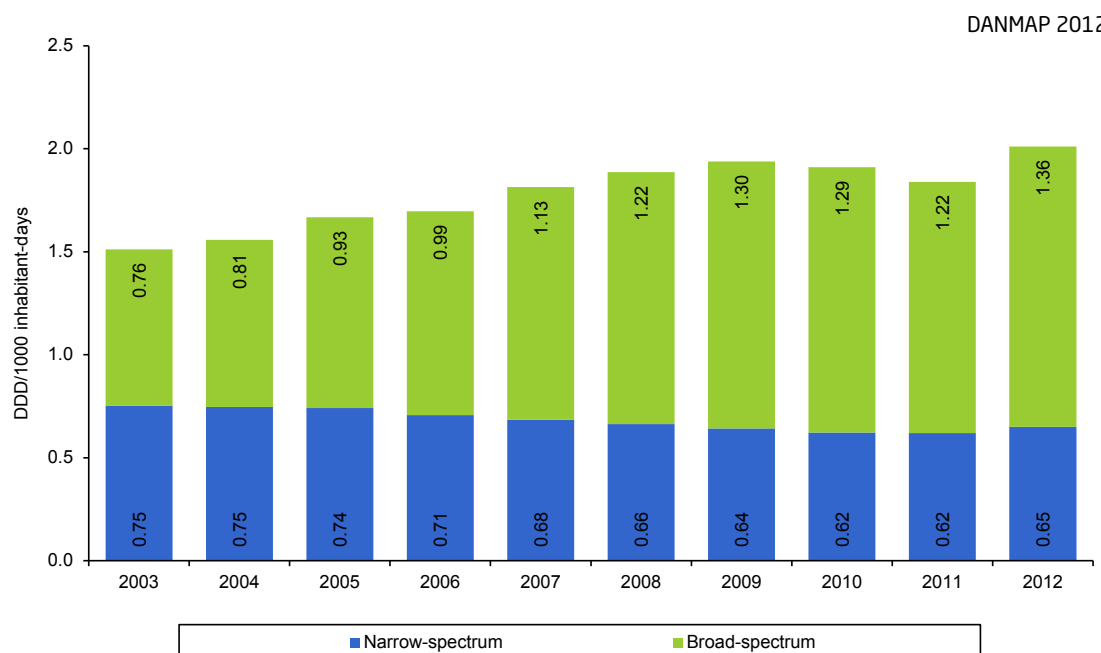


Figure 5.16. Consumption of antimicrobial agents (J01) in hospital care by narrow-spectrum and broad-spectrum agents, Denmark



Note: "Narrow-spectrum" antibiotics includes: beta-lactamase sensitive penicillins, first-generation cephalosporins, beta-lactamase resistant penicillins, monobactams, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofurantoin derivatives, and 'other antibiotics'

"Broad-spectrum" includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, second-generation cephalosporins, third-generation cephalosporins, carbapenems, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones, and polymyxins

Citizen-centered website on how and when to use antimicrobial agents

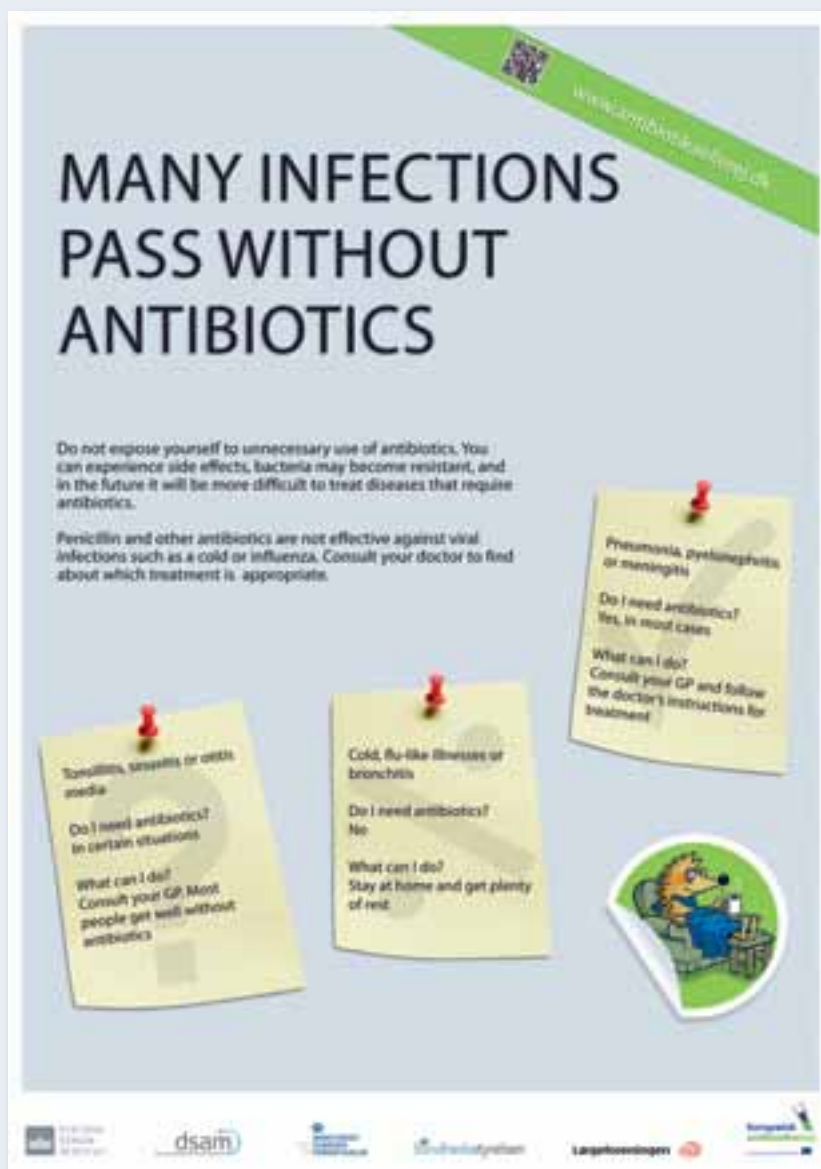
At the European Antibiotic Awareness Day in November 2012, the website antibiotikaellerej.dk about antimicrobial agents and resistance was launched in cooperation between the Ministry of Health, the Danish Health and Medicines Authority, the Danish College of General Practitioners, the Danish Medical Association, and Statens Serum Institut. The purpose of the website is to focus on our use of antimicrobial agents and help to raise awareness about when it is appropriate to get antimicrobial agents.

On the citizen-centered website one may find answers on when and when not there is a need for antimicrobial agents as well as why it is important to only use antimicrobial agents when necessary.

Furthermore, the website describes two case stories with patients infected with multi-resistant bacteria. The website has information about what the patient can do to get well, as well as a sub-site with frequently asked questions.

Line Skjøl-Rasmussen, Katrin G. Kuhn, Berit Jørgensen, Lars Bjerrum, Tove Rønne, Grith Strøbæk, Anette M. Hammerum and Robert L. Skov

For further information: Robert L. Skov (rsk@ssi.dk)



Excessive use of tetracyclines for acne treatment among young Danish adults

Background: Acne vulgaris is a disease of the sebaceous glands, caused either by infection with *Propionibacterium acnes*, sebaceous gland hyperplasia or excessive production of sebum. In Northern Europe, acne affects between 80-85% of teenagers and the most severe cases are observed in 17–19 year old males. Tetracyclines are used as treatment for acne vulgaris in adolescents worldwide.

In Denmark, a large increase in consumption of antimicrobial agents in primary health care was recently shown to be driven by 15–19 year olds, with particular increases for tetracyclines [Textbox 3, DANMAP 2011].

In this study, we investigated the consumption of tetracyclines in young adults and the population as a whole by collecting consumption data and codes of indication from tetracycline prescriptions from 2008 to 2011.

Methods: Consumption data for antimicrobial agents and indication codes from general practitioner tetracycline prescriptions from 2008 to 2011 were obtained from National Register of Medicinal Products Statistics. Prescription indication codes specify the disease/infection for which the antimicrobial agent has been prescribed, including the two broad options 'against infection' and 'against inflammation'. Completing the indication code field on a prescription was not mandatory for Danish general practitioners in the period 2008–2011.

Data were analysed by descriptive statistics and two-tailed t-tests using the STATA™ software 11.0 (Statacorp., Lakeway, TX, USA).

Results: In 2008, tetracyclines accounted for 4.5 DID (29%) of all antimicrobial agents prescribed to 15–19 year olds (Figure 1). By 2011, this had increased to 5.4 DID (33%). For all age groups (including 15–19 year olds), tetracyclines accounted for 1.6 DID (10%) of all prescribed antimicrobial agents in 2008 and 1.7 DID (9.6%) in 2011. For 15–19 year olds, penicillins accounted for 7 DID (45%) of all prescribed antimicrobial agents in 2008 and 7.3 DID (45%) in 2011. In the whole population, penicillins accounted for 10 DID (63%) in 2008 and 11 DID (63%) in 2011 (Figure 1).

In 2008, 47% of tetracycline for 15–19 year olds were prescribed with an indication code of acne and 55% with codes indicating any skin disorder (acne, 'skin problems', 'unclean skin' or 'skin disorders') (Figure 2). By 2011, 53% of tetracyclines in 15–19 year olds were prescribed against acne and 59% against skin disorders in total. During 2008–2011, indication codes were missing on 21–28% of the tetracycline prescriptions (Figure 2).

Discussion: From 2008 to 2011, tetracyclines accounted for 29–33% of all antimicrobial consumption in young Danish adults but only around 10% in the general population. In comparison, penicillins, which are the most common used antimicrobial agents in Denmark, accounted for 45% of the consumption in 15–19 year olds and 63% of antimicrobial consumption in all age groups during the study period. These numbers confirm that tetracyclines are used to a great extent among adolescents, reaching almost the same consumption level as penicillins. Further, the consumption of tetracyclines remained at a constantly high level during the four years of interest. Even though all prescriptions did not have codes of indication, many of the prescriptions with tetracyclines were given for skin disorders like acne.

An important problem with the widespread use of the broad-spectrum antimicrobial agents, such as tetracyclines, for acne treatment is the development of resistant bacteria. Treatment regimens for acne are generally long, varying between 8 to 24 weeks with an average of 12 weeks which strongly increases the selection for resistant bacteria.

Katrin G. Kuhn, Maja Laursen and Anette M. Hammerum

For further information: Anette M. Hammerum (ama@ssi.dk)

Figure 1. Consumption of tetracyclines (J01A), penicillins (J01C) and other antimicrobial agents in 15-19 year olds and all age groups in Denmark, 2008-2011

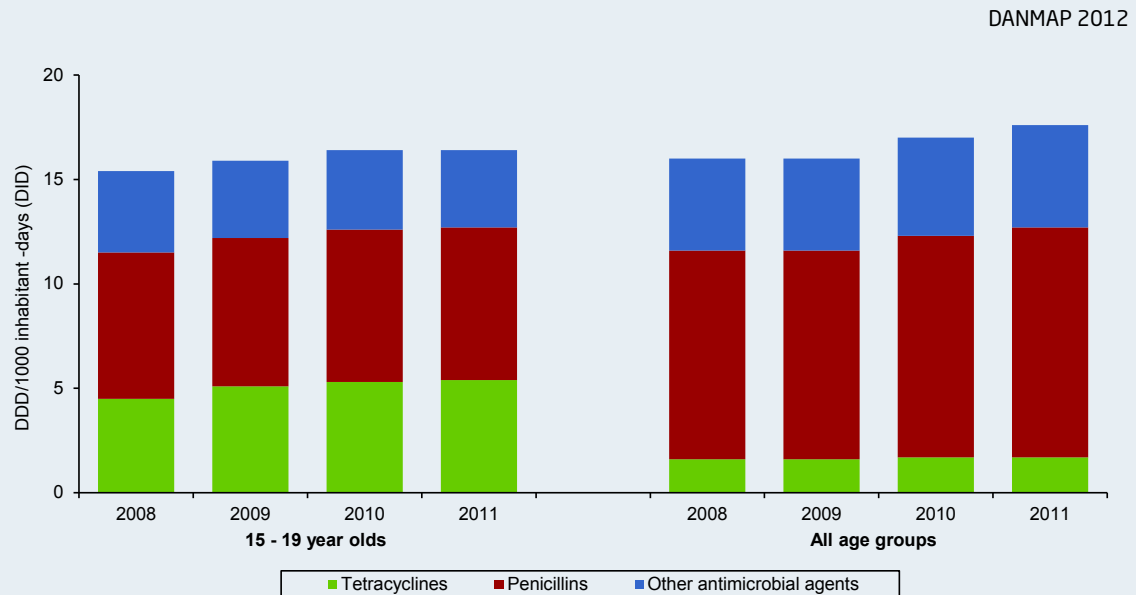
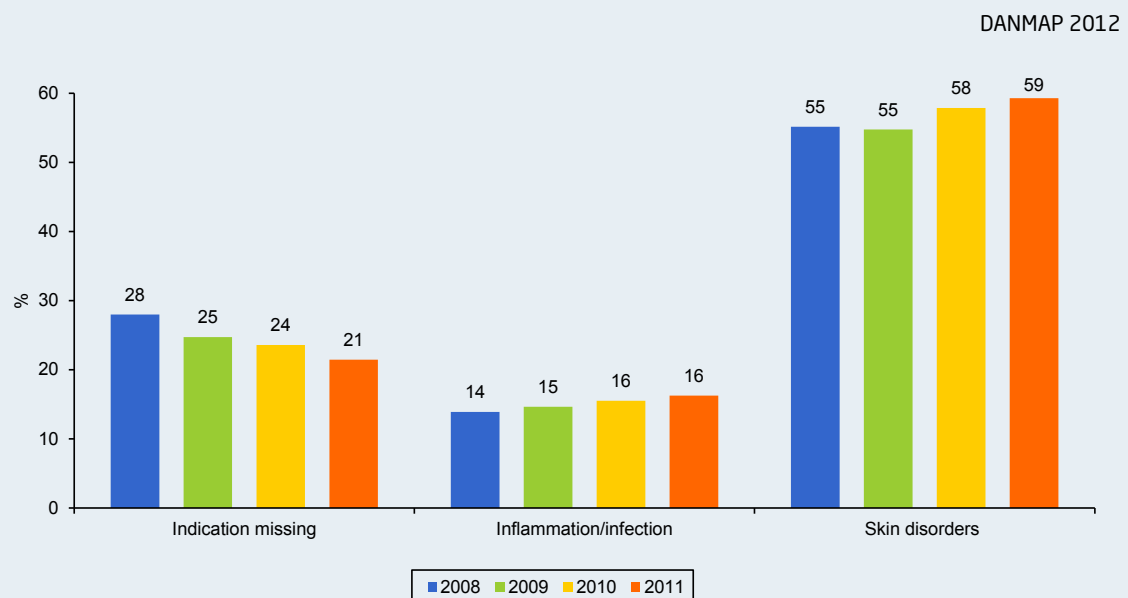


Figure 2. Distribution (%) of indication codes for tetracycline prescriptions in 15-19 year olds in Denmark, 2008-2011





6. Resistance in zoonotic bacteria

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact or indirectly by contaminated food. Zoonotic bacteria, such as *Salmonella* and *Campylobacter*, can develop resistance towards antimicrobial agents as a result of treatment of both animals and humans, which subsequently may lead to limited treatment possibilities or even treatment failure of human infectious diseases. A more 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 2012 [www.food.dtu.dk].

6.1 *Salmonella*

Salmonella is the second most important zoonotic bacterial pathogen in Denmark and can have a severe economic impact on both animal production and human productivity.

In Denmark and the rest of European Union, *S. Enteritidis* and *S. Typhimurium* are the serovars most frequently associated with human illness. Human cases caused by *S. Enteritidis* are most commonly associated with the consumption of contaminated eggs or poultry meat, whereas

S. Typhimurium cases are mostly associated with the consumption of contaminated pork, beef or poultry meat.

For *Salmonella*, DANMAP 2012 includes isolates from broiler, layer and cattle farms reported infected during 2012 as well as isolates from Danish and imported broiler meat, turkey meat, beef and pork collected as part of national surveillance and control programmes. From pigs, *Salmonella* isolates recovered from sow herds as well as multiplier and breeding herds as part of the *Salmonella* control programme are included. In addition, faecal samples from healthy pigs were collected at the slaughterhouses and cultured for *Salmonella* as part of the DANMAP programme. Isolates from all reported human cases are included. Only one isolate per farm, meat sample or human case was included, and data are presented in the report where a sufficient number of isolates were obtained (>15). For details on methodology see Chapter 10, Material and Methods.

In DANMAP 2012, we primarily present resistance among *S. Typhimurium*. During the last ten years, the numbers of *S. Enteritidis* isolates from infected poultry flocks and meat thereof have been decreasing, and therefore resistance in *S. Enteritidis* will not be presented in this report. This year however, the occurrence of resistance among *Salmonella* spp. from pigs and Danish pork is presented (Table 6.1).

Table 6.1. Resistance (%) among *Salmonella* Spp. from pigs and Danish pork, Denmark

Antimicrobial agent	DANMAP 2012	
	Pigs	Pork
	%	Danish %
Tetracycline	41	33
Chloramphenicol	5	2
Florfenicol	3	2
Ampicillin	31	28
Ceftiofur	0	0
Cefotaxime	0	0
Sulfonamide	36	35
Trimethoprim	9	7
Apramycin	2	0
Gentamicin	3	1
Neomycin	2	2
Spectinomycin	11	8
Streptomycin	37	34
Ciprofloxacin	0	0
Nalidixic acid	0	0
Colistin	1	2
Fully sensitive	46	52
Multi-resistant	34	32
Number of isolates	374	120

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

Among all the *Salmonella* isolates from Danish pigs (n = 374) and pork (n = 120), we observed high levels (28% to 41%) of resistance to ampicillin, streptomycin, sulfonamide, and tetracycline. None of the isolates were resistant to cephalosporins (ceftiofur, cefotaxime) or quinolones (ciprofloxacin, nalidixic acid).

The most common serotypes from Danish pigs and pork were *S. Derby* (47% and 38%, respectively), *S. Typhimurium* including the monophasic variants (39% and 34%, respectively) and *S. Infantis* (5% and 7%, respectively).

Note that the isolates from pigs in Table 6.1 and 6.2 include isolates from pen faecal samplings from the national control programme as well as isolates from the DANMAP sampling of healthy pigs. Thus the serotype distributions is not exactly as presented for the slaughter pig herds in Textbox 6.

As the majority of the *S. Derby* isolates were fully sensitive, the overall occurrences of resistance among *Salmonella* spp. in pigs and Danish pork were lower than observed among the *S. Typhimurium* isolates only.

Even though *S. Derby* is very common among pigs, relatively few human *S. Derby* cases are reported in Denmark [Annual Report on Zoonoses in Denmark 2012].

In DANMAP, *S. Typhimurium* includes the monophasic variants with antigenic formulas *S.* 4,5,12:i:- and *S.* 4,12:i:-, as recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826]. In the text, generic *S. Typhimurium* indicates results only covering isolates of the non-monophasic variants. Since 2012, routine analyses of *S. Typhimurium* phage types are no longer performed in Denmark.

MIC distributions for *S. Typhimurium* from pigs, Danish pork and humans, as well as for *Salmonella* spp. from pigs and Danish pork in 2012 are presented in the web annex (Tables A6.1-A6.5). Data for each of the figures are also presented in the web annex.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the ten included antimicrobial classes (see Table 10.3).

6.1.1 *S. Typhimurium* in pigs and pork

S. Typhimurium isolated from pigs (*n* = 144) had very high levels of resistance to ampicillin (65%), streptomycin (67%), sulfonamide (67%), and tetracycline (65%, Table 6.2), and the occurrence of resistance for all four antimicrobial agents increased compared with 2011 (Figure 6.1). Co-resistance of these four antimicrobial agents is often called the ASSuT resistance-profile, also when resistant to additional antimicrobial agents.

Since 2007, ASSuT resistance among *S. Typhimurium* in pigs has been increasing, partly due to an increase in the prevalence of the monophasic variants of *S. Typhimurium* often carrying the ASSuT resistance-profile (Figure 6.2). In 2012, 73% of the monophasic variants were ASSuT resistant compared with 27% among the generic (e.g. non-monophasic) *S. Typhimurium* isolates.

In 2012, the monophasic *S. Typhimurium* variants constituted 56% of the total number of *S. Typhimurium* isolates from pigs, representing 72% of the multi-resistant isolates.

Table 6.2. Resistance (%) among *Salmonella Typhimurium*^(a) from pigs, Danish pork and human cases^(b), Denmark

DANMAP 2012

Antimicrobial agent	Pigs	Pork	Human			
	%	Danish %	Domestic sporadic %	Domestic outbreak %	Travel abroad %	Unknown origin %
Tetracycline	65	51	54	92	73	64
Chloramphenicol	9	2	9	0	20	11
Florfenicol	8	2	4	0	19	6
Ampicillin	65	56	58	92	71	61
Ceftiofur	0	0	1	0	5	2
Cefotaxime	0	0	-	-	-	-
Sulfonamide	67	61	65	92	71	64
Trimethoprim	6	5	6	0	12	9
Apramycin	5	0	1	0	0	0
Gentamicin	5	0	2	0	7	2
Neomycin	3	0	2	0	2	2
Spectinomycin	13	2	8	0	17	11
Streptomycin	67	59	62	94	71	60
Ciprofloxacin	0	0	2	0	24	6
Nalidixic acid	0	0	1	0	14	5
Colistin	0	0	1	0	2	1
Fully sensitive	22	29	25	6	20	24
Multi-resistant	66	59	62	92	76	62
Number of isolates	144	41	177	48	59	127

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

a) Include isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas *S.* 4,[5],12:i:-

b) An isolate was categorised as 'domestic sporadic' if the patient did not travel outside Denmark one week prior to the onset of the disease and was not reported as being part of an outbreak

The prevalence of *S. Typhimurium* infected slaughter pig herds was 7.6% in 2012 (Textbox 6.), and as 66% of the *S. Typhimurium* isolates were multiresistant, the prevalence of pig herds with multi-resistant *S. Typhimurium* amounts to approximately 5%.

In Danish pork (n = 41), 56% of the isolates were of the monophasic variants and high levels (34%) of ASSuT resistance were observed. Levels of resistance were comparable among *S. Typhimurium* isolates from pigs and Danish pork (Table 6.2). Thus, the unexplained differences in resistance levels among isolates from pigs and pork observed in 2011 were not observed in 2012.

Resistance to the other tested antimicrobial agents was similar to levels reported in 2011. However, results showed a decrease in resistance to neomycin in isolates from pigs and to spectinomycin in pork, compared with 2011. None of the *S. Typhimurium* isolates from Danish pigs and pork were resistant to cephalosporins (ceftiofur, cefotaxime), quinolones (ciprofloxacin, nalidixic acid) or colistin (Table 6.2).

It is also important to note that the occurrence of *S. Typhimurium* isolates fully sensitive to all included antimicrobial agents have been decreasing since 2008, this is the case for isolates both from pigs and Danish pork.

Among the generic *S. Typhimurium* isolates from pigs, 48% of the isolates were fully sensitive isolates, a level comparable to previous years, whereas the proportion have been slightly decreasing in the monophasic isolates, and only one (4%) of the monophasic isolates from pigs were fully sensitive in 2012.

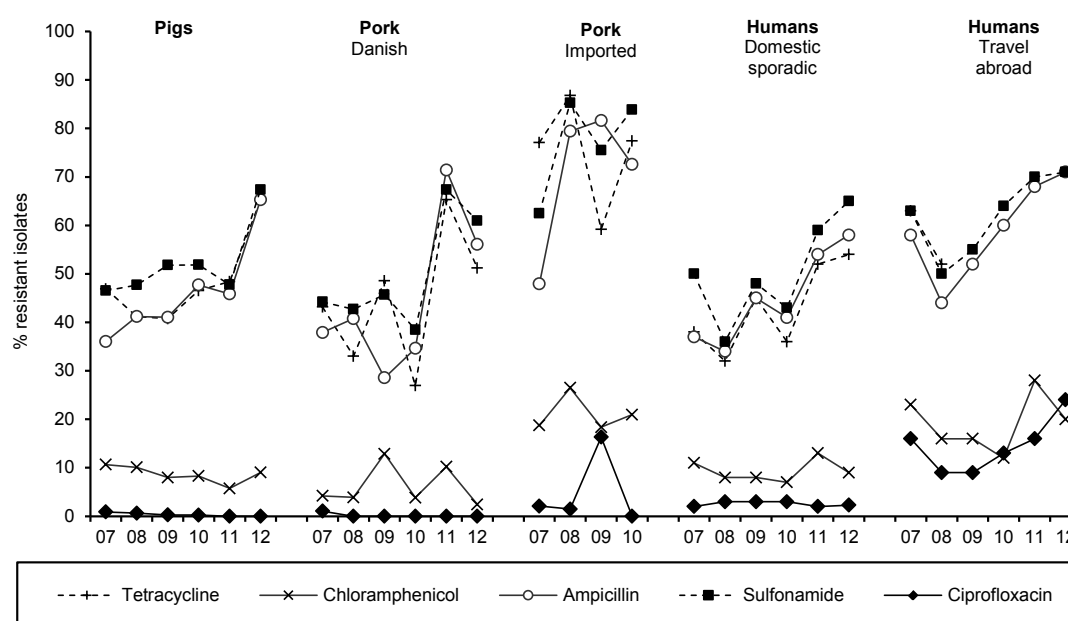
The increased occurrence of monophasic *S. 4,[5],12:i:-* are not an isolated Danish phenomenon, but these variants are considered new pandemic strains of *Salmonella* in Europe [Hopkins et al. 2010, Eurosurveillance 3;1].

In the EFSA Summary Report on antimicrobial resistance 2011 [EFSA, 2013], four to seven Member states, including Denmark, report resistance in generic *S. Typhimurium* and the monophasic variants *S. 4,[5],12:i:-* from pigs and pork. The levels of ASSuT resistance are comparable between the Member States.

In 2004, monophasic variants constituted 3% of the *S. Typhimurium* from pigs and pork in the EU but 20% in 2011 [EFSA, 2013]. Likewise, in 2010 monophasic variants accounted for 1.7% of all human cases in EU, increasing to 4.7% in 2011 [EFSA, 2013].

Figure 6.1. Resistance (%) in *Salmonella Typhimurium* in^(a) pigs, pork and human cases^(b), Denmark

DANMAP 2012



Note: The number of isolates varies between years (pigs: n = 144–563, Danish pork: n = 26–103, imported pork: n = 48–68, domestic sporadic human cases: n = 98–269 and travel related human cases: n = 55–117). Data for imported pork in 2011 and 2012 are not presented due to insufficient number of isolates

a) Include isolates verified as monophasic variants of *S. Typhimurium* with antigenic formulas *S. 4,[5],12:i:-*

b) An isolate is categorised as 'domestic sporadic' if the patient did not travel outside Denmark one week prior to the onset of disease and was not reported as being part of an outbreak

6.1.2 *Salmonella* in humans

In 2012, *Salmonella* continued to be the second most frequent cause of bacterial intestinal infections in Denmark. A total of 1,198 human laboratory-confirmed cases of salmonellosis were reported (21.4 cases per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2012]. This year, *S. Enteritidis* isolates from humans have not been susceptibility tested. A total of 242 confirmed *S. Enteritidis* cases were reported (4.3 cases per 100,000 inhabitants), of which more than 75% were associated with travel outside Denmark.

As in previous years, SSI collected information on travel history through phone interviews. Patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to the onset of the disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as “domestically acquired” if the patients had not travelled within the last week prior to the onset of the disease. Patients were categorised as of ‘unknown origin’ if no telephone interview was conducted and travel information had not been reported to the general practitioners. In 2012, travel information was obtained for 69% of the *Salmonella* cases.

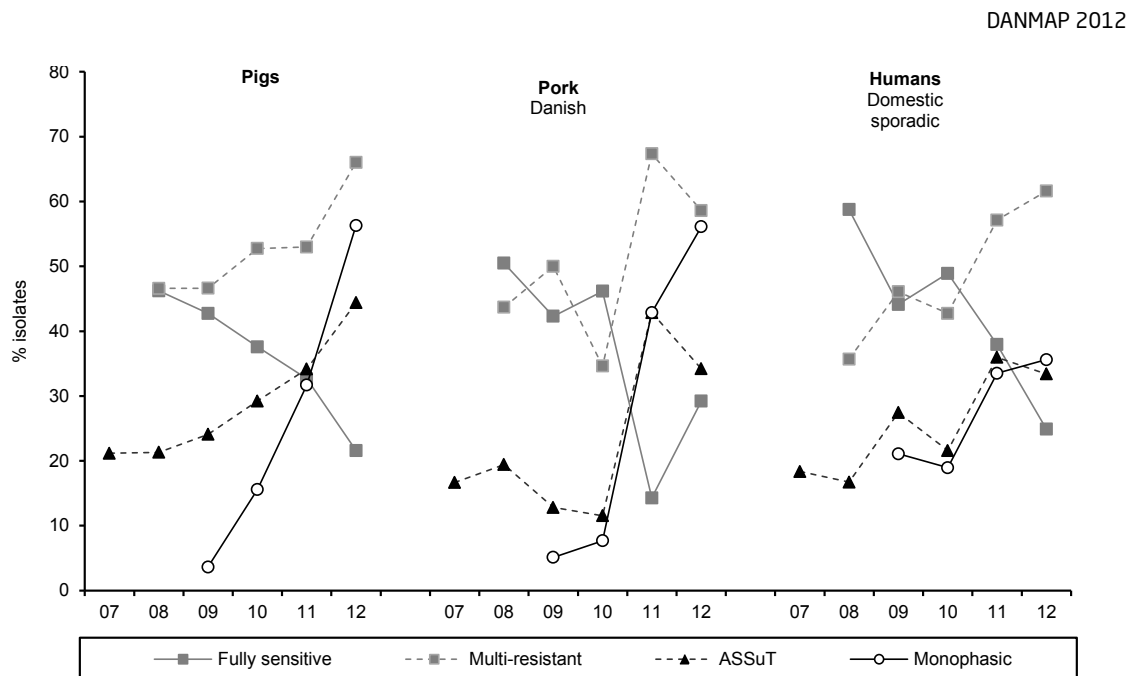
Outbreaks of human salmonellosis are reported in the Annual Report on Zoonoses in Denmark in 2012 [www.food.dtu.dk]. All human cases associated with a detected outbreak were considered ‘outbreak-related’ and all other domestic cases were considered ‘sporadic domestic’ in this report.

Salmonella Typhimurium in humans

S. Typhimurium was the most common serotype among the human cases (415 cases), and isolates with valid results from susceptibility testing of all antimicrobial agents included in the test panel were included (n = 411).

Among the reported human *S. Typhimurium* isolates included in DANMAP, 14% of the cases were categorised as travel-associated and whereas 55% most likely had acquired their infection in Denmark as sporadic incidences or as part of detected outbreaks (Table 6.2). Among the cases where the origin of infection was unknown (31%), the occurrence of resistance was for most antimicrobial agents comparable with the levels found among domestic cases.

Figure 6.2. Occurrence (%) of multi-resistance^(a,b) and monophasic variants^(c) in *Salmonella* Typhimurium in pigs, pork and human cases^(d), Denmark



Note: The number of isolates varies between years (pigs: n = 144–563, Danish pork: n = 26–103, domestic sporadic human cases: n = 98–269)

a) An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3). Data on resistance to colistin and trimethoprim are not available for 2007, thus the proportions of multi-resistant or fully sensitive are not calculated

b) ‘ASSuT’ isolates are resistant to ampicillin, streptomycin, sulfonamide and tetracycline, but can include resistant to other antimicrobial agents also chloramphenicol

c) Recording of the monophasic variants of *S. Typhimurium* with antigenic formulas S. 4,[5],12:i:- in the database was not fully implemented in 2007 and 2008, thus data are not presented

d) An isolate is categorised as ‘domestic sporadic’ if the patient did not travel outside Denmark one week prior to the onset of disease and was not reported as being part of an outbreak

Among the *S. Typhimurium* isolates from domestic as well as travel-associated cases, we observed very high levels of resistance to ampicillin, streptomycin, sulfonamide, and tetracycline. High levels of ASSuT resistance occurred among isolates from sporadic domestic cases (42%, $n = 177$) as well as and travel-associated cases (63%, $n = 59$).

Among the domestic sporadic cases, resistance to fluoroquinolones (ciprofloxacin) and amphenicols (florfenicol) increased compared with 2011, whereas resistance to spectinomycin decreased. Occurrences of the resistance to the other antimicrobial agents were similar to levels reported in 2011 for the domestic sporadic cases and similar for all antimicrobial agents for the travel-associated cases (Figure 6.1). The increase in fluoroquinolone (ciprofloxacin) resistance among the sporadic domestic cases is probably linked to an undetected outbreak, as there was a cluster of cases with similar resistance and MLVA profiles. In general, the levels of resistance for the domestic cases were similar to those of Danish pork, except for the low occurrence of fluoroquinolone resistance, a resistance not observed among the isolates from Danish pork.

Since 2008, the proportion of multi-resistant isolates from sporadic domestic cases has increased from 36% in 2008 to 62% in 2012, again probably as a consequence of an increasing occurrence of multi-resistant monophasic variants of *S. Typhimurium* (Figure 6.2). The monophasic variants represented 36% of all domestic sporadic isolates and 48% of all multi-resistant isolates. Also in line with this, the proportion of fully sensitive *S. Typhimurium* isolates of domestic sporadic origin decreased from 59% in 2008 to 25% in 2012, and as for the pigs, only 2% of the monophasic isolates was fully sensitive.

There were several domestic foodborne outbreaks with *S. Typhimurium* detected, and most of the cases ($n = 48$) were of the multi-resistant monophasic variants (92%).

Among the *S. Typhimurium* from travel-associated cases, resistance to cefotaxime, chloramphenicol, ciprofloxacin, florfenicol, nalidixic acid, spectinomycin and tetracycline, as well as multi-resistance was more frequent than among isolates of domestic sporadic origin. This is reflecting that a relatively larger proportion of the cases were of the monophasic variants among the travel-associated cases (51%) compared with the domestic sporadic cases (36%).

Resistance to cephalosporins (ceftiofur) was low and only found in isolates from three travel-associated cases, two sporadic domestic cases and three cases of unknown origin.

As also observed in 2011, a marked difference in fluoroquinolone (ciprofloxacin) resistance was found between domestically acquired infections (2%) and travel-associated infections (24%). The higher level of ciprofloxacin resistance in the travel-associated *S. Typhimurium* infections may reflect a higher prescription of fluoroquinolones in production animals in the countries of destination. In Denmark, fluoroquinolones are rarely used in animal husbandry (except for pet animals) since the implementation of legal restrictions in 2002-2003 [DANMAP 2010].

Although the MIC of the observed fluoroquinolone resistant isolates was below the clinical breakpoint, it should be noted that ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with severe bacterial gastroenteritis.

**Karl Pedersen, Vibe Dalhoff Andersen,
Helle Korsgaard, Lars Stehr Larsen and Mia Torpdahl**

Surveillance of *Salmonella* and *Campylobacter* in Denmark

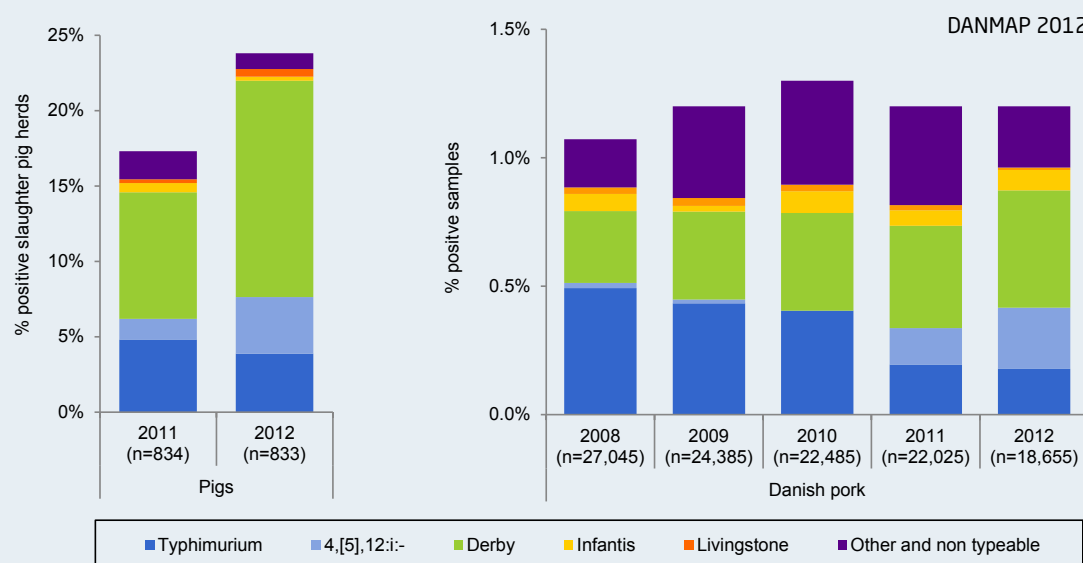
Background: In Denmark, all flocks of laying hens, broilers and turkeys, including breeder flocks, are monitored for *Salmonella* according to the EU requirements and the Danish legislation. A *Salmonella* surveillance and control programme is also running in the Danish pig production, and *S. Dublin* in cattle is monitored on a voluntary basis. Since January 2010, a mandatory surveillance of *Campylobacter* in broiler flocks at the farm has also been in place. *Salmonella* and *Campylobacter* in fresh meat are surveyed at the slaughterhouses and at the retail level and finally, an intensified control of *Salmonella* and *Campylobacter* in fresh meat, based on a case-by-case risk assessment, has been in place for Danish and imported meat ready for retail, since 2007. Human salmonellosis and campylobacteriosis are notifiable illnesses in Denmark, and all cases are reported to SSI and recorded in a national database. More information regarding trends and sources of zoonotic infections in humans and animals is available in the Annual Reports on Zoonoses at www.food.dtu.dk.

Salmonella: In broiler and layer flocks, the *Salmonella* prevalence has been low for more than a decade and only 0.8% and 0.6% of flocks were positive in 2012. A level well below the EU targets [Regulation (EC) No 1168/2006 and 646/2007]. From 2008, all Danish broiler flocks that test positive at the farm are heat treated at slaughter, and none of the slaughter batches tested at slaughter were *Salmonella* positive in 2012.

The prevalence of *Salmonella* in cattle is low, and in 2012 only 0.3% of the cattle carcasses tested at slaughter were positive. Furthermore, 94% of the non-milk producing herds and 92% of the milk-producing herds were, according to the monitoring programme, classified as “probably *S. Dublin* free”. Among pig herds sampled prior to slaughter, 24% were found *Salmonella* positive, while 1.2% of the pig carcasses tested at slaughter were positive (Figure 1). As in previous years, the most common serotype in pigs and pork in 2012 was *S. Derby* (60% and 38%, respectively) followed by *S. Typhimurium* (32% and 35%, respectively) when including the monophasic variants with antigenic formulas *S. 4,[5],12:i-* (Figure 1).

In 2012, the number of cases of human salmonellosis (21.4 cases per 100,000 inhabitants) increased slightly after several years of decrease. The increase in cases is mainly due to an increase in monophasic variants of *S. Typhimurium*. Cases due to *S. Enteritidis* continue to decrease. In total, 11 *Salmonella* outbreaks were reported, and 7 of these were caused by *S. Typhimurium*, primarily the monophasic variants. Two of the outbreaks were caused by Danish beef and one by Danish pork. As in previous years, the *Salmonella* source account estimated that almost half of the human cases of salmonellosis were acquired during international travel. More than 75% of the *S. Enteritidis* cases were acquired abroad whereas the majority of the *S. Typhimurium* cases were acquired in Denmark. For the sporadic cases not related to travel, Danish pork was estimated to be the most important source, which is similar to previous years. Danish beef was the second most common source in 2012. This is unusual and a result of an increased number of sporadic cases as well as the two outbreaks related to Danish beef. As in 2011, no human cases were attributed to Danish broiler meat in 2012 (Figure 2).

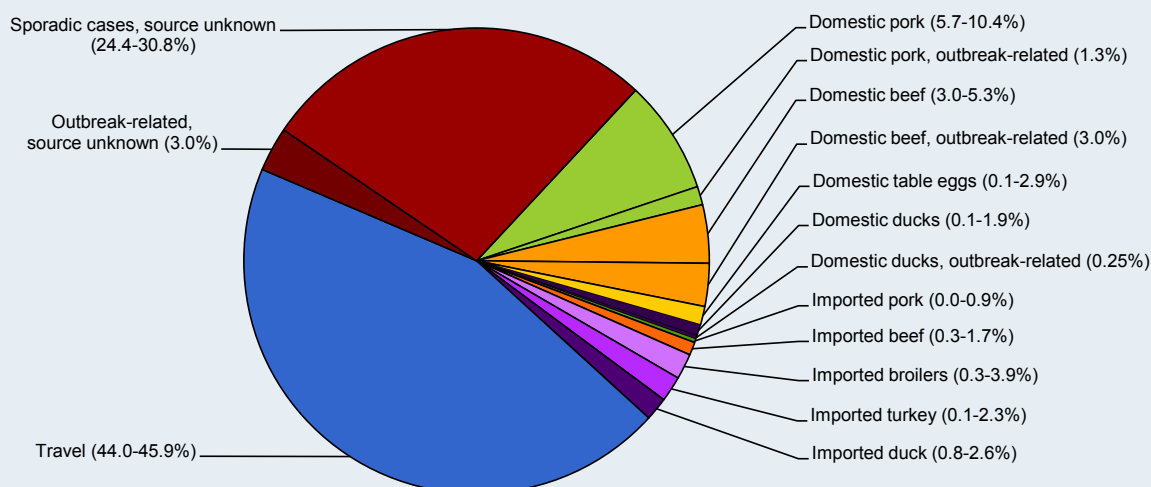
Figure 1. Occurrence (%) of *Salmonella* serovars in pigs at farm^{a)} and in Danish pork, Denmark



a) Faecal samples from healthy pigs collected at the slaughterhouses and cultured for *Salmonella* as part of the DANMAP programme

Figure 2. Estimated sources^(a) of 1,198 cases of human salmonellosis, Denmark

DANMAP 2012



Source: Danish Zoonosis Centre, National Food Institute

a) Sporadic and outbreak-related cases with unknown source include all sources not in the model

Campylobacter: The proportion of *Campylobacter* positive broiler flocks has decreased significantly since 2010, and 12% of all broiler flocks tested *Campylobacter* positive in 2012. At retail, *Campylobacter* was detected in chilled (10%) and frozen (6%) fresh broiler meat (Figure 3).

The most common *Campylobacter* species in broilers are *C. jejuni* (94% in 2012). *Campylobacter* is also found in cattle and pigs, where *C. jejuni* is dominant in cattle and *C. coli* is dominant in pigs.

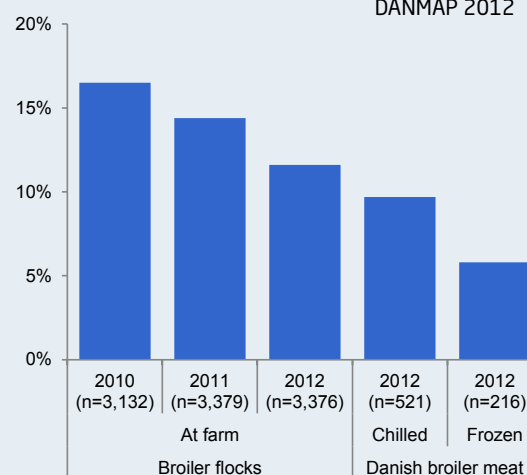
Campylobacter is the most frequently reported foodborne pathogen in Denmark, however, the number of human campylobacteriosis cases in 2012 (66.5 cases per 100,000 inhabitants) was 8% lower than in 2011. Since 2007, approximately one-third of the cases have been associated with travel outside Denmark. Three *Campylobacter* outbreaks were reported during 2012, where the suspected food source was broiler meat and raw milk. Consumption and handling of broiler meat is assumed to be the most important source of human campylobacteriosis (estimated source for more than 50% of domestic sporadic cases), however other sources such as contaminated water, vegetables and direct contact to farm animals exist.

Helle Korsgaard and Birgitte Helwich

For further information: Birgitte Helwich (bhel@food.dtu.dk)

Figure 3. Occurrence of *Campylobacter* in broilers^(a) and fresh chilled and frozen broiler meat^(b), Denmark

DANMAP 2012



a) Boot swabs collected in the stable 7-10 days before slaughter (detection limit: <10 cfu/g)

b) Non-heat treated chilled and frozen broiler meat at retail (detection limit: <10 cfu/g). The prevalence was calculated as a mean of quarterly prevalences

6.2 *Campylobacter*

Thermotolerant *Campylobacter* are the most commonly reported causes of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union [ECDC, 2013]. The species most commonly associated with human infections is *C. jejuni*, but other species may also cause infections in humans. In Denmark, 85%-95% of the human campylobacteriosis cases are caused by *C. jejuni*.

Campylobacter are widespread in nature and the most important reservoirs are the alimentary tract of wild and domesticated birds and mammals. Among sporadic human cases, broilers have been identified as a primary source of infection, though other sources also exist, e.g. water from untreated water sources, and other infected animals.

For *Campylobacter*, DANMAP 2012 includes randomly collected isolates from healthy pigs, broilers and cattle at slaughter and from fresh broiler meat sold at wholesale and retail outlets. Isolates from human cases originate from three out of five geographical regions in Denmark. Only one isolate per farm, meat sample or human case is included, and data are only presented if a sufficient number of isolates were obtained (>15). For details see Chapter 10, Materials and Methods.

MIC distributions for *C. jejuni* from broilers and cattle, broiler meat and humans, as well as for *C. coli* from pigs and broiler meat in 2012 are presented in the web annex (Tables A6.6-A6.10). Data for each of the figures are also presented in the web annex.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the six included antimicrobial classes (see Table 10.3).

6.2.1 *C. jejuni* in broilers and Danish broiler meat

In 2012, we observed moderate levels of resistance (15%) to both fluoroquinolones (ciprofloxacin) and tetracycline among *C. jejuni* isolates from broilers (n = 41, Table 6.3).

The consumption of antimicrobial agents in broilers is generally low, and tetracycline has been one of the most commonly used antimicrobial agents in Danish broilers over the last five years (Figure 4.6). The consumption of tetracycline increased considerably from 2008 to 2010, but decreased from 2011 to 2012. Nonetheless, for *C. jejuni* isolates from broilers, the resistance and consumption patterns for tetracycline appear to follow each other (Figure 4.6 and Figure 6.3). We did not observe a similar agreement between the consumption and resistance patterns for fluoroquinolones (ciprofloxacin) in *C. jejuni* broiler isolates. Even though fluoroquinolones have not been used in the broiler production since 2009, the resistance level has remained at a moderate-to-high level (12%-23%) during the last five years. However, the increasing trend observed since 2007 does not appear to continue (Figure 6.3).

In *C. jejuni* isolates from Danish broiler meat (n = 65), we observed high levels of resistance to ciprofloxacin (29%) and moderate (14%, Table 6.3) levels of resistance to tetracycline in 2012. The resistance to ciprofloxacin and tetracycline has fluctuated over the last five years, however the ciprofloxacin resistance level observed in 2012 is the highest since 2007. Macrolide (erythromycin) resistance has remained at a very low level for a decade. The levels of antimicrobial resistance were comparable between *C. jejuni* isolated from Danish broilers and Danish broiler meat (Figure 6.3).

Table 6.3. Resistance (%) in *Campylobacter jejuni* from animals, meat of Danish and imported origin and human cases^(a), Denmark
DANMAP 2012

Antimicrobial agent	Cattle	Broilers	Broiler meat		Humans	
	Danish %	Danish %	Danish %	Import %	Domestic %	Travel abroad %
Tetracycline	1	15	14	58	20	52
Chloramphenicol	0	0	0	0	0	0
Erythromycin	1	0	0	4	1	2
Gentamicin	0	0	0	0	3	4
Streptomycin	0	0	0	0	5	9
Ciprofloxacin	16	15	29	46	35	80
Nalidixic acid	16	15	29	46	36	80
Fully sensitive	84	78	64	31	61	17
Multiresistant	1	0	0	4	4	7
Number of isolates	89	41	66	26	80	46

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the six antimicrobial classes (see Table 10.3)

a) An isolate is categorised as 'domestic sporadic' if the patient did not travel outside Denmark one week prior to the onset of disease

6.2.2 *C. jejuni* from imported meat

In *C. jejuni* isolates from imported broiler meat ($n = 26$), the levels of resistance to tetracycline (58%) and quinolones (nalidixic acid and ciprofloxacin, 46%, Table 6.3) remained high, and from 2007 to 2012 there has been an overall decrease in the proportion of fully sensitive *C. jejuni* isolates among isolates from imported broiler meat. Also, over the past five years, the level of resistance to tetracycline and ciprofloxacin has generally been higher in isolates from imported broiler meat compared with Danish broiler meat. This corresponds with the data reported by EFSA, where Denmark has reported the lowest proportions of resistance among *C. jejuni* isolates from broiler meat [EFSA, 2013].

6.2.3 *C. jejuni* in cattle

In *C. jejuni* isolates from cattle ($n = 89$), we observed moderate levels (16%) of resistance to fluoroquinolones (ciprofloxacin) and low levels of resistance to tetracycline (Table 6.3).

Resistance to fluoroquinolones (ciprofloxacin) among *C. jejuni* from cattle has remained at a moderate-to-high level (16%-20%) since 2008 (Figure 6.4). As described in previous DANMAP reports, we observed an increase in the level of fluoroquinolone resistance in 2005 despite low consumption of fluoroquinolones in cattle. In 2012, only one of the fluoroquinolone-resistant isolates was also resistant to tetracycline, indicating that co-selection by tetracycline (one of the major drugs for treatment of calves) was not the explanation for the observed levels of fluoroquinolone resistance. It has been discussed [DANMAP 2007] that clonal spread, particularly between farms, could be an explanation for the observed resistance to fluoroquinolones.

Initially, fluoroquinolone-resistant *C. jejuni* isolates came from cattle farms in Southern Jutland, but in 2012 isolates were obtained from farms distributed throughout Jutland and Funen.

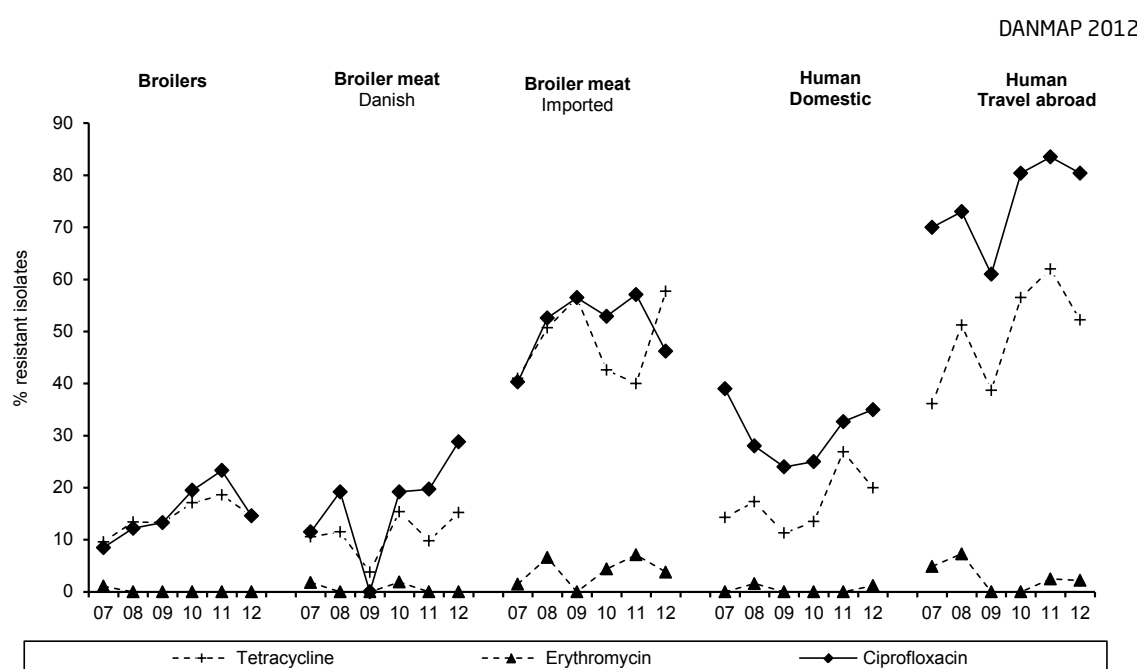
From 2003 to 2010, we observed a general increase in the resistance to tetracycline. However, this trend was discontinued in 2011, and in 2012 the level of tetracycline resistance was very low (1%, Figure 6.4).

6.2.4 *C. coli* in pigs

In *C. coli* isolates from pigs ($n = 103$), we observed very high levels (57%) of resistance to the aminoglycoside streptomycin. The level of streptomycin resistance in *C. coli* isolates from pigs has remained at the same high level since 2009 and may reflect that isolates originated from producers with high occurrence of diseases typically treated with streptomycin (limb, joint, CNS and skin). While the increase in streptomycin consumption represents only a very small fraction of the total consumption, it is still noteworthy that the consumption of penicillin-streptomycin combinations for finisher has increased continuously from 2009-2012 (web annex, Table A4.2).

In 2012, we observed moderate (12%) levels of resistance to fluoroquinolones (ciprofloxacin) in *C. coli* isolates from pigs. Since 2007 there has been an increasing trend in the level of tetracycline resistance in *C. coli* isolates from pigs, and in 2012 the resistance level was moderate (15%, Figure 6.5 and web annex, Table A4.2). The increasing trend in the tetracycline resistance has generally complied well with the development in consumption of tetracycline over the past years. However, the

Figure 6.3. Resistance (%) in *Campylobacter jejuni* from broilers, broiler meat and human cases^a, Denmark



Note: The number of isolates varies between years (broilers: $n = 41-94$, Danish broiler meat: $n = 26-113$, imported broiler meat: $n = 26-152$, domestic sporadic human cases: $n = 52-185$ and travel-associated human cases: $n = 31-79$)

a) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

dramatic decrease (27%) in the use of tetracycline in the pig production in 2011 is not reflected in the tetracycline resistance levels of *C. coli* isolates from pigs.

Until 2007, a continuous decrease in macrolide (erythromycin) resistance in *C. coli* was observed following the withdrawal of the macrolide growth promoter tylosin from the Danish pig production in 1998–1999. However, since then the resistance to erythromycin has fluctuated, but remains at a low level (7%, Figure 6.5).

6.2.5 *C. jejuni* in humans

In 2012, *Campylobacter* continued to be the most frequent cause of bacterial intestinal infections in Denmark. A total of 3,728 human laboratory confirmed cases of campylobacteriosis were reported (66.5 per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2012].

A subset (n = 126) of the *C. jejuni* isolates submitted to SSI were selected for susceptibility testing continuously over the year. The isolates were randomly selected from all of the *Campylobacter* isolated from stool samples in the three geographical regions included in the surveillance. Among the tested isolates, 37% were from travel-associated cases and 63% were considered to be domestically acquired. As in previous years, SSI collected information on travel history through phone interviews. Travel history was collected only for patients where isolates had been submitted to susceptibility testing. Patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to the onset of disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as “domestically acquired” if the patients had not travelled within the week prior to the onset of disease.

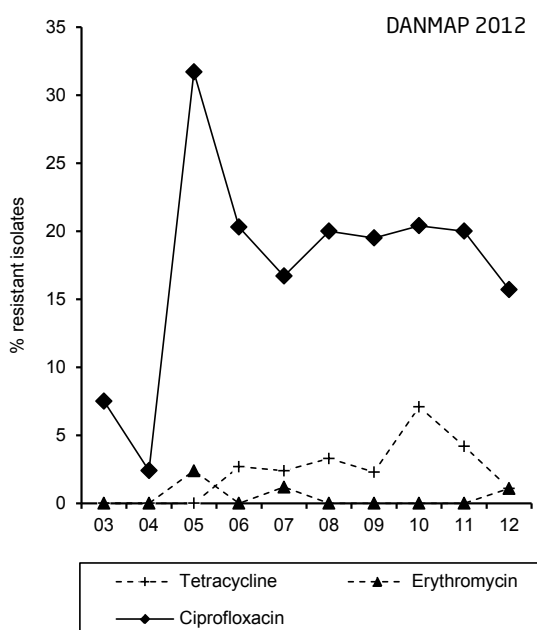
Among the domestically acquired infections, 61% were fully sensitive to the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower (17%) among isolates from travel associated cases (Table 6.3). Among the cases with resistant *C. jejuni*, more than 90% were resistant to fluoroquinolones (ciprofloxacin), while multi-resistance was found in 3% of the isolates from infections acquired in Denmark and in 7% of the infections associated with travel.

The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel-associated *C. jejuni* isolates (80% and 52%, respectively) compared with isolates from domestically acquired infections (35% and 20%, respectively, Table 6.3 and Figure 6.3). For the other antimicrobial agents tested, resistance levels were comparable.

Ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with severe bacterial gastroenteritis, and the level of resistance to these antimicrobial agents is therefore of major importance. In Denmark, fluoroquinolones are rarely used in animal husbandry (except for pet animals) since the implementation of legal restrictions in 2002–2003 [DANMAP 2010]. Travelling to, or eating meat from countries where fluoroquinolone restrictions are not implemented can be associated with a higher risk of acquiring infection with ciprofloxacin-resistant *C. jejuni*.

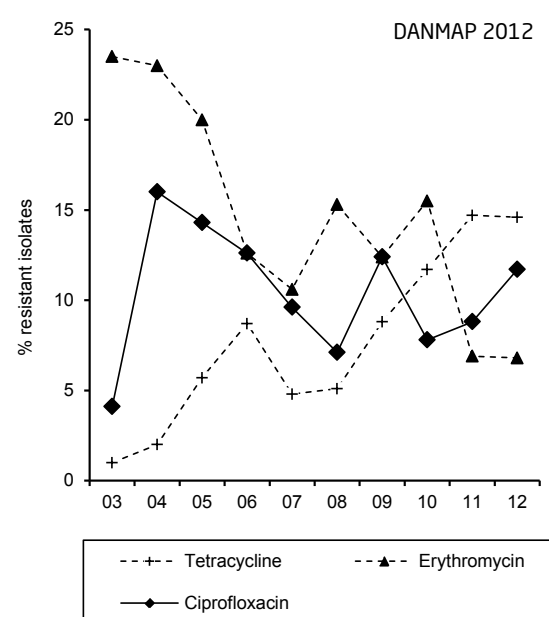
Birgitte Borck Høg, Lars Stehr Larsen,
Mia Torpdahl and Anne Mette Seyfarth

Figure 6.4. Resistance (%) in *Campylobacter jejuni* from cattle, Denmark



Note: The number of isolates varies between years (n = 41–98)

Figure 6.5. Resistance (%) in *Campylobacter coli* from pigs, Denmark



Note: The number of isolates varies between years (n = 98–113)

Occurrence of *Clostridium difficile* in Danish pig farms, cattle at slaughter and meat

Background: *Clostridium difficile* is causing intestinal infection in humans and have caused outbreaks in hospitals in Denmark and other countries [DANMAP 2009]. In, 2010, *C. difficile* was isolated from pig farms (15% (15/99)), cattle at slaughter (15% (29/192)) and broiler flocks at slaughter (3% (6/197)). Isolates from broiler flocks contained the toxin genes *tcdA* and *tcdB*. Isolates from cattle and pigs were more virulent as some contained toxin genes, *tcdA* and *tcdB*, and the *cdtA* and *cdtB* genes encoding the binary toxin. Moreover, among isolates with all four toxin genes deletions of either 39 bp or 54 bp in the *tcdC* gene were observed. These deletions are used as evolutionary markers and indicate highly virulent types. In both cattle and pigs these suspected highly virulent isolates belonged to PCR ribotypes previously involved in human infections (see DANMAP 2010, Textbox 3).

Even though *C. difficile* can be isolated from animals and meat its role as a zoonotic agent is not fully understood [Rupnik. 2007. Clin. Microbiol. Infect. 13:457-9]. The aim of this study was to investigate the occurrence of *C. difficile* in pig farms, cattle at slaughter and in meat samples collected from retail and outlets to determine if humans are exposed through meat to virulent *C. difficile* isolates originating from production animals.

Materials and Methods: During February through October 2011, 71 stool samples from primarily slaughter pig pens at 71 farms and 186 faecal samples from cattle at slaughter were tested for *C. difficile* in the same way as in 2010. One gram of faecal sample were added to 9 ml CDMN broth supplemented with 0.1% natrium taurocholate and incubated anaerobically at 37°C for 7 days. Two ml were transferred to 2 ml 99% ethanol and left at room temperature for one hour. After centrifugation, 10 µl of pellet was transferred to a CDMN agar plate. The plate was incubated for 44 to 48 hours anaerobically at 37°C. Presumptive *C. difficile* were re-streaked at CDMN agar plates. *C. difficile* toxin genes (*tcdA*, *tcdB* and the binary toxin genes *cdtA* and *cdtB*) were identified by PCR as previously described [Persson *et al.* 2008. Clin. Microbiol. Infect. 14: 1057-64]. Isolates positive for all four toxin genes were furthermore typed using Tandem Repeat Sequence Typing (TRST) (<http://pubtrst.org/>) and tested for deletions in *tcdC*. *C. difficile* isolates with no toxin genes were verified by 16s sequencing.

During February through December 2012 meat samples were randomly collected from retail and outlets in all regions of Denmark; a total of 972 meat samples were collected: Broiler meat (205 Danish and 179 imported), beef (121 Danish and 100 imported) and pork (188 Danish, 179 imported). The same procedure as for faecal samples was followed for isolation of *C. difficile* except that 15 gram of meat was added to 50 ml CDMN broth supplemented with 0.1% natrium taurocholate.

Results and discussion: Two (2.8%) of the pig farm samples were positive for *C. difficile*. One isolate had all four toxin genes (not TRST typed) and one had *tcdA* and *tcdB*. The occurrence of *C. difficile* was significantly lower than in 2010, where 15% of the samples from pig farms were positive. *C. difficile* are intrinsic resistant to several classes of antimicrobial agents including cephalosporins, therefore the reduction in cephalosporin consumption in the pig production close to zero since July 2010 and to some extent the total reduction of antimicrobials in pigs, may be the reason for the reduction of *C. difficile* in pig farms as suggested for cephalosporinase producing *E. coli* in the same period [Agersø and Aarestrup. 2013. J. Antimicrob. Chemother. 68: 569-72]. The occurrence in cattle was at the same level as in 2010 (15% (27/186)) and no major changes in the cephalosporin consumption or in the total consumption has been observed in cattle in this period. All isolates were tested for toxin genes and 23 had the toxin genes *tcdA* and *tcdB*, one had in addition *cdtA*, two had in addition both genes (*cdtA* and *cdtB*) encoding the binary toxin, and in one isolate no toxin genes were detected. One of the isolates containing all toxins was tr070 with a 39 bp deletion in *tcdC* and one was tr016 with a 54 bp deletion. Isolates with these deletions are previously observed in cattle [DANMAP 2010]. The TRST types found in isolates from cattle are types found in humans in Denmark and tr070 are commonly involved in human infections [DANMAP 2010].

From the meat samples, the highest occurrence was observed in broiler meat, where *C. difficile* was found in 7% of the tested samples from both Danish (14/205) and imported (13/179) broiler meat. In Danish pork an occurrence of 2% (4/188) was observed and no *C. difficile* was found in imported pork (n = 179), or in Danish (n = 121) and imported beef (n = 100). Thirteen isolates from Danish broiler meat, eight isolates from imported broiler meat and two isolates from Danish pork contained *tcdA* and *tcdB* toxin genes. Five from imported broiler meat, one from Danish broiler meat and two from pork did not contain any toxin genes.

Most of the isolates from animals and from meat samples contained *tcdA* and *tcdB*, but whether these are of zoonotic importance is unknown. In 2009 approx. 1/3 of the human cases were caused by *C. difficile* with these toxins [DANMAP 2009].

Conclusion: In conclusion, the decrease of *C. difficile* in pig farms may be explained by a reduction close to zero of cephalosporin consumption in the same period. The occurrence of *C. difficile* was generally low in meat and none of the most virulent types containing the binary toxin were observed in the meat although present in cattle and pigs. The highest occurrence in meat was observed in the broiler meat, but whether this was due to differences in the slaughter processes for broilers compared to pigs and cattle or differences in occurrence in the animals requires further investigation. Although none of the most virulent types were detected in the meat samples, the types observed with *tcdA* and *tcdB* may contribute to human infections so the zoonotic importance of these types should be further investigated.

Yvonne Agersø, Mia Torpdahl and Katharina E. P. Olsen

For further information: Yvonne Agersø (yvoa@food.dtu.dk)



7. Resistance in indicator bacteria

Indicator bacteria (*Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli*) have been included in the DANMAP programme since 1995. Enterococci are included to monitor resistance in Gram-positive bacteria and *E. coli* as representative of Gram-negative bacteria. These bacteria were selected as indicators for occurrence of antimicrobial resistance for several reasons: they are ubiquitous and present as major commensals in both the animal and human reservoirs, they can acquire antimicrobial resistance as response to selective pressures and finally they have the potential for transferring resistance to pathogenic bacteria and between reservoirs.

7.1 Enterococci

For Enterococci, DANMAP 2012 includes randomly collected isolates from healthy pigs and broilers at slaughter and from domestic fresh broiler meat, pork and beef sold at wholesale and retail outlets. In addition, enterococci from imported broiler meat, beef and pork were included. We included only one isolate per farm or meat sample, and data are presented in the report where a sufficient number of isolates were obtained (>15). For details on methodology, see Chapter 10, Materials and Methods.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the ten included antimicrobial classes (see Table 10.3).

The MIC distributions and occurrence of resistance among *E. faecium* and *E. faecalis* are presented in the web annex (Tables A7.1 - A7.4).

7.1.1 *E. faecium* from Danish broilers and broiler meat

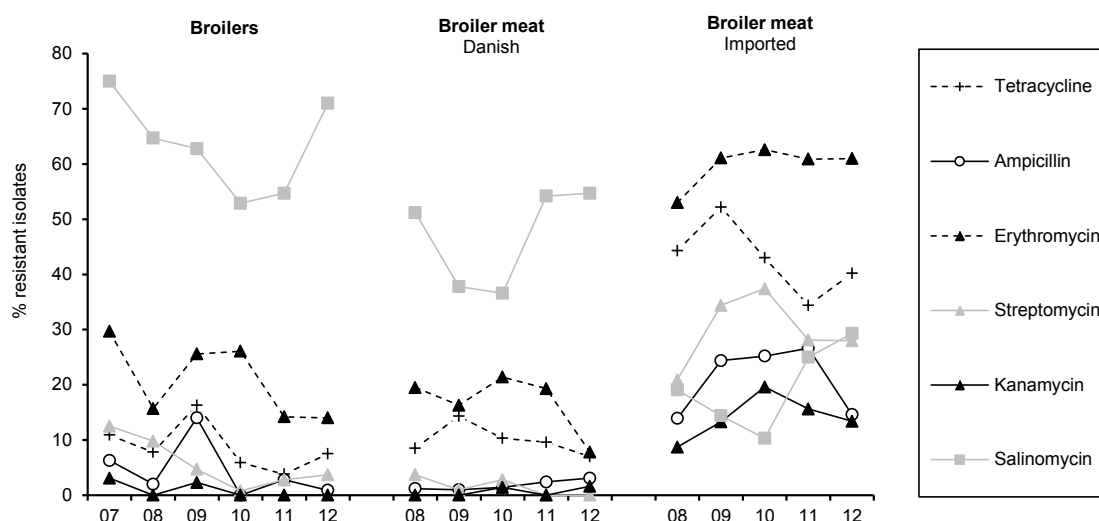
In the *E. faecium* isolates from broilers (n = 107), we found very high levels (71%) of salinomycin resistance in 2012 (Table 7.1), a deviation from the decreasing trend 2007-2011 (Figure 7.1). Salinomycin is a coccidiostat commonly used in the broiler production, but information on consumption is not currently available and we could, therefore, not relate the occurrence of resistance to the usage. The levels of resistance were similar to levels reported in 2011 (Figure 7.1); however since 2007, resistance to the aminoglycoside streptomycin has been decreasing. Resistance to the growth promoter virginiamycin (quinupristin/dalfopristin resistance) persisted at a low level (1%), even though the usage has been banned for more than a decade. The majority of resistant isolates (72%) were only resistant to salinomycin, and only four isolates were multi-resistant.

We also observed a very high occurrence of salinomycin resistance (55%) in *E. faecium* isolates from broiler meat (n = 128, Table 7.1). The antimicrobial resistance to macrolides (erythromycin) decreased from 19% in 2011 to 8% in 2012 (Figure 7.1). Most of the resistant isolates from broiler meat were only resistant to salinomycin (76%), and only one isolate was multi-resistant.

None of the isolates from broilers or domestic broiler meat were resistant to fluoroquinolone (ciprofloxacin). In general, the levels of antimicrobial resistance were comparable between *E. faecium* isolated from Danish broilers and broiler meat. However, in isolates from Danish broiler meat the level of salinomycin resistance was lower than in isolates from Danish broilers.

Figure 7.1. Resistance (%) in *Enterococcus faecium* from broilers and broiler meat, Denmark

DANMAP 2012



Note: The number of isolates varies between years (broilers: n = 43–119, Danish broiler meat: n = 82–145, imported broiler meat: n = 64–115). Data from broiler meat are not available from 2007

Table 7.1. Resistance (%) among *Enterococcus faecium* from animals and meat of Danish and imported origin, Denmark

DANMAP 2012

Antimicrobial agent	Broilers	Broiler meat		Beef meat	Pigs	Pork meat	Pork meat
	Danish %	Danish %	Imported %	Danish %	Danish %	Danish %	Imported %
Tetracycline	7	7	40	7	62	3	14
Tigecycline	0	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0	0
Penicillin	2	3	16	0	20	6	0
Ampicillin	1	3	15	0	12	6	0
Erythromycin	14	8	61	13	24	3	0
Gentamicin	0	0	1	0	2	0	0
Kanamycin	0	2	13	7	21	16	0
Streptomycin	4	0	28	7	42	6	0
Ciprofloxacin	0	0	1	0	0	0	0
Vancomycin	0	0	0	0	0	0	0
Quinupristin/dalfopristin	1	0	16	0	12	0	0
Salinomycin	71	55	29	0	0	0	0
Linezolid	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0
Fully sensitive	23	35	20	87	37	84	86
Multi-resistant	4	1	34	7	37	6	0
Number of isolates	107	128	82	15	112	32	22

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

Table 7.2. Resistance (%) among *Enterococcus faecalis* from animals and meat of Danish and imported origin, Denmark

DANMAP 2012

Antimicrobial agent	Broilers	Broiler meat		Beef		Pigs	Pork meat	
	Danish %	Danish %	Imported %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	43	47	69	13	33	87	11	46
Tigecycline	0	0	0	0	0	0	0	0
Chloramphenicol	1	3	3	3	2	21	3	1
Penicillin	0	0	0	0	0	0	0	0
Ampicillin	0	0	0	0	0	0	0	0
Erythromycin	20	21	54	3	7	56	5	6
Gentamicin	0	0	1	0	0	9	1	3
Kanamycin	2	3	29	3	5	26	3	5
Streptomycin	3	11	34	8	9	34	5	5
Ciprofloxacin	0	0	2	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0	0
Salinomycin	0	1	1	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0	0
Fully sensitive	52	49	22	87	67	12	88	54
Multi-resistant	4	8	35	3	7	37	4	6
Number of isolates	100	75	93	38	43	119	104	108

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

7.1.2 *E. faecium* from Danish pigs and pork

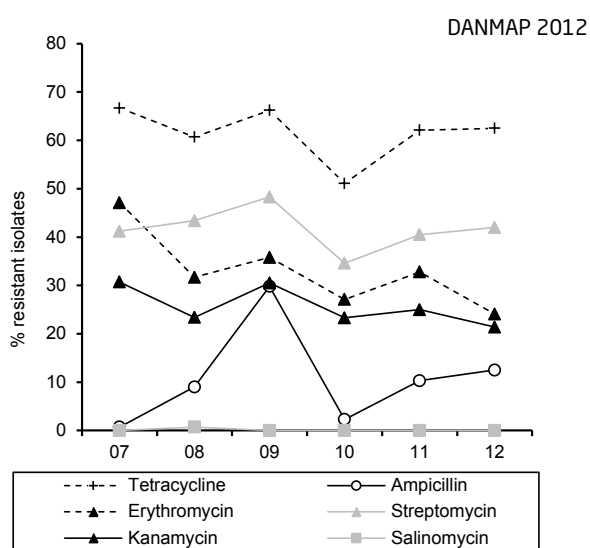
Among the *E. faecium* isolates from pigs ($n = 112$), we found high occurrences of resistance to tetracycline (62%) and streptomycin (42%). Tetracycline has been the most frequently used antibiotic in Danish pig production for a decade. Beta-lactam (ampicillin and penicillin) resistance in *E. faecium* isolates from pigs stayed at the level found in 2011, whereas macrolide (erythromycin) resistance has declined since 2007 and now stands at 24% (Figure 7.2), a trend that does not correlate to changes in usage of macrolides (Figure 4.5). All isolates resistant to streptomycin and/or erythromycin were also resistant to tetracycline, and overall 37% of the isolates were multi-resistant (Table 7.1).

Even though virginiamycin (quinupristin/dalfopristin) has not been used as growth promoter for more than a decade, resistance remained among *E. faecium* isolated from pigs, and even increased, compared with 2011. None of the isolates were resistant to glycopeptides (vancomycin) in 2012 (Table 7.1).

Among the five resistant *E. faecium* isolates (16%) from Danish pork, all were resistant to kanamycin (aminoglycosid) and two isolates were multi-resistant (Table 7.1). Occurrences of the resistance were similar to levels reported in 2011.

Fluoroquinolone (ciprofloxacin) resistance was not observed in *E. faecium* isolates from Danish pigs or pork. Resistance to tetracycline, erythromycin and streptomycin, as well as multi-resistance was more frequent in *E. faecium* from Danish pigs compared with isolates from Danish pork.

Figure 7.2. Resistance (%) in *Enterococcus faecium* from pigs, Denmark



Note: The number of isolates varies between years (Pigs: $n = 112$ –153). Data from pork are not presented due to insufficient number of isolates

7.1.3 *E. faecium* from imported meat

Compared with Danish broiler meat, resistance to tetracycline, penicillin, ampicillin, erythromycin, kanamycin, streptomycin, quinupristin/dalfopristin as well as multi-resistance was more frequent in *E. faecium* from imported broiler meat ($n = 82$), while resistance to salinomycin was higher in Danish broiler meat compared with imported (Table 7.1). Overall, 34% of the isolates were multi-resistant, and among the resistant isolates, 18% of the isolates were resistant to erythromycin and tetracycline. Only 20% of the *E. faecium* isolates from imported broiler meat were fully sensitive to the antimicrobial agents tested (Table 7.1).

In imported pork, 86% of the *E. faecium* isolates ($n = 22$) were fully sensitive to the tested antimicrobial agents (Table 7.1), and only resistance to tetracycline (14%) was detected. Occurrences of resistance were similar between Danish and imported pork.

7.1.4 *E. faecalis* from Danish broilers and broiler meat

In *E. faecalis* isolates from broilers ($n = 100$), antimicrobial resistance to tetracycline increased from 17% in 2011 to 43% in 2012, contradictory to the reduced usage of tetracycline for broilers (Figures 7.3 and 4.7). Only four isolates were multi-resistant (Table 7.2).

As in previous years, the level of salinomycin resistance was significantly lower in *E. faecalis* than in *E. faecium* isolates from broilers, while antimicrobial resistance to tetracycline and erythromycin was higher in *E. faecium* than in *E. faecalis*.

The levels of resistance in *E. faecalis* isolates from broiler meat were similar to *E. faecalis* isolates from broilers (Figure 7.3).

7.1.5 *E. faecalis* from Danish pigs and pork

We found very high occurrences of resistance to tetracycline (87%) and erythromycin (56%) in *E. faecalis* isolates from pigs ($n = 119$), and only 12% of the isolates was fully sensitive to all the antimicrobial agents tested. The levels of resistance were comparable with 2011, except for resistance to the aminoglycoside gentamicin, which declined from 21% in 2011 to 9% in 2012. This decrease did not correlate with the general trend in usage of aminoglycosides (Figure 4.5).

Since 2010 the occurrence of macrolide (erythromycin) resistance among *E. faecalis* isolated from pigs has increased from 44% to 56%, in contrast to a decline among the *E. faecium* isolates (Figures 7.2 and 7.4). We can offer no explanation for this with the information available.

All the chloramphenicol resistant isolates from pigs ($n = 25$) were resistant to both tetracycline and erythromycin, while all kanamycin resistant isolates ($n = 31$) were also resistant to erythromycin, 97% to tetracycline and 94% to streptomycin. All gentamicin resistant isolates ($n = 11$) were resistant to kanamycin, tetracycline and erythromycin. Overall, 37% of the isolates were multi-resistant (Table 7.2).

Most of the *E. faecalis* isolates from Danish pork ($n = 104$) were fully sensitive to all of the antimicrobial agents tested (Table 7.2), and the proportion of fully sensitive isolates have increased since 2007. Among the 12 resistant isolates, 92%

were resistant to tetracycline, and only 4 isolates were multi-resistant. Occurrences of the resistance were similar to levels reported in 2011.

Fluoroquinolone (ciprofloxacin) resistance was not observed in *E. faecalis* isolates from Danish pigs or pork. The levels of resistance were much lower in the *E. faecalis* isolates from Danish pork compared with isolates from Danish pigs (Figure 7.4).

7.1.6 *E. faecalis* from imported meat

Compared with Danish broiler meat, *E. faecalis* from imported broiler meat ($n = 93$) had higher levels of resistance to tetracycline, erythromycin, kanamycin and streptomycin and were more often multi-resistant (Figure 7.3). Among the resistant isolates, only one isolate was not resistant to either tetracycline or erythromycin, and 58% of the resistant isolates were resistant to at least both antimicrobial agents.

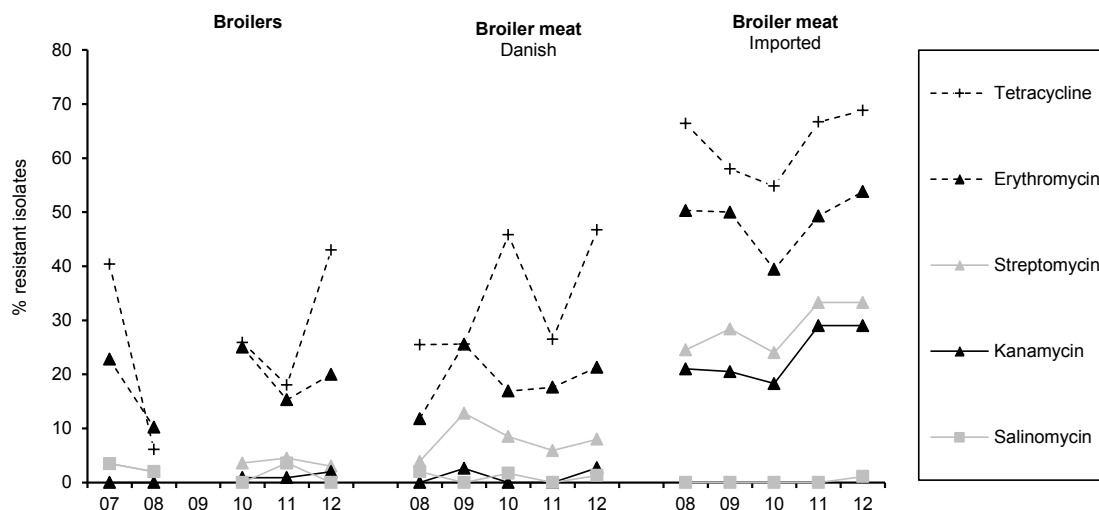
Overall, 35% of the *E. faecalis* from imported broiler meat were multi-resistant (Table 7.2).

Compared with Danish pork, *E. faecalis* isolates from imported pork ($n = 108$) were more frequently resistant to tetracycline (Table 7.2), whereas resistance levels to the other antimicrobial agents were comparable. Approximately half of the *E. faecalis* isolates from imported meat were fully sensitive to all of the antimicrobial agents tested, and all resistant isolates were at least resistant to tetracycline. Only 6% were multi-resistant (Table 7.2).

The levels of resistance in *E. faecalis* isolates in Danish ($n = 38$) and imported beef ($n = 43$) were comparable. The highest occurrence of resistance was found for tetracycline, where 13% and 33%, respectively, were resistant. The majority of isolates were fully sensitive to the antimicrobial agents tested, and only a few isolates were multi-resistant (Table 7.2).

Figure 7.3. Resistance (%) in *Enterococcus faecalis* from broiler and broiler meat, Denmark

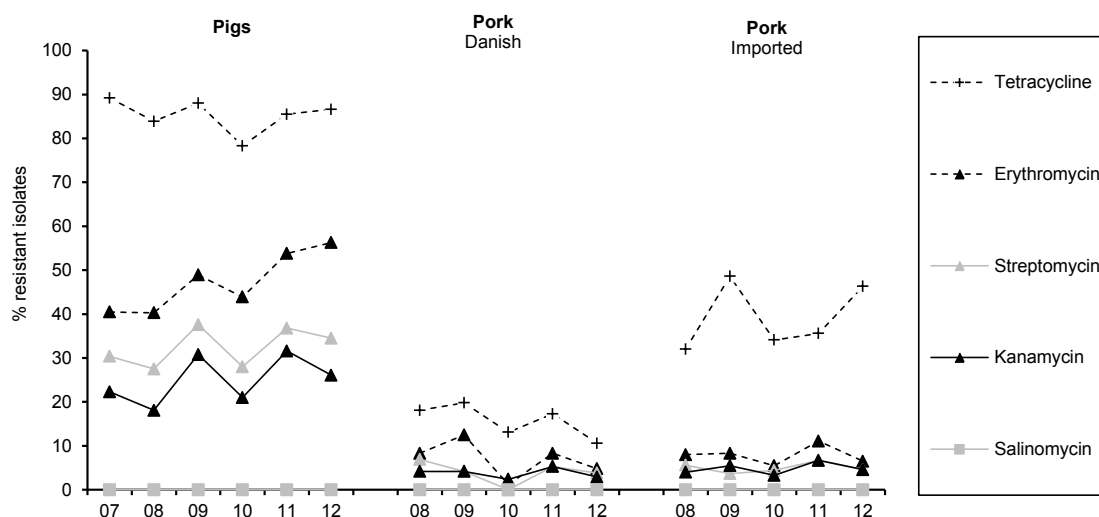
DANMAP 2012



Note: The number of isolates varies between years (broilers: $n = 49$ –112, Danish broiler meat: $n = 34$ –75, imported broiler meat: $n = 69$ –143). Data from meat is not available from 2007, and broiler data from 2009 are not presented due to insufficient number of isolates

Figure 7.4. Resistance (%) in *Enterococcus faecalis* from pigs and pork, Denmark

DANMAP 2012



Note: The number of isolates varies between years (Pigs: $n = 117$ –157, Danish pork: $n = 72$ –133 and imported pork: $n = 45$ –125). Data from meat are not available from 2007

7.1.9 One Health perspective

Occurrence of antimicrobial resistance in enterococci isolated from production animals and food are used as indicators for prevalence and transmission of antimicrobial resistance through the food chain to the consumer. Changes in occurrences of antimicrobial resistance in enterococci from production animals are thought to be directly related to the use of antimicrobial agents.

The level of resistant enterococci is generally higher in pigs than in broilers, indicating that pig production is a potential greater reservoir for resistance genes.

The higher level of resistance to salinomycin in *E. faecium* from broilers compared to pigs is expected since these coccidiostats are only used in the poultry production. Salinomycin is not used for treating humans, so salinomycin resistance in itself does not pose a public health problem. However, co-resistance with other antimicrobial agents can be of importance, and in 2012, 27% of the salinomycin-resistant isolates were also resistant to other antimicrobial agents, especially erythromycin.

We do not always find close associations between trends in usage of specific antimicrobials and resistance. One possible

explanation for this observed discrepancy could be cross- and multi-resistance as indicated above. It is, however, clear that the occurrence of antimicrobial resistance in enterococci from pigs is significantly higher than in enterococci from poultry, and that these significant differences in resistance occurrence reflect differences in usage of specific antimicrobial agents.

When looking at trends in antimicrobial resistance in *E. faecalis* from Danish broilers and broiler meat, the levels of antimicrobial resistance are comparable, and in *E. faecium* they follow the same tendencies for most used antimicrobials (salinomycin, erythromycin and tetracycline). In contrast, we see much lower levels of antimicrobial resistance in enterococci in pork compared with pigs.

These results may indicate that enterococcal populations in the live animal and on pork constitute different sub populations. Pork cuts for sampling are collected from wholesale and retail outlets. Possibly, enterococci on the product may reflect the processing environment, rather than direct contamination of the meat during slaughter and dressing. In contrast, cutting of broilers is done in slaughter plants, which may explain why the enterococcal populations from live broilers and from broiler meat do not appear too dissimilar.

Lars Bogø Jensen and Lars Stehr Larsen

7.2 Indicator *Escherichia coli*

For indicator *E. coli*, DANMAP 2012 includes randomly collected isolates from healthy pigs, broilers and cattle at slaughter and from fresh broiler meat, beef and pork sold at wholesale and retail outlets. We included only one isolate per farm or meat sample, and present only data where a sufficient number of isolates were obtained (>15). For details on methodology see Chapter 10, Materials and Methods.

The MIC distributions and occurrence of resistance among *E. coli* are presented in the web annex (Tables A7.5 and A7.6). Data for each of the figures are also presented in the web annex.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the ten included antimicrobial classes (see Table 10.3).

7.2.1 Indicator *E. coli* from broilers and broiler meat

E. coli isolates from broilers (n = 115) were most often resistant to sulfonamide (21%) and ampicillin (20%, Table 7.3). Resistance to ampicillin and streptomycin has increased compared to 2011, whereas the occurrence of the other resistances was comparable to the levels observed in 2011 (Figure 7.5).

Ampicillin resistance conveys cross-resistance to amoxicillin which has been the most frequently used antimicrobial agent in the broiler production for at least a decade.

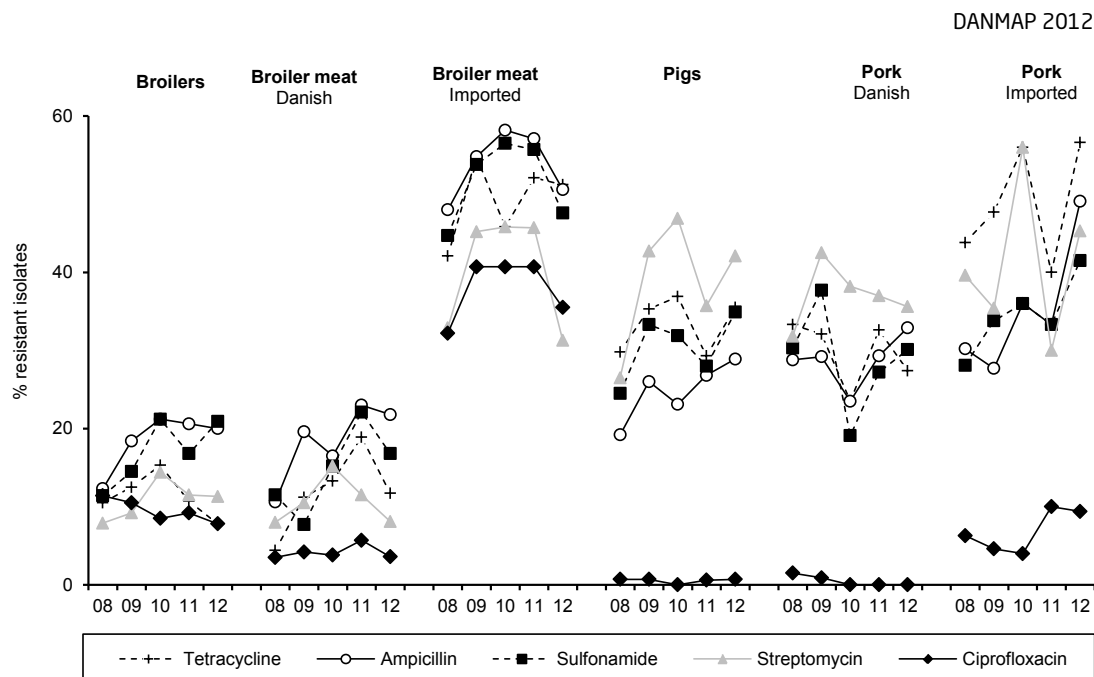
Sulfonamides have only been used in the last 2-3 years, but resistance to sulfonamide has been high the past decade (Figure 7.5). Historical use of sulfonamide and co-selection by ampicillin may partly explain the high occurrence of sulfonamide resistance. Among the sulfonamide resistant isolates, 42% were also resistant to ampicillin.

Among *E. coli* isolates from broilers, the majority of multi-resistant isolates (10/15) were resistant to both ampicillin and sulfonamide, often in combination with tetracycline and/or streptomycin resistance. It is noteworthy that the level of multi-resistance has more than doubled within the past five years, while the level of fully sensitive strain has remained stable (Figure 7.6).

In addition, we found 8% of the *E. coli* broiler isolates resistant to both nalidixic acid and ciprofloxacin (fluoroquinolone). During the period 2003-2007, fluoroquinolone consumption in poultry was significantly higher than for the other production animal species in Denmark, because antimicrobial agents approved for poultry were limited to amoxicillin and fluoroquinolones. However, since 2008, the fluoroquinolone usage in the broilers has been very low (Figure 4.6).

Fluoroquinolone (ciprofloxacin) resistance in *E. coli* from broilers has varied between 7% and 13% over the past decade with a decreasing trend in recent years. The majority of fluoroquinolone resistant isolates (78%) were not resistant to the more frequent resistance types.

Figure 7.5. Resistance (%) in *Escherichia coli* from animals and meat of Danish and imported origin, Denmark



Note: The number of isolates varies between years (broilers: n = 114–152, Danish broiler meat: n = 113–197, imported broiler meat: n = 140–304, pigs: n = 150–160, Danish pork: n = 66–106, imported pork: n = 30–96)

Among *E. coli* isolates (n = 197) from domestic broiler meat, we found the highest levels of resistance for ampicillin (22%) and sulfonamide (17%) while the occurrence of fluoroquinolone (ciprofloxacin) resistance remained at a low level (4%). The levels of resistance in *E. coli* from Danish broiler meat were similar to what was found among isolates from Danish broilers, except for lower levels of resistance to spectinomycin in the isolates from broiler meat (Table 7.3).

Resistance to cephalosporin (ceftiofur) was observed in two *E. coli* isolates from broilers and two isolates from Danish broiler meat. Based on a more sensitive selective enrichment method high level of cephalosporinase producing *E. coli* was also observed in broiler meat (see Textbox 8).

During the period 2007–2012, we have observed increasing trends in resistance to ampicillin, while resistance to spectinomycin decreased. The increasing trend in sulfonamide resistance observed from 2009 through 2011 (8%–22%) did not appear to continue in 2012 (Figure 7.5).

The occurrence of multi-resistance increased from 2008 to 2011, however this trend was discontinued in 2012 (Figure 7.6). This appears to reflect increasing total antimicrobial consumption (mainly ampicillin and tetracycline) in 2009 and a decrease in 2012 (Figure 4.6). The majority (91%) of multi-resistant isolates were resistant to ampicillin, sulfonamide and trimethoprim, frequently in combination with tetracycline resistance.

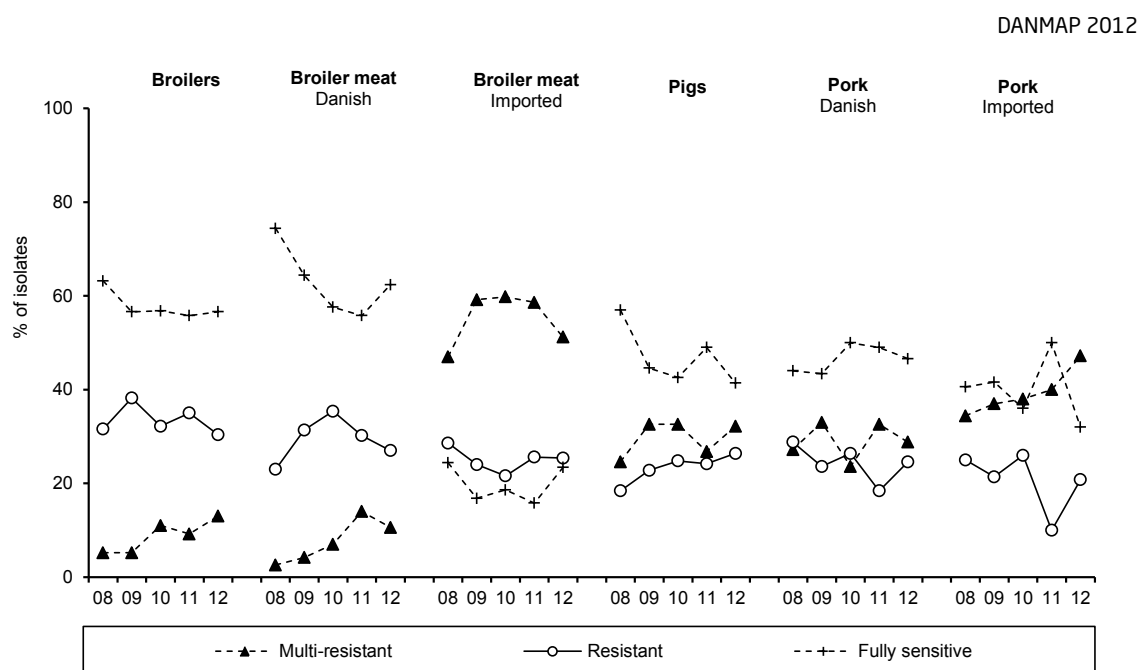
7.2.2 Indicator *E. coli* from cattle and beef

In cattle, we found similar levels of resistance in *E. coli* isolates (n = 98) as in 2011. The highest levels of resistance was found for tetracycline (7%), sulfonamide (6%) and streptomycin (6%), and among the resistant isolates 86% (4/7) were resistant to all three (Table 7.3). The level of multi-resistance has remained at the same level, fluctuating between 2% and 7% during the past five years.

As in cattle, the levels of resistance in *E. coli* isolates (n = 46) from Danish beef were very low, and at the same level as in 2011.

We found 91% of the isolates were fully sensitive whereas only two isolates were multi-resistant (Table 7.3). None of the *E. coli* isolates from cattle or Danish beef were resistant to cephalosporins (ceftiofur and cefotaxime). We detected one fluoroquinolone (ciprofloxacin) resistant isolate in Danish beef, while no isolate from cattle was resistant to fluoroquinolones. The use of fluoroquinolones in cattle has been close to zero since 2003, and the use of cephalosporins has been gradually decreasing since 2008 (Figure 4.6).

Figure 7.6. Occurrence (%) of multi-resistant and fully sensitive *Escherichia coli* ^(a) from animals and meat of Danish and imported origin, Denmark



Note: The number of isolates varies between years (broilers: n = 114–152, Danish broiler meat: n = 113–197, imported broiler meat: n = 140–304, pigs: n = 150–160, Danish pork: n = 66–106, imported pork: n = 30–96)

a) An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

Table 7.3. Resistance (%) among *Escherichia coli* from animals and meat of Danish and imported origin, Denmark

DANMAP 2012

Antimicrobial agent	Broilers	Broiler meat		Cattle	Beef		Pigs	Pork meat	
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	8	12	51	7	7	10	36	27	57
Chloramphenicol	0	1	10	2	0	2	3	1	15
Florfenicol	0	1	1	2	0	2	1	0	2
Ampicillin	20	22	51	5	4	10	29	33	49
Ceftiofur	2	1	8	0	0	0	1	1	2
Cefotaxime	2	1	9	0	0	0	1	1	2
Sulfonamide	21	17	48	6	4	8	35	30	42
Trimethoprim	10	12	37	2	2	8	22	22	34
Apramycin	0	0	1	0	0	0	0	0	2
Gentamicin	0	0	2	0	0	2	1	0	2
Neomycin	1	2	7	0	2	6	3	3	4
Spectinomycin	4	1	24	1	0	2	14	18	21
Streptomycin	11	8	31	6	4	8	42	36	45
Ciprofloxacin	8	4	36	0	2	2	1	0	9
Nalidixic acid	8	4	36	0	2	2	1	0	6
Colistin	0	1	3	0	0	0	0	0	2
Fully sensitive	57	62	23	93	91	87	41	47	32
Multi-resistant	13	11	51	7	4	8	32	29	47
Number of isolates	115	197	166	98	46	52	152	73	53

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 10.3)

7.2.3 Indicator *E. coli* from pigs and pork

Resistance to ampicillin (29%), streptomycin (42%), sulfonamide (35%) and tetracycline (36%) was common in *E. coli* isolates (n = 152) from pigs (Table 7.3). We found 86% of streptomycin resistant isolates co-resistant to tetracycline and/or sulfonamides in 2012. Streptomycin, sulfonamide and tetracycline resistance increased compared to 2011, and trend analysis over the period 2007–2012 shows an increasing occurrence of resistance to ampicillin and sulfonamide (Figure 7.5). Resistance to spectinomycin decreased compared to 2011, however the level has been fluctuating between 14% and 25% during the past five years.

Overall, we found 32% of the isolates from pigs were multi-resistant (Table 7.3), and most of multi-resistant isolates (78%) were co-resistant to ampicillin, sulfonamide and streptomycin (ASSu) and more than half of these (21/38) were also resistant to tetracycline (ASSuT). Thirteen isolates were only resistant to tetracycline (9% of all isolates). The level of multi-resistance in *E. coli* from Danish pigs has been similar during 2007–2012. The level of fully sensitive isolates in 2012 was similar to the level in 2010, with a temporary increase (not statistically significant) of fully sensitive isolates in 2011, coinciding with the low consumption of antimicrobial agents in 2011. Prior to 2011, the proportion of fully sensitive *E. coli* decreased significantly (Figure 7.6).

The occurrence of resistance in the *E. coli* isolates (n = 73) from Danish pork was comparable to 2011 (Figure 7.5), and the highest occurrence of resistance was found for streptomycin (36%), tetracycline (27%), ampicillin (33%) and sulfonamide (30%, Table 7.3). Multi-resistance was seen among 29% of the isolates, and of the multi-resistant isolates, 67% where ASSu and more than half of these were also ASSuT. The occurrence of resistance in *E. coli* from Danish pork and pigs was comparable to 2011.

The level of fluoroquinolone (ciprofloxacin) resistance remained low, only one isolate from pigs and none from Danish pork was observed. This likely reflects the low consumption of fluoroquinolones since 2002–2003 (web annex, Table A4.2), when the use in production animals was restricted by legislation. Also, occurrence of cephalosporin (ceftiofur and cefotaxime) resistance remained very low; we observed only one isolate from pigs and one isolates from Danish pork. The use of cephalosporins in pigs has been close to zero since July 2010, when a voluntary ban was adopted by the industry. Surveillance based on selective methods has shown a decrease in the number of cephalosporin resistant (ESC) in samples from pigs at slaughter in 2011–2012 compared to 2009–2010 (see Text box 8).

7.2.4 Indicator *E. coli* from imported meat

The level of multi-resistance (51%) in imported broiler meat was considerably higher compared to Danish broiler meat (11%, Table 7.3), and also compared to other Danish meat types included in the DANMAP programme. The observed level of multi-resistance in imported pork in 2012, reached a level similar to that of imported broiler meat (Figure 7.6).

The highest occurrences of resistance in *E. coli* isolates (n = 166) from imported broiler meat were found for ampicillin (51%), sulfonamide (48%) and tetracycline (51%). Resistance to colistin was observed in 3% of the isolates, however in contrast to previous years, this level was not higher than observed in the Danish broiler meat (1%). Compared to Danish broiler meat, we found a higher occurrence of resistance for 13 of the 16 antimicrobial agents in the test panel in *E. coli* from imported broiler meat (Figure 7.5, Table 7.3).

In imported beef, 87% of the *E. coli* isolates (n = 52) were fully sensitive and four isolates (8%) multi-resistant (Table 7.3). The level of fully sensitive and multi-resistance has not changed during the past 5 years, and is not higher than what we find in Danish beef. One isolate was resistant to quinolones (ciprofloxacin and nalidixic acid) and none of the isolates were resistant to cephalosporins (ceftiofur or cefotaxime).

The highest occurrence of resistance in *E. coli* isolates (n = 53) from imported pork was for tetracycline (57%), ampicillin (49%), sulfonamide (42%), trimethoprim (34%) and streptomycin (45%, Table 7.3). Overall, 47% of the isolates were multi-resistant, and during the past five years the occurrence of multi-resistance has been increasing in *E. coli* isolates from imported pork (Figure 7.6).

Resistance to fluoroquinolones (ciprofloxacin) was found in three isolates and cephalosporin (ceftiofur) resistance was observed in one isolate only. Compared to Danish pork, resistance to ampicillin, chloramphenicol, ciprofloxacin and tetracycline was higher in imported pork (Figure 7.5). In contrast to prior years, we detected resistance to all of the 16 antimicrobial agents in the test panel in *E. coli* from imported pork meat, including resistance to apramycin, gentamycin and colistin.

7.2.5 One Health Perspective

We use *E. coli* as an indicator organism for antimicrobial resistance because it is a commensal in both mammals and birds and commonly present on raw meat. Using phenotypic resistance as a marker, our data indicate that, *E. coli* in slaughter animals and in the derived meat constitute overlapping bacterial populations (Table 7.3). Therefore, meat has the potential to act as a vehicle transferring antimicrobial resistance from food animals to humans. Furthermore, some of these *E. coli* strains are virulent with a zoonotic potential for disease in humans [DANMAP 2010, Textboks 6].

Transfer of genes coding for antimicrobial agents that are critically important in human medicine such as 3rd generation cephalosporins is especially worrisome, as is transmission through food of *E. coli* resistant to fluoroquinolones. Resistance to fluoroquinolones is generally low in Danish animals, but higher in imported meats (Figure 7.5). Resistance to cephalosporins is presently increasing internationally in the animal reservoir, causing great concern both nationally and internationally (see further in Textbox 8). Cephalosporin resistance commonly resides on mobile genetic elements, e.g. plasmids, and therefore may be transferred between bacteria, in addition to clonal spread of *E. coli* strains.

Multi-resistance is of importance, because high levels of resistance decrease the number of good first choice antibiotics available for humans to treat infections and because it increases the risk of selection of antimicrobial resistance. As an example, if resistance to fluoroquinolone (chromosomal) or ESBL (plasmid) develops, the risk of co-selection through use of “old antimicrobial agents” (such as tetracycline, sulfonamide and penicillin) in the same bacterium increases with the occurrence of multi-resistance. Resistance to these antimicrobial agents is common in *E. coli* from Danish pigs and pork, as well as imported broiler meat, where we also observed high levels (>20%) of multi-resistance (Figure 7.5 and 7.6).

With co-resistance to critically important antimicrobial agents, the risk of maintenance and spread of the critical important antimicrobial resistance through use of un-related antimicrobial agents increases markedly. High levels of resistance and multi-resistance contribute to a reduced number of antimicrobial agents available for use in human medicine, thus limiting the solutions for treating human illness.

Vibeke Frøkjær Jensen, Vibe Dalhoff Andersen
and Lars Stehr Larsen

Occurrence of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in meat and slaughter pigs after selective enrichment with ceftriaxone, but no sign of carbapenemase producing *E. coli*

Background: Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide in both humans and production animals. Lately, several studies have found the same ESBL genes, plasmids and clones of *E. coli* isolates originating from animals and involved in human infections, suggesting a zoonotic link. The occurrence of *Enterobacteriaceae* resistant to carbapenems is a growing threat in human medicine as carbapenems are the last line antimicrobial agents for treatment of infections caused by multidrug resistant Gram-negative bacteria in humans; this has been pointed out by both EFSA and ECDC. The presence of carbapenemase producing bacteria in food-producing animals is not known, but lately carbapenemase producing *E. coli* has been detected in livestock pigs in Germany, and carbapenemase producing *Salmonella enterica* subsp. *enterica* has been detected in both livestock pigs and poultry in Germany [Fischer *et al.* 2012. J. Antimicrob Chemother. 67:1793-5]. The presence and possible spread of carbapenemase producing bacteria in production animals is thus considered extremely important for the assessment of potential zoonotic risks.

Carbapenems are not used in the Danish animal production. Both ESBL and carbapenem resistance can be driven by usage of cephalosporins. In July 2010, the use of cephalosporins in the Danish pig production was discontinued, but it is still used for systemic and intramammary treatment in cattle. Cephalosporins have not been used in the Danish broiler production for at least a decade. The aim of this study was to investigate the occurrence of ESBL-producing *E. coli* in pigs and in meat at retail and to investigate if carbapenemase producing *E. coli* could be present in meat and production animals (see section 11. for definition of ESBL).

Materials and methods: During February through December 2012, faecal samples were taken from pigs at slaughter (n = 787). One animal represented one herd and no herds were sampled more than once in the same month. In the same period, broiler meat (Danish: n = 206, imported: n = 178), beef (Danish: n = 121, imported: n = 98) and pork (Danish: n = 188, imported: n = 178) samples were collected randomly in retail stores and outlets in all regions of Denmark. *E. coli* was isolated from 1 g of faecal sample or 5 g of meat after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL-resistance was revealed by use of whole genome sequencing (WGS). The reads were assembled de novo prior to prediction of genes. The web-server ResFinder (www.genomicepidemiology.org) was used to identify acquired ESBL and carbapenemase genes in the WGS data. For isolates where no genes were detected, the sequences were investigated for up-regulation of chromosomal *ampC* by use of CLC bio 6. Moreover, a study of extended spectrum cephalosporinase (ESC)-producing *E. coli* collected from pigs (n = 28) and cattle (n = 19) at slaughter and from meat (n = 138) collected in 2011 (see textbox 7, DANMAP 2011) were tested for reduced susceptibility towards carbapenems by use of discs containing meropenem, ertapenem and imipenem, respectively.

Results: For pigs at slaughter, 8% (64/787) contained ESC-producing *E. coli*, which was significantly higher than in 2011 but significantly lower than in 2010. The most commonly detected gene was, as in previous years, CTX-M-1. One TEM-55 producing *E. coli* and one TEM-135 producing *E. coli* were detected in pigs at slaughter which has not previously been detected. CTX-M-14 and CTX-M-15 producing *E. coli* were detected in a few isolates (n = 5 and n = 1, respectively) from pigs as well (Figure 1). From meat samples, the highest prevalence of ceftriaxone resistant *E. coli* was found among imported broiler meat (61%, 110/178) and had increased significantly compared with 2011. The prevalence (36%, 75/206) of ceftriaxone resistant *E. coli* in Danish broiler meat was at the same level as in 2011. The occurrence of ceftriaxone resistant *E. coli* was significantly higher in imported broiler meat when compared to Danish broiler meat. The most commonly detected gene in Danish broiler meat was, as found in previous years, CMY-2, whereas CTX-M-1 and CMY-2 were almost equally present in imported broiler meat. Eight isolates of the ESBL-producing isolates from broiler meat contained two ESBL genes. In the other meat types, the prevalence was generally low (0-2%, Figure 2). None of the isolates from 2012 contained any known carbapenemase genes and none of the isolates from 2011 that were phenotypically tested showed reduced susceptibility towards any of the three carbapenems tested.

Discussion: Despite a usage of cephalosporins close to zero (approximately 1 kg), the results showed that even though the prevalence of cephalosporinase producing *E. coli* in slaughter pigs was significantly higher than in 2011, it is still significantly lower than in 2010 where the voluntary ban of cephalosporin usage in the Danish pig production was effectuated. The prevalence of cephalosporinase producing *E. coli* in Danish and imported broiler meat was still high. The occurrence of cephalosporinase producing *E. coli* in Danish broiler meat has not decreased significantly although the consumption of amoxicillin, which may select for ESBL-producing *E. coli*, has decreased in the broiler production. This is probably due to continuous introduction of ESBL-producing *E. coli* from imported parent animals, but other factors such as horizontal gene transfer, persistence of certain clones or cross contamination at slaughter may influence the occurrence of ESC-producing *E. coli* in the meat. An increasing trend of ESBL-producing *E. coli* from human infection has been observed the last years. CTX-M-15 is the most commonly gene detected in *E. coli* isolates from human infections, but CTX-M-1 occur in 7-8% of the ESBL-producing *E. coli* from human infections and to a lesser extent AmpC enzymes [Textbox 9, DANMAP 2011; Textbox 9, DANMAP 2012].

Testing for reduced susceptibility to carbapenems among isolates from food and food-producing animals has not been included as part of DANMAP until now. The detection of carbapenem resistance is not straightforward since carbapenemases belong to several different classes of beta-lactamases and no single test is likely to give high sensitivity as well as high

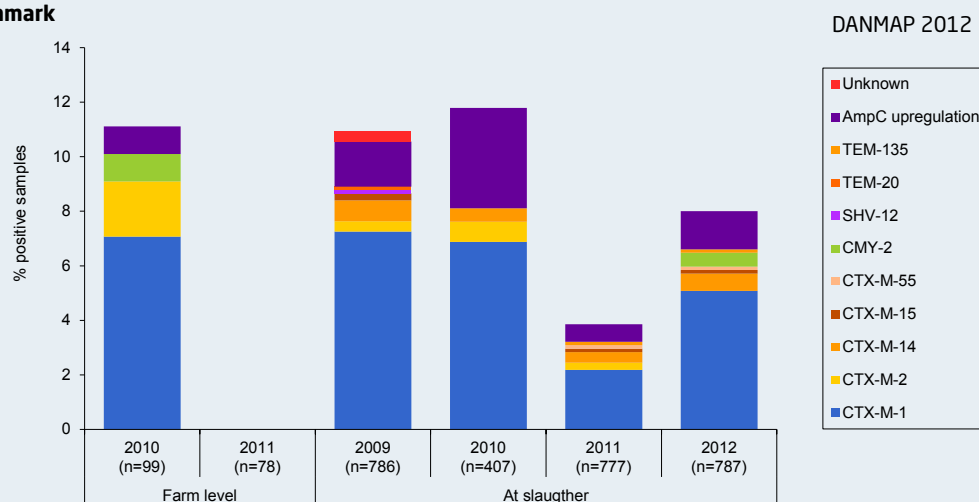
specificity for all types of enzymes. Moreover, several types are not always expressed phenotypically. Since none of the tested ESC-producing *E. coli* isolates showed reduced susceptibility towards any of the three tested carbapenems or contained known carbapenemase genes, meat or food-producing animals in Denmark are most likely not a source to carbapenemase producing bacteria causing human infections in Denmark, but monitoring of carbapenemase producing bacteria in animals and meat is still important as the situation may change over time.

Conclusion: In conclusion, it is important to maintain the voluntary ban of cephalosporins in the pig production to limit occurrence of ESBL-producing *E. coli*. ESBL genes found in ESBL-producing *E. coli* from human infections are still present in meat and pigs, and broiler meat from both Danish and imported origin still seems to be the most important meat sources to ESBL-producing *E. coli*. Carbapenemase producing *E. coli* from meat or food-producing animals are considered a low if any risk to human health.

Yvonne Agersø, Rolf Sommer Kaas, Henrik Hasman

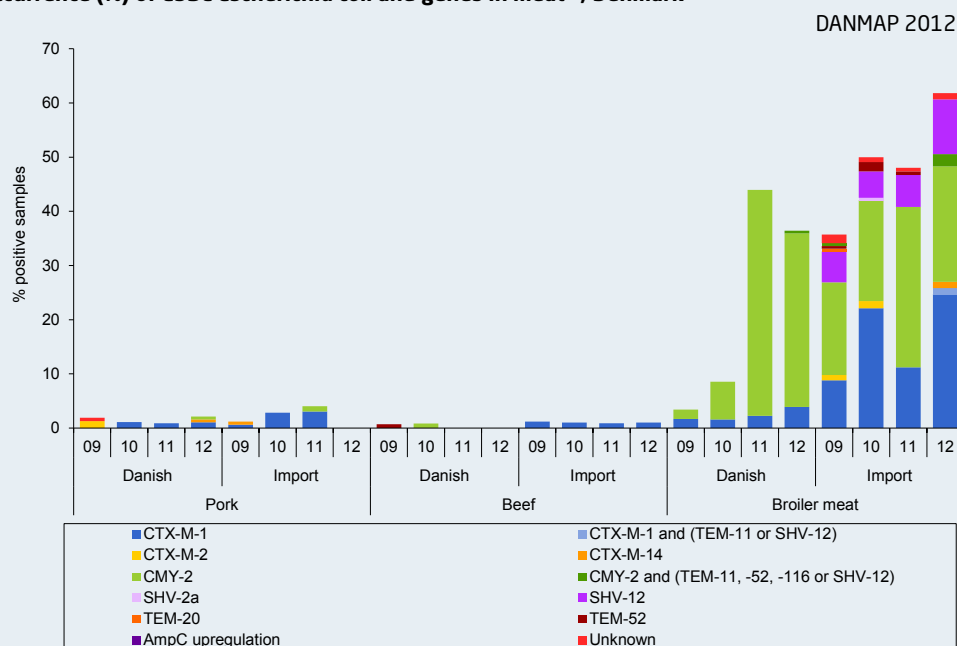
For further information: Yvonne Agersø (yvoa@food.dtu.dk)

Figure 1. Occurrence (%) of ESBL *Escherichia coli* and genes in pig from samples collected at farm and slaughterhouse level, Denmark



Note: *E. coli* was isolated after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL resistance was revealed by use of PCR, micro array and DNA sequencing

Figure 2. Occurrence (%) of ESBL *Escherichia coli* and genes in meat^{a)}, Denmark



Note: *E. coli* was isolated after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL resistance was revealed by use of PCR, micro array and DNA sequencing

a) Each year approximately 1,000 samples are collected evenly distributed between the six categories of meat



8. Resistance in human clinical bacteria

8.1 Escherichia coli

Escherichia coli is part of the normal intestinal flora of both humans and animals but also cause infections. In humans, *E. coli* is the most frequent cause of bacteraemia and community- and hospital-acquired urinary tract infections. For *E. coli*, DANMAP 2012 includes data from 12 out of 13 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. Eleven of the 12 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *E. coli* from primary health care (Table 8.1).

Blood isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 3,900 *E. coli* isolates from blood (Table 8.1 and Figure 8.1).

In 2012, resistance to 2nd generation cephalosporins (cefuroxime) was 9% and resistance to 3rd generation cephalosporins 7%. Both levels are similar to the levels reported in 2011.

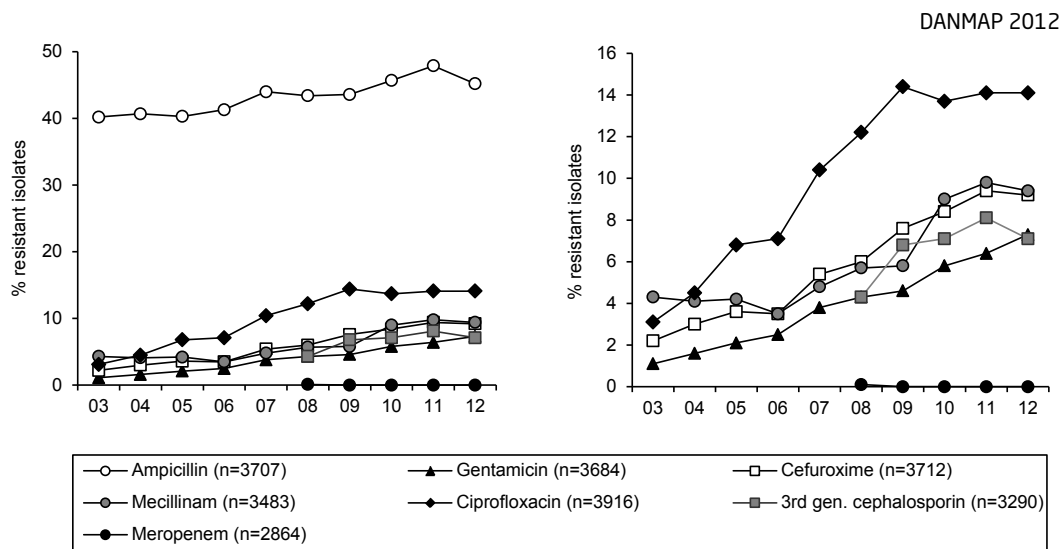
One carbapenem (meropenem) resistant *E. coli* blood isolate was reported in 2012. The mechanism behind the carbapenem

Table 8.1. Resistance (%) in *Escherichia coli* isolates from humans, Denmark DANMAP 2012

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Ampicillin	45 #	41	40 #
Mecillinam	9	7	6
Sulfonamide		33 #	33 #
Gentamicin	7	5 *	
Ciprofloxacin	14	12 #	10
Cefuroxime	9	6 *	4 *
3rd generation cephalosporins ^(a)	7	6 *	4 *
Meropenem	<1	<1	<1
Max. number of isolates tested	3916	38610	36475

*) An asterisk indicates a significant increase from 2011 to 2012
#) A number sign indicates a significant decrease from 2011 to 2012
a) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

Figure 8.1. Resistance (%) in *Escherichia coli* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2012

resistance in this isolate is not known since the isolate was not sent to SSI for further analysis, but another meropenem resistant *E. coli* isolate from the same patient was analysed at SSI. The carbapenem resistance in this isolate was due to ESBL-production and reduced permeability in combination [Textbox 11].

In 2012, ciprofloxacin resistance was 14%, which is similar to the level reported in 2011. The level of quinolone and 3rd generation cephalosporin resistance in Denmark was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by other European countries in 2011 [EARS-Net 2011].

Aminoglycoside (gentamicin) resistance was 7%, similar to the level in 2011. Again, this was higher than the level reported to EARS-Net by the other Nordic countries in 2011 [EARS-Net 2011]. Mecillinam resistance was 9% in 2012, which is at the same level as in 2011.

The occurrence of ampicillin resistance decreased from 48% in 2011 to 45% in 2012.

Since 2003, resistance in *E. coli* blood isolates has increased steadily: resistance to 2nd generation cephalosporins from 2% to 9%; ciprofloxacin resistance from 3% to 14%; aminoglycoside (gentamicin) resistance from 1% to 7%. These increases parallel the increased antimicrobial consumption which has been seen until 2012 (Table 5.4).

Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 38,000 *E. coli* isolates from hospital patients with a urinary tract infection (Table 8.1 and Figure 8.2).

The occurrence of resistance to both 2nd generation cephalosporin (cefuroxime) and 3rd generation cephalosporin increased from 5% in 2011 to 6% in 2012 [Textbox 9].

In 2012, carbapenem (meropenem) resistance was observed in six *E. coli* urine isolates from hospitalised patients. One of these isolates produced Verona integron-encoded metallo- β -lactamase (VIM)-4, however the mechanism behind the carbapenem resistance in the remaining five isolates is not known, since the isolates were not further analysed [Textbox 11].

The occurrence of ciprofloxacin resistance decreased from 13% in 2011 to 12% in 2012. This is a change to the steady increase seen in ciprofloxacin resistance from 2% in 2003 to 12% in 2012 (Figure 8.2).

Aminoglycoside (gentamicin) resistance increased from 4% in 2011 to 5% in 2012.

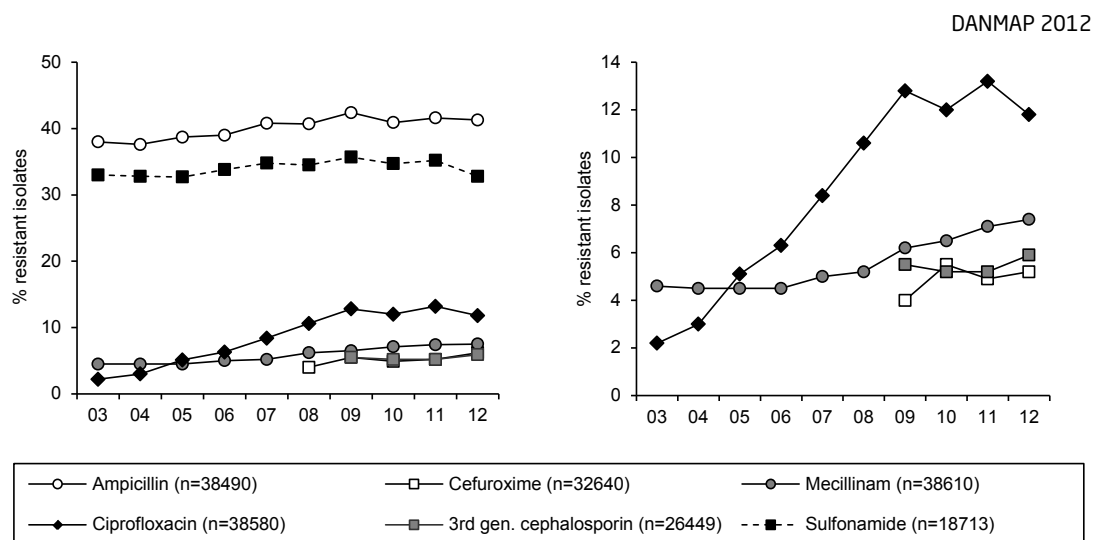
The occurrence of sulfonamide resistance decreased from 35% in 2011 to 33% in 2012.

Urine isolates from primary health care

DANMAP received data on the antimicrobial susceptibility of approximately 36,000 *E. coli* isolates from urinary tract infection in patients from primary health care (Table 8.1 and Figure 8.3).

The occurrence of resistance to both 2nd generation cephalosporin (cefuroxime) and 3rd generation cephalosporin increased from 3% in 2011 to 4% in 2012 [Textbox 9].

Figure 8.2. Resistance (%) in *Escherichia coli* urine isolates from humans in hospitals, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2012

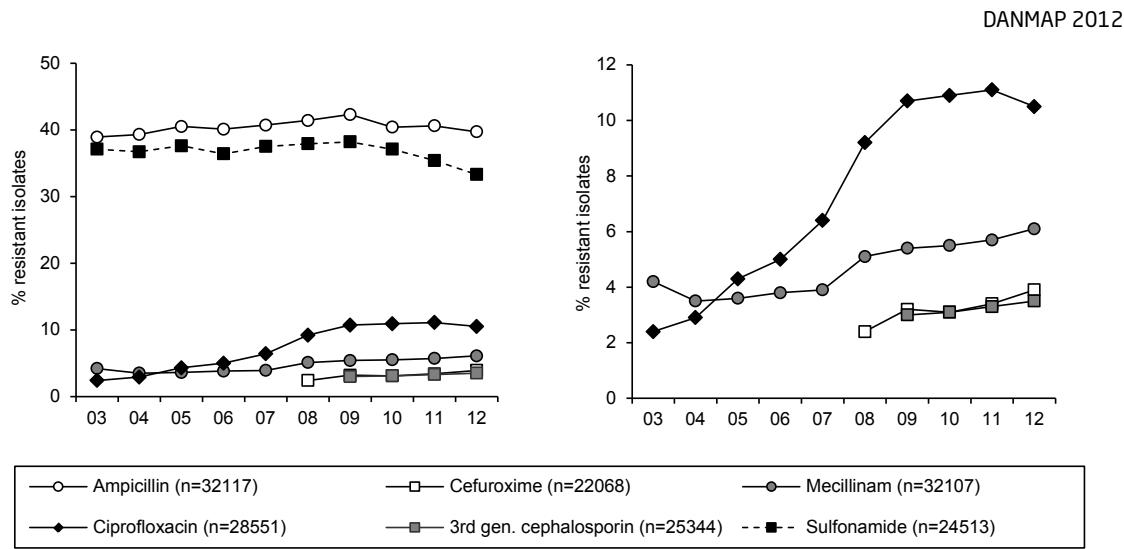
Since 2003, the level of ciprofloxacin resistance has increased steadily from 2% in 2003 to 10% in 2012 (Figure 8.3), which parallels the increasing trend observed in the consumption of fluoroquinolones during the last decade (Table 5.2).

The occurrence of ampicillin resistance decreased from 41% in 2011 to 40% in 2012. Sulfonamide resistance decreased from 35% in 2011 to 33% in 2012, which follows the decreasing trend observed in the consumption of sulfonamides during the last years (Table 5.2).

In 2012, carbapenem (meropenem) resistance was observed in nine *E. coli* urine isolates from primary health care. The isolates were not further investigated [Textbox 11].

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Figure 8.3. Resistance (%) in *Escherichia coli* urine isolates from humans in primary health care, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2011

Increased occurrence of 3rd generation cephalosporin resistance among *Escherichia coli* isolates from urinary tract infections

Background: Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria are not reportable in Denmark. However, 3rd generation cephalosporin resistance can be used as a marker for ESBL-producing bacteria, since 3rd generation cephalosporin resistance is most often due to production of ESBL-enzymes and to a lesser extent AmpC enzymes.

Methods: Data on 3rd generation cephalosporin (ceftriaxone, cefpodoxime and cefotaxime) resistance in *E. coli* urine isolates from hospitalised patients in 2011 and 2012 were collected from 9 of 13 Departments of Clinical Microbiology (DCM) in Denmark; 8 DCM working with primary health care isolates contributed data on 3rd generation cephalosporin resistance in urine isolates of *E. coli*.

Results: In 2012, 938 out of 26,633 (4%) tested patients from primary health care had a UTI with a 3rd generation cephalosporin resistant *E. coli*, as compared to 835 out of 25,344 (3%) tested patients in 2011. In 2012, 1,573 out of 26,449 (6%) tested hospitalised patients had a urinary tract infection (UTI) with a 3rd generation cephalosporin resistant *E. coli*, as compared to 1,539 out of 29,451 (5%) tested patients in 2011. The increases observed from 2011 to 2012 in 3rd generation cephalosporin resistance in *E. coli* from UTI in both primary health care and hospitalised patients were statistically significant (Table 8.1).

Discussion: An increasing trend has been observed for 3rd generation cephalosporin resistant *E. coli* from UTI during the last years. In 2012, 2,511 patients had a UTI with 3rd generation cephalosporin resistant *E. coli*. Furthermore, 235 patients had a bloodstream infection with a 3rd generation cephalosporin resistant *E. coli* in 2012, which was at the same level as in 2011 (Table 8.1). The genes encoding the 3rd generation cephalosporin resistance were not detected in the isolates from 2012, but earlier studies have shown that the ESBL-production is most often due to production of CTX-M-15, and to a lesser extent CTX-M-14, CTX-M-1 and other enzymes [Focus Area, DANMAP 2009; Textbox 9, DANMAP 2011]. The epidemiology behind the increasing trend in 3rd generation cephalosporin resistant *E. coli* from UTI is not known. Further studies are needed to investigate why 3rd generation cephalosporin resistant *E. coli* isolates from UTI episodes are detected with increasing frequency.

Anette M. Hammerum, Stefan S. Olsen and Line Skjøl-Rasmussen

For further information: Anette M. Hammerum (ama@ssi.dk)

8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the normal intestinal flora in humans but also cause infections such as urinary tract-, respiratory tract-, wound- and bloodstream infections. Many of these infections are hospital acquired and can be life threatening, especially if the strains are resistant to antimicrobial agents. *K. pneumoniae* is intrinsically resistant to aminopenicillins (e.g. ampicillin). Therefore, infections caused by *K. pneumoniae* are treated with broad spectrum antimicrobial agents such as ciprofloxacin, gentamicin, cephalosporins and carbapenems. For *K. pneumoniae*, DANMAP 2012 includes data from 12 out of 13 DCM, representing 95% of the Danish population. Eleven of the 12 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *K. pneumoniae* from primary health care (Table 8.2).

Blood isolates from hospital patients

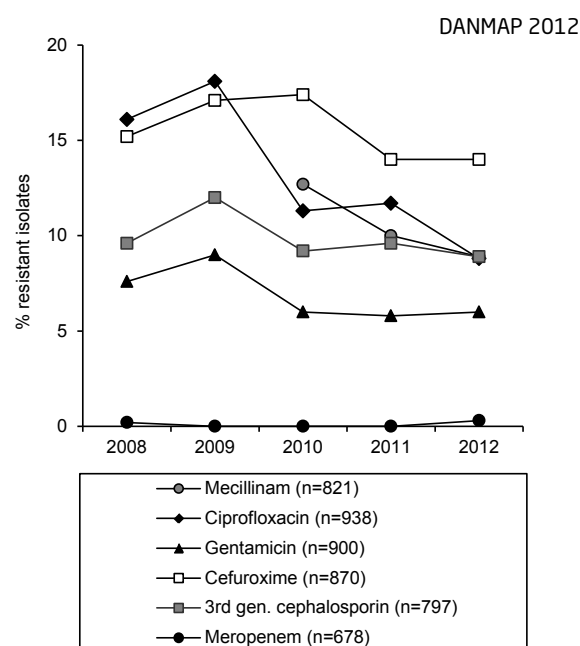
DANMAP received data on the antimicrobial susceptibility of approximately 900 *K. pneumoniae* isolates from blood (Table 8.2 and Figure 8.4).

In general, the level of antimicrobial resistance in 2012 was similar to 2011 (Figure 8.4).

Resistance to 2nd generation cephalosporins (cefuroxime) was 14% and resistance to 3rd generation cephalosporins 9%. Both levels were similar to the levels reported in 2011 (14% and 10%, respectively). In 2011, 3rd generation cephalosporin resistance was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by other European countries in 2011 [EARS-Net 2011]. Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes. The genetic background was not reported for the 3rd generation cephalosporin resistant *K. pneumoniae* isolates in 2012. The prevalence study from October 2011 showed that most of the ESBL production was due to production of CTX-M-15 [Textbox 9, DANMAP 2011].

In 2012, two carbapenem (meropenem) resistant *K. pneumoniae* blood isolates were detected. One of these isolates was sent to SSI for further analysis; the reduced susceptibility

Figure 8.4. Resistance (%) in *Klebsiella pneumoniae* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2012

to carbapenems was due to production of ESBL and reduced permeability. The mechanism behind the carbapenem resistance in the other isolate was not known [Textbox 11].

Ciprofloxacin resistance decreased from 12% in 2011 to 9% in 2012 (Figure 8.4). From 2011 to 2012, a decreased consumption of fluoroquinolones has been observed (Table 5.4). Resistance to aminoglycoside (gentamicin) was 6%, similar to the level in 2011. The levels of resistance to ciprofloxacin and gentamicin were both above the levels reported from the other Nordic countries and the same as reported to EARS-Net by other European countries in 2011 [EARS-Net 2011].

Table 8.2. Resistance (%) in *Klebsiella pneumoniae* isolates from humans, Denmark

DANMAP 2012

Substance	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	9	11	11 #
Sulfonamide		24 #	26 #
Gentamicin	6	6	
Ciprofloxacin	9 #	9 #	8
Cefuroxime	14	11	
3rd generation cephalosporins ^(a)	9	8	5
Meropenem	<1	<1	<1
Max. number of isolates tested	938	5982	3142

#) A number sign indicates a significant decrease from 2011 to 2012

a) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 5,900 *K. pneumoniae* isolates from hospital patients with a urinary tract infection (Table 8.2 and Table 8.3).

Resistance to 2nd generation cephalosporins (cefuroxime) was 11% and resistance to 3rd generation cephalosporins 8%. Both levels were similar to the levels reported in 2011 (11% and 10%, respectively). The level of resistance to 3rd generation cephalosporins has decreased from 13% in 2009, when it was first reported to DANMAP, to 8% in 2012.

In 2012, carbapenem (meropenem) resistance was observed in three *K. pneumoniae* urine isolates from hospitalised patients. One of the three isolates was sent to SSI for further analysis; the reduced susceptibility to carbapenems was due to production of ESBL and reduced permeability. The mechanism behind the carbapenem resistance in the other two isolates was not known [Textbox 11].

Ciprofloxacin resistance decreased from 11% in 2011 to 9% in 2012.

Sulfonamide resistance decreased from 33% in 2011 to 24% in 2012. Mecillinam resistance (11%) was at the same level as reported in 2011.

Urine isolates from primary health care

DANMAP received data on the antimicrobial susceptibility of approximately 3,100 *K. pneumoniae* isolates from urinary tract infection in patients from primary health care (Table 8.2 and Table 8.4).

In 2012, resistance to 3rd generation cephalosporins was 5%, which is similar to the level reported in 2011. Resistance to 3rd generation cephalosporins in *K. pneumoniae* from urinary tract infection in patients from primary health care was lower than the occurrence of resistance detected in isolates from both blood and urine from hospital patients.

In 2012, two carbapenem (meropenem) resistant *K. pneumoniae* urine isolates from patients in primary health care were found. Both isolates were sent to SSI for further analysis; the reduced susceptibility to carbapenems was probably due to production of ESBL and reduced permeability.

Resistance to ciprofloxacin was 8%, which is similar to the level reported in 2011. Ciprofloxacin resistance in *K. pneumoniae* from urinary tract infections in patients from primary health care was lower than the occurrence of resistance detected in isolates from urine from hospital patients.

Sulfonamide resistance decreased from 35% in 2011 to 26% in 2012, and mecillinam resistance decreased from 12% in 2011 to 11% in 2012.

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Table 8.3. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark

Substance	DANMAP 2012			
	2009 %	2010 %	2011 %	2012 ^(a) %
Mecillinam	13.3	14.3	11.9	11.1
Sulfonamide	27.0	28.6	33.4	24.3
Gentamicin		7.3	6.4	5.7
Ciprofloxacin	17.3	13.9	11.5	9.1
Cefuroxime		12.8	10.9	10.6
3rd gen. cephalosporins ^(b)	12.8	12.0	9.6	8.3
Max. number of isolates tested	6394	5740	5746	5982

a) Susceptibility to mecillinam was tested in 5971 isolates, sulfonamide susceptibility in 3115 isolates, gentamicin susceptibility in 4469 isolates, ciprofloxacin susceptibility in 5982 isolates, cefuroxime susceptibility in 4943 isolates, and 3rd gen. cephalosporin susceptibility in 4106 isolates

b) Tested 3rd generation cephalosporins were cefpodoxime and cefotaxime

Table 8.4. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark

Substance	DANMAP 2012			
	2009 %	2010 %	2011 %	2012 ^(a) %
Mecillinam	15.1	16.3	12.5	10.6
Sulfonamide	30.2	33.9	34.9	25.7
Ciprofloxacin	13.2	11.9	8.6	7.8
3rd gen. cephalosporins ^(b)	8.1	7.0	5.3	4.7
Max. number of isolates tested	3200	3200	3489	3142

a) Susceptibility to mecillinam was tested in 3,142 isolates, sulfonamide susceptibility in 2,825 isolates, ciprofloxacin susceptibility in 2,968 isolates, and 3rd generation cephalosporin susceptibility in 2,163 isolates

b) Tested 3rd generation cephalosporins were cefpodoxime and cefotaxime

Reduced susceptibility in the *Bacteroides fragilis* group isolated from blood cultures in Denmark

Background: Bacteraemia with *Bacteroides fragilis* group species is associated with high mortality rates if appropriate antimicrobial therapy is not administered. Increasing resistance in the *B. fragilis* group has been reported worldwide, especially towards clindamycin and piperacillin-tazobactam. Lately, resistance towards the carbapenems and metronidazole have been reported in Europe [Nagy *et al.* 2011. Clin. Microbiol. Infect. 17: 371-9; Hartmeyer *et al.* 2012. J. Med. Microbiol. 61: 1784-8]. At Odense University Hospital, 10.2% (9 of 88) of *B. fragilis* group blood culture isolates from a 3-year period (November 2007 to November 2010) had reduced susceptibility (intermediate susceptible or resistant) towards meropenem (MIC >2 mg/L).

The antimicrobial susceptibility of the *B. fragilis* group in Denmark was until recently largely unknown. We are here reporting on the antimicrobial susceptibility of piperacillin-tazobactam, meropenem, metronidazole and clindamycin in the *B. fragilis* group isolated from blood cultures in Denmark [Justesen *et al.* 2013. Int J Antimicrob Agents, 42: 188-90].

Methods: A national survey was performed from January to the end of May 2012, including 11 of 13 Departments of Clinical Microbiology in Denmark. The study included 118 consecutive blood culture isolates (Table 1) from the *B. fragilis* group. EUCAST clinical breakpoints were used for SIR categorization.

Results: None of the isolates were resistant to metronidazole. Overall, high rates of reduced susceptibility towards piperacillin-tazobactam and clindamycin were seen in *B. thetaiotaomicron* and towards meropenem in *B. fragilis* (Table 1).

Conclusion: The perspectives from this study are worrying, as the blood culture isolates from our study only represent “the-tip-of-the-tip of the iceberg”. Considering the astronomical numbers of *Bacteroides fragilis* group bacteria in the gut, the percentage of patients that are harbouring isolates with decreased susceptibility could be much higher than the percentages reported from this study. This study emphasizes the need for more simple and inexpensive antimicrobial susceptibility testing methods for anaerobic bacteria, especially the *B. fragilis* group. It is no longer enough to rely on local surveillance data to treat patients with serious infections with the *B. fragilis* group. Metronidazole is still the drug of choice for anaerobic infections. However, although no resistant strains were detected in this study, the emergence of resistant strains is a concern and should be monitored closely.

Ulrik Stenz Justesen

For further information: Ulrik Stenz Justesen (Ulrik.Stenz.Justesen@rsyd.dk)

Table 1. *Bacteroides fragilis* group isolates from the study and percentage with reduced susceptibility (I or R) according to the EUCAST clinical MIC breakpoints

DANMAP 2012			
MIC breakpoint (mg/L)	Piperacillin-tazobactam S≤8 and R>16	Meropenem S≤2 and R>8	Clindamycin S≤4 and R>4
Total (118)	8.5% (9/1) ^(a)	3.4% (3/1)	24.6% (-/29) ^(b)

Note: I: intermediate susceptible. R: resistant
a) Numbers of I/R
b) There is no intermediate susceptible category for clindamycin

Carbapenemase producing bacteria in Denmark

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant 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 β -lactamase (OXA), Verona integron-encoded metallo- β -lactamase (VIM), and New Delhi metallo- β -lactamase (NDM).

Currently there is no systematical monitoring of carbapenemase producing bacteria in Denmark and these bacteria are not mandatory reportable. In recent years, Danish Departments of Clinical Microbiology (DCM) have, on a voluntary basis, submitted carbapenem resistant isolates for verification and genotyping at the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut. The results of these investigations have been reported in Textbox 11 in DANMAP 2011 where attention was given to the emergence of carbapenemase producing *Enterobacteriaceae* (CPE) including *K. pneumoniae* and *E. coli*. The present textbox describes the carbapenemase producing *P. aeruginosa* and *A. baumannii* in addition to *Enterobacteriaceae*.

Enterobacteriaceae: During 2008–2011, 15 CPE cases were detected in Denmark [Textbox 11, DANMAP 2011]. In 2012 four new cases were detected, among these the first NDM-4 producing *E. coli* in Denmark [Jakobsen *et al.* in press]. Prior to detection the patient had been to Vietnam. Furthermore, an NDM-1 producing *Citrobacter freundii* and two VIM-4 producing *E. coli* were detected. None of these patients had a prior history of travel and the origin of these carbapenemase producing isolates was unknown.

When comparing the number of CPE cases registered at the Antimicrobial Resistance Reference Laboratory with DANMAP 2012 resistance data collected from the Danish DCM, it was clear that not all carbapenem resistant *E. coli* and *K. pneumoniae* isolates detected at the hospitals had been submitted to SSI. In addition to the four isolates received at the Antimicrobial Resistance Reference Laboratory, nine carbapenem resistant isolates were reported (eight *E. coli* from urinary tract infections and one *K. pneumoniae* isolate).

A. baumannii: During 2012, the Antimicrobial Resistance Reference Laboratory received 20 carbapenem resistant *A. baumannii* isolates from an outbreak at one of the major hospitals in the Capital Region. All isolates tested positive for the presence of the OXA-23 carbapenemase. In previous years the hospital has had outbreaks with OXA-23 producing *A. baumannii* isolates. It is under investigation if the outbreak seen in 2012 could be related to earlier outbreaks. Furthermore, three OXA-23 producing *A. baumannii* were received from three other hospitals, one was from a patient who had been in Egypt prior to the detection and the other two were from patients who had been hospitalized in Turkey.

P. aeruginosa: In most studies, *P. aeruginosa* carbapenem resistance mechanisms have been shown primarily to include loss of outer membrane porin channels and increased drug-targeted efflux pump activity with consequential reduced permeability of antimicrobial agents, but carbapenemases such as VIMs can also be detected in *P. aeruginosa*. In recent years, an increasing trend has been observed for carbapenem resistance in *P. aeruginosa* from bloodstream infections in Denmark. A Danish study of 116 *P. aeruginosa* carbapenem non-susceptible (R+I) isolates from 2011 showed that carbapenemases (VIM-2) were present in 7% of the isolates [Hansen *et al.* 2012, ECCMID, P1697]. During 2012, four VIM producing *P. aeruginosa* isolates were detected at four different hospitals. One of the patients with a VIM producing *P. aeruginosa* had been in Pakistan prior to the detection of the isolate, whereas none of the other patients had a prior travel history. The origin of these VIM producing isolates was unknown.

Conclusion: Currently, surveillance of carbapenemase producing bacteria in Denmark is not mandatory and not all isolates are sent to the Antimicrobial Resistance Reference Laboratory for investigation. The increasing occurrence of CPE and carbapenemase producing *A. baumannii* and *P. aeruginosa* in Denmark is worrying. The carbapenemase producing bacteria detected in Denmark were all multi-resistant which makes infections caused by these bacteria extremely difficult to treat with antimicrobial agents.

Anette M. Hammerum, Lotte Jakobsen and Frank Hansen

For further information: Anette M. Hammerum (ama@ssi.dk)

8.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen of immunocompromised individuals. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other bloodstream infections. It is the most frequent coloniser of medical devices (e.g. catheters). *P. aeruginosa* infection is a serious problem in patients hospitalised with cancer, cystic fibrosis and burns. 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 (e.g. ciprofloxacin and levofloxacin), aminoglycosides (e.g. gentamicin, tobramycin and amikacin), some beta-lactams (piperacillin-tazobactam, ceftazidime, and carbapenems) and colistin.

P. aeruginosa blood isolates obtained from hospitalised patients

For *P. aeruginosa*, DANMAP 2012 includes data from 12 out of 13 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. DANMAP received data on the antimicrobial susceptibility of approximately 370 *P. aeruginosa* isolates from blood. Resistance to all the tested antimicrobial agents was not significantly different from the level in 2011, but an increasing trend has been observed for gentamicin during 2007–2012 and for meropenem during 2007–2011 (Figure 8.5). The occurrence of resistance to fluoroquinolones, carbapenem, ceftazidime and piperacillin/tazobactam was at the same level or lower than most of the other countries reporting to EARS-Net in 2011 [EARS-NET 2011].

Meropenem resistance was observed for 4% (n = 13) of the *P. aeruginosa* isolates in 2012. A Danish study of *P. aeruginosa* carbapenem non-susceptible isolates from 2011 showed that carbapenemases were present in a minority of the isolates (7%) [Textbox 11]. As in previous years, presumable carbapenemase producing *P. aeruginosa* isolates were sent on a voluntary basis from the DCM to SSI for national surveillance on carbapenemase producing bacteria, including not only isolates from bloodstream infections but also from other origins. During 2012, 4 Verona integron-encoded metallo- β -lactamase (VIM)-producing *P. aeruginosa* isolates were identified at SSI [Textbox 11].

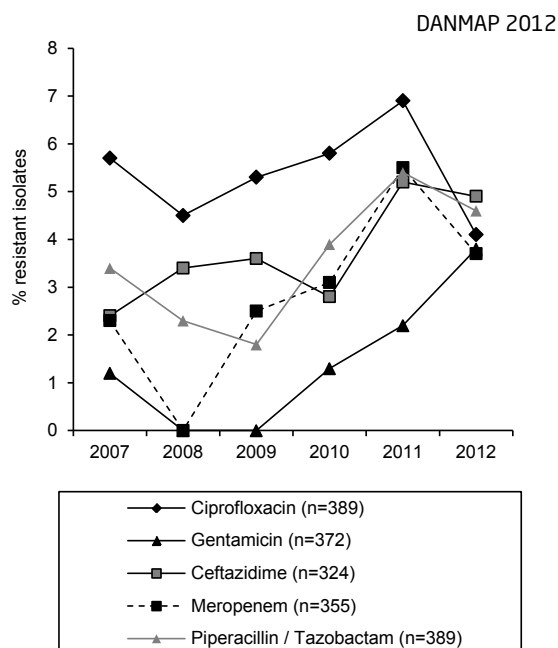
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and Line Skjøl-Rasmussen

8.4 Streptococci

Streptococci are part of the normal commensal flora of the mouth, skin, intestine, and upper respiratory tract of humans, but streptococci also cause infections such as otitis media, tonsillitis, bacterial pneumonia, bacteraemia/sepsis, endocarditis and meningitis.

In this report, data on resistance in invasive (from blood or cerebrospinal fluid) streptococcal isolates were obtained from the Neisseria and Streptococcus Reference laboratory covering all DCM in Denmark. In Denmark, penicillins and macrolides are often used for treatment of infections caused by streptococci. All invasive non-duplicate *Streptococcus pneumoniae* and

Figure 8.5. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2012

group A, B, C and G streptococci were susceptibility tested against erythromycin and penicillin.

Streptococcus pneumoniae

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, otitis media, bacteraemia and meningitis. In 2012, susceptibility testing was performed on 872 non-duplicate *S. pneumoniae* isolates from invasive infections (Figure 8.6).

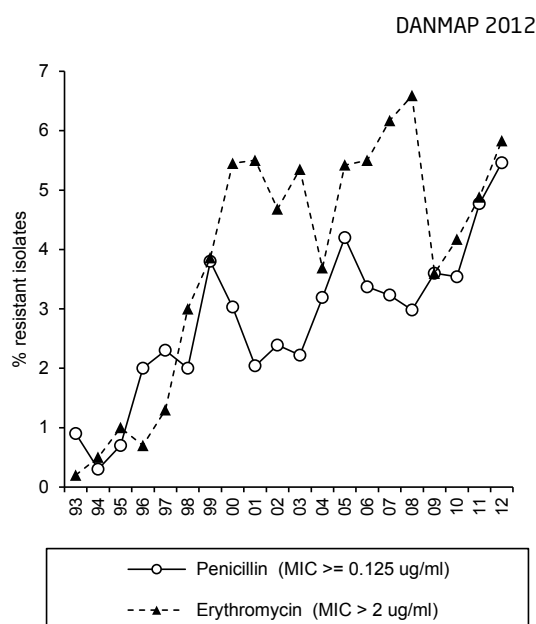
Erythromycin resistance in *S. pneumoniae* isolates from blood and cerebrospinal fluid was 5.4% (n = 47) in 2012 compared to 4.9% in 2011 (Figure 8.6). These 47 isolates belonged to 12 different serotypes and the most commonly found resistant serotypes were type 15A (n = 10), 19A (n = 9), 33F (n = 8) and 6C (n = 5).

The percentage of *S. pneumoniae* invasive isolates being non-susceptible (resistant and intermediary resistant) to penicillin was 5.1% (n = 44) in 2012 compared to 4.8% in 2011 (Figure 8.6). These 44 isolates belonged to 15 different serotypes and the most commonly found penicillin non-susceptible serotypes were type 15A (n = 10), 19A (n = 9), 23B (n = 17) and 6C (n = 5). Two of the 872 tested isolates (0.2%) were resistant to penicillin (MIC > 2 µg/ml) and both of these were serotype 14.

As can be seen from above, four serotypes were particularly non-susceptible to either penicillin or erythromycin. These were serotypes 6C, 14, 15A and 19A. Of the six received serotype 14 isolates, four were resistant to either penicillin, erythromycin or both. All of the erythromycin resistant isolates of serotypes 6C, 15A and 19A were also non-susceptible to penicillin.

The level of erythromycin and penicillin resistance in Denmark was similar to the level reported to EARS-Net by the other Nordic countries but lower than reported by other European countries in 2011 [EARS-Net 2011].

Figure 8.6. Nonsusceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark



Group A streptococci

In 2012, 160 invasive GAS (*Streptococcus pyogenes*) isolates were susceptibility tested. As in previous years, no resistance to penicillin in GAS isolates from invasive infections was reported in 2012. Erythromycin resistance was detected in one isolate (0.6%) as compared to two of 164 (1.2%) in 2011.

Group B, C and G streptococci

Two isolates (2.7%) were reported resistant to penicillin from group C invasive infections in 2012, while as in previous years no resistance to penicillin was reported in isolates from group B or G invasive infections in 2012.

In 2012, 134 invasive group B streptococci (*Streptococcus agalactiae*) isolates from invasive infections were tested. Erythromycin resistance was detected in 17 isolates (12.7%) compared to 20.2% in 2011. Seventy-three isolates of invasive group C streptococci were tested in 2012. Four isolates (5.5%) were resistant to erythromycin compared to one isolate (2.0%) in 2011. Seventeen (10.6%) of the tested 160 invasive group G streptococci were resistant to erythromycin compared to 8.0% in 2011.

Tine Dalby, Hans-Christian Slotved and Steen Hoffmann

8.5 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but also cause infections. Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteraemia and bacterial endocarditis. *E. faecalis* and *E. faecium* can cause life-threatening infections in humans, especially in the hospital environment. The naturally high level of antimicrobial resistance found in *E. faecalis* and *E. faecium* makes infections difficult to treat. Antimicrobial therapy for serious enterococcal infections requires the use

of synergistic combinations of a cell-wall-active agent such as penicillin (ampicillin) and an aminoglycoside (gentamicin) or a glycopeptide (vancomycin).

For *E. faecalis* and *E. faecium*, data from 12 of the 13 DCM were obtained, representing 95% of the Danish population.

Enterococcus faecium and *Enterococcus faecalis* blood isolates obtained from hospitalised patients

DANMAP received data on the antimicrobial susceptibility of 647 *E. faecium* isolates and 595 *E. faecalis* isolates from blood.

As in previous years, most of the *E. faecium* isolates from bloodstream infections were ampicillin resistant. In 2012, 94% of the *E. faecium* isolates were resistant to ampicillin. Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as a risk factor for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has been observed in hospitals in Denmark during the last decade. The antimicrobial selection pressure in a hospital environment might be a reason for the high level of ampicillin resistant *E. faecium* as a cause of bloodstream infections.

Two of the DCM (Aalborg and Aarhus) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates from the two DCM, 27% were HLGR, whereas 62% of the tested *E. faecium* isolates were HLGR. The occurrence of HLGR *E. faecalis* and HLGR *E. faecium* was similar to or higher than the occurrence detected in many countries reporting to EARS-Net in 2011 [EARS-Net 2011].

In 2012, vancomycin resistance was detected in 1.8% of the *E. faecium* isolates ($n = 11$) and 0.2% ($n = 1$) of the *E. faecalis* isolates from bloodstream infections. Six of the vancomycin resistant *E. faecium* isolates were detected at DCM Aarhus and three at DCM Hvidovre. These bloodstream isolates were part of outbreaks with vancomycin resistant (*vanA*) *E. faecium*. The occurrence of vancomycin resistant *E. faecium* and vancomycin resistant *E. faecalis* was at the same level or lower compared to most other European countries [EARS-Net 2011].

Since 2005, presumable vancomycin resistant enterococcal isolates from invasive and non-invasive infections and screening samples for national surveillance on vancomycin resistant enterococci have been sent from the DCM the Antimicrobial Resistance Reference Laboratory at SSI. During 2012, 50 *vanA* *E. faecium*, two *vanB* *E. faecium* and one *vanB* *E. faecalis* isolates were received at SSI.

As described above, most of the *E. faecium* isolates from bloodstream infections were resistant to ampicillin; these infections can therefore not be treated with ampicillin but will often be treated with vancomycin or linezolid instead. This might in part, together with the increased number of MRSA infections, explain the increased consumption of glycopeptides (vancomycin) and linezolid in hospitals which has been observed during the last years.

Anette M. Hammerum, Stefan S. Olsen, Lotte Jakobsen and Line Skjøl-Rasmussen

***Neisseria gonorrhoeae* 2012**

Background: *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 and sometimes the pharynx or rectum. Infection in the two latter sites is usually asymptomatic. Complicated cases include prostatitis, orchitis, epididymitis, and salpingitis. Conjunctivitis may occur in newborns after transmission from the mother during labour and rarely in adults, e.g. following auto-inoculation.

Methods: Through decades, all Departments of Clinical Microbiology in Denmark have submitted their isolates of *N. gonorrhoeae* (gonococci) to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance. Most of the received isolates are from urethra or cervix, while specimens from rectum and pharynx are only rarely obtained by clinicians. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

At the NSR laboratory, antimicrobial susceptibility testing towards ceftriaxone and ciprofloxacin is performed by a gradient diffusion technique, and the isolates are tested for penicillinase production.

Since 2009, as part of NSR's participation in ECDC's surveillance of sexually transmitted infections, approximately 60 consecutive gonococcus isolates are investigated twice yearly for susceptibility to an expanded panel of antimicrobial agents. In addition to ceftriaxone and ciprofloxacin, this panel includes azithromycin, cefixime, spectinomycin, and gentamicin.

Results and discussion: The number of strains received in the years 2008, 2009, 2010, 2011, and 2012 were 357, 445, 370, 339, and 505, respectively.

The ciprofloxacin resistance rate increased steadily from 30% in 2003 reaching a peak of 75% in 2009, followed by a decrease to 57% in 2012 (Figure 1). The percentage of strains producing penicillinase fluctuated between 12% and 24%.

Occurrence of ceftriaxone resistant gonococci (MIC ≥ 0.25 mg/L), as well as ceftriaxone treatment failure in patients with gonorrhoea, has been reported from several countries during recent years. No cases from Denmark have been reported. However, since 2008 the ceftriaxone MIC distribution has shown a drift towards higher values than previously (Figure 2).

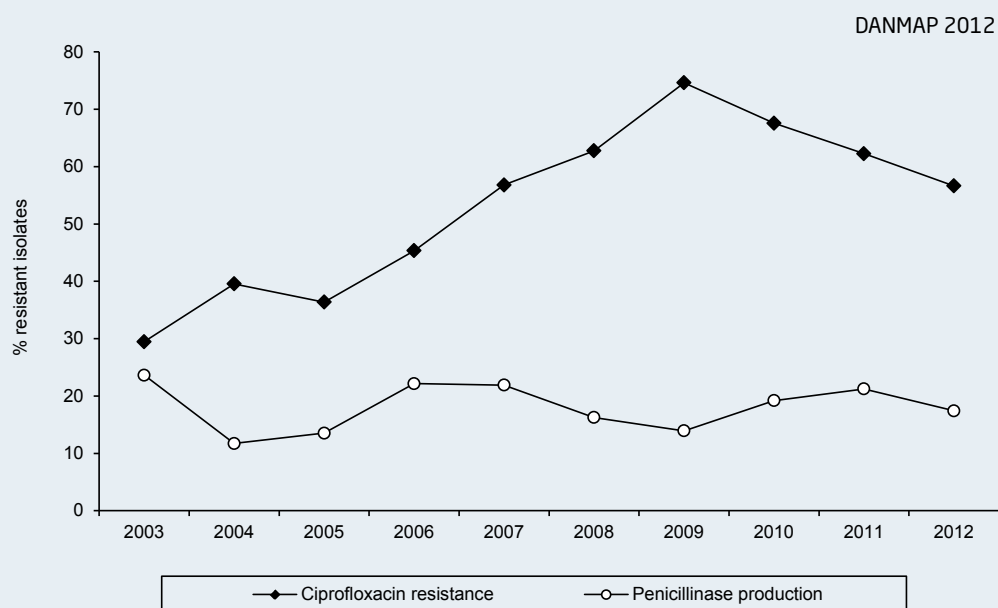
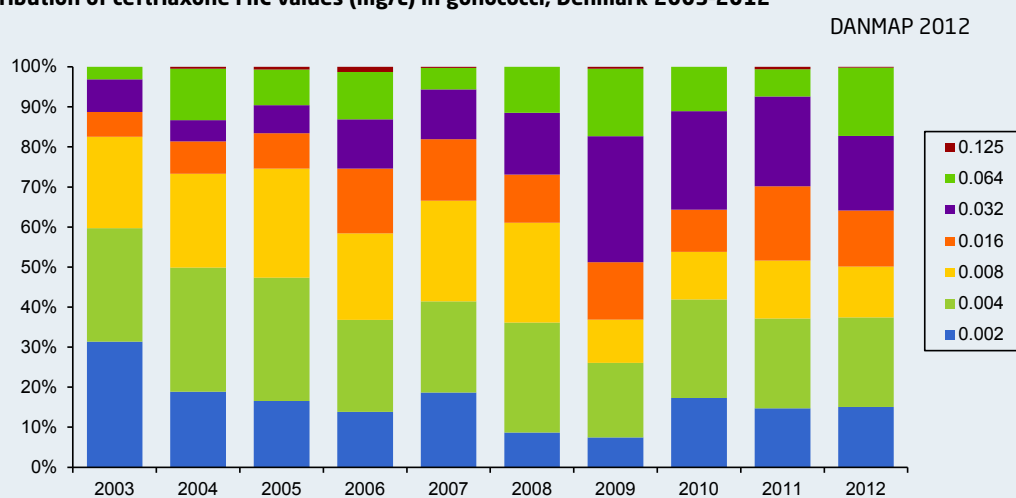
Results obtained by examining strains against an expanded panel of antimicrobial agents are shown in Table 1. In 2012, azithromycin resistance in gonococci was 12%, a decrease since 2009 where 46% of the isolates were resistant. Resistance to cefixime increased from 15% in 2009 to 21% in 2011, and it was 11% in 2012. Cefixime is an oral cephalosporin which has never been used in Denmark. Throughout 2009–2012 all strains were susceptible to spectinomycin; however, this parenteral drug is not marketed in Denmark and is not readily accessible in most countries. Gentamicin has been used successfully for the treatment of gonorrhoea in several countries in Africa but no randomized clinical trials have been published. Gentamicin breakpoints for gonococci have not been determined. In 2011 and 2012, 99% of the examined strains from Denmark had gentamicin MIC ≤ 4 mg/L.

Conclusions:

The centralised national surveillance of antimicrobial resistance in gonococci should be continued.

Steen Hoffmann

For further information: Steen Hoffmann (hof@ssi.dk)

Figure 1. Ciprofloxacin resistance and penicillinase production in gonococci, Denmark 2003-2012**Figure 2. Distribution of ceftriaxone MIC values (mg/L) in gonococci, Denmark 2003-2012****Table 1. Resistance rates (%) against azithromycin, cefixime and spectinomycin in gonococci, Denmark 2009-2012**

DANMAP 2012

	2009	2010	2011	2012
	(n = 119)	(n = 111)	(n = 126)	(n = 114)
Azithromycin	46	23	12	12
Cefixime	15	18	21	11
Spectinomycin	0	0	0	0

8.6 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora from skin and mucosa in approximately 50% of humans. Some people only carry *S. aureus* intermittently whereas others carry *S. aureus* for longer time. However, in addition *S. aureus* also cause infections ranging from superficial skin infections i.e. impetigo and boils, to invasive infections such as post-operative wound infections, infections related to intravenous catheters and prosthetic devices, bacteraemia, osteomyelitis, endocarditis and arthritis.

In Denmark, a voluntary surveillance programme of all *S. aureus* bacteraemia cases was established in 1957. Methicillin-resistant *S. aureus* (MRSA) has been both laboratory and clinical notifiable since November 2006. MRSA belonging to clonal complex 398 (CC398) has in recent years attracted special attention as this type has been closely connected to livestock animals, especially pigs, and increasingly affects people in direct contact with pigs. The cases have in the

past years constituted an increasing part of the community-acquired (CA) cases and due to these increasing numbers, cases belonging to CC398 will be treated as a separate group as both epidemiology and exposition are different.

Surveillance of bacteraemia

In 2012, 1,528 *S. aureus* bacteraemia cases corresponding to 27.4 per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. Nineteen (1.2%) of the cases were caused by MRSA. This is at the same level as in previous years and very low compared to most other countries participating in EARS-Net [EARS-Net 2011]. Antimicrobial resistance in *S. aureus* bacteraemia isolates from 2007–2012 is presented in Table 8.5. The highest frequency of resistance other than to penicillins was observed for fusidic acid (14%), erythromycin (6%), clindamycin (6%) and norfloxacin (4%). Susceptibility to all tested antimicrobials was at the same level as in 2011. Resistance to at least 1, 2 or 3 other antimicrobials in addition to penicillin was demonstrated in 25%, 9% and 2% of the cases, respectively.

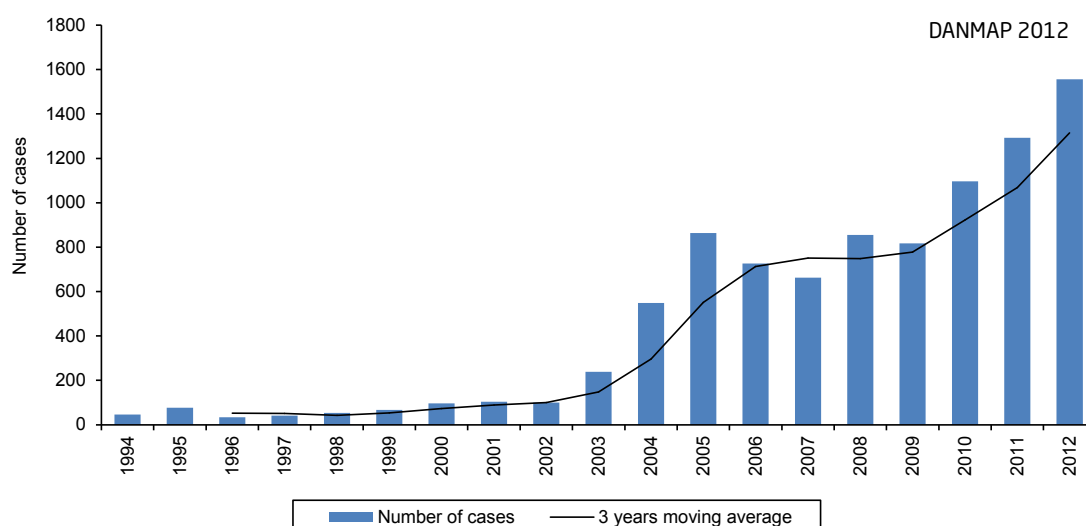
Table 8.5. Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark

DANMAP 2012

Antimicrobial agent	2007 %	2008 %	2009 %	2010 %	2011 %	2012 %
Methicillin	0.6	1.3	1.6	1.4	1.4	1.2
Penicillin	78	77	77	75	77	74
Erythromycin	4	5	7	5	7	6
Clindamycin	3	4	6	4	6	6
Tetracycline	2	3	2	3	2	2
Fusidic acid	9	9	9	13	13	14
Rifampicin	<1	<1	<1	<1	<1	<1
Norfloxacin	1	2	2	3	4	4
Kanamycin	<1	1	1	1	<1	1
Linezolid	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	nt	<1	1
Number of isolates	1345	1344	1480	1418	1525	1528

Note: nt = not tested

Figure 8.7. Number of MRSA cases, with a three years moving average, Denmark



Surveillance of methicillin-resistant *S. aureus*

In 2012, 1,556 new MRSA cases were detected (27.9 per 100,000 inhabitants). This is the highest number of cases observed in over 25 years (Figure 8.7). A case was a person found positive for the first time with a specific MRSA strain regardless whether the patient was infected or colonised.

In 2012, the number of MRSA increased by 20% compared to 2011. The number of new cases has more than doubled (135%) since 2007. In 2012, fourteen persons were found with their second case of MRSA (i.e. MRSA of a new subtype). At the time of diagnosis, 838 (54%) of the new cases were found due to infection which is similar to 2011 (53%). The proportion of bloodstream infections with MRSA was 1.2% in 2012 (see surveillance of *S. aureus* bacteraemia).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.6. Most of the cases (79%) were acquired

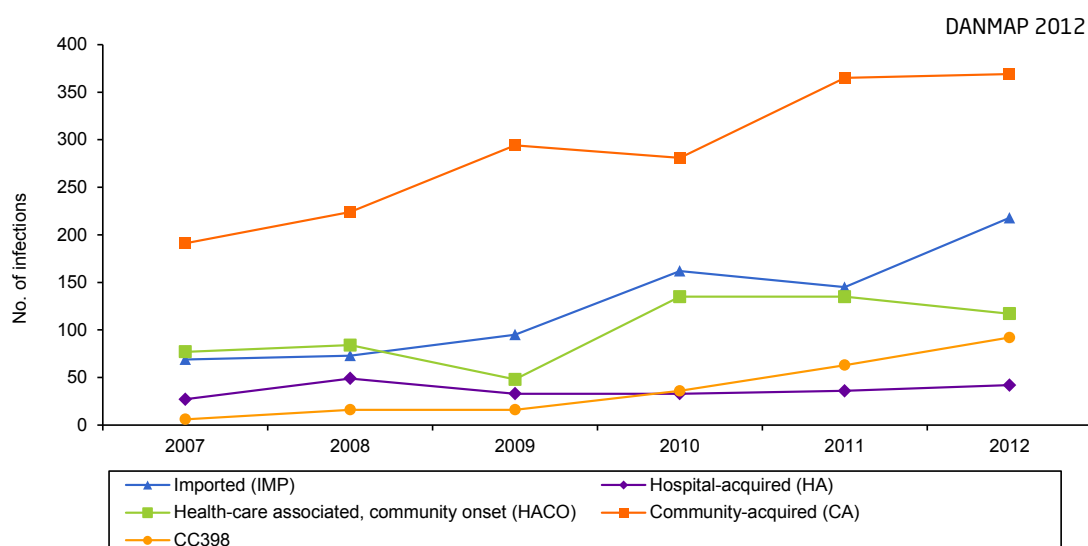
in Denmark. The epidemiological classification of MRSA infections 2007–2012 is shown in Figure 8.8. The figure has been updated from 2007 to include CC398 infections. Twenty-seven of the healthcare-associated with a community onset (HACO) infections (22%) could be associated with a known exposition, 16 from hospitals, 5 from nursing homes and the remaining 6 from other social institutions. The remaining 99 cases of infection classified as HACO were registered with a possible association to health-care institutions (within the last 12 months) but without known exposition; of these, 68 cases were with an association to hospitals and 24 cases with an association to nursing homes and private home care. The remaining 7 cases could not be associated to any particular institution. The number of infections classified as CA was 369 in 2012 (Figure 8.8). The proportion of CA infections with known exposure was at the same level (19%) as in 2011 (both years adjusted for CC398 cases). CC398 cases constituted 15% of new MRSA cases in 2012 (Table 8.6). See also Textbox 13.

Table 8.6. Epidemiological classification of new MRSA cases, Denmark

Epidemiologic classification	Exposure	2011		2012	
		No. of cases (% of total)	No. (%) of cases with infections	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		252 (20)	145 (58)	324 (21)	218 (67)
Hospital-acquired (HA)		58 (4)	29 (50)	67 (4)	42 (63)
Health-care associated, community onset (HACO)	with health care risk	182 (14)		178 (11)	
	with known exposure	44	10 (23)	60	24 (40)
	without known exposure	138	117 (85)	118	88 (75)
Health care worker		40 (3)	7 (18)	29 (2)	5 (17)
Community-acquired (CA)	without health care risk	596 (46)		726 (47)	
	with known exposure	311	64 (21)	378	69 (18)
	without known exposure	285	246 (86)	348	300 (86)
CC398		164 (13)	63 (38)	232 (15)	92 (40)

Note: Numbers shown in bold are totals

Figure 8.8. Number of MRSA infections according to epidemiological classification, Denmark



Molecular typing of the MRSA strains

In total, *spa* typing revealed 232 different strain types. The number of isolates belonging to the 10 dominating *spa* types isolated in 2012 is shown in Table 8.7. They constituted 57% of the total number of MRSA isolates. Seven *spa* types constituted 49% of the 838 clinical infections with MRSA (out of 180 different *spa* types associated with clinical infection). Most prevalent *spa* types causing clinical infections at time of presentation were t002 (n = 88), t034 (n = 75), t019 (n = 74), t008 (n = 73), t032 (n = 45), t044 (n = 34) and t304 (n = 23). The PVL gene was demonstrated in 42% of the infections and in 20% of the asymptomatic carriers.

Resistance among MRSA isolates

The resistance pattern varied considerably between *spa* types (Table 8.8). In 2012, 100% of CC398 *spa* type t034 isolates were resistant to tetracycline and 100% of CC22 *spa* type t032 were resistant to norfloxacin. In contrast, the majority of t019, a primarily community-acquired *spa* type, and t304 were susceptible to all tested antimicrobial agents except for beta-lactams.

Table 8.7. The ten most prevalent *spa* types demonstrated in MRSA cases, Denmark

DANMAP 2012

<i>spa</i> type	CC group ^(a)	No. of cases	No. causing infections (%)
t034	CC398	185	75 (41)
t002	CC5	141	88 (62)
t008	CC8	130	73 (56)
t019	CC30	97	74 (76)
t032	CC22	89	45 (51)
t304	CC8	74	23 (31)
t044	CC80	45	34 (76)
t127	CC1	42	19 (45)
t024	CC8	42	20 (48)
t223	CC22	37	13 (35)

a) CC = Clonal complex

Even though differences in antimicrobial resistance were demonstrated between *spa* types, the success of antimicrobial treatment cannot be predicted based on *spa* type or epidemiological classification. Resistance to at least 1, 2 or 3 other antimicrobials in addition to cefoxitin/penicillin was demonstrated in 68%, 53% and 35% of the cases, respectively. In Table 8.9 are shown the most common resistance patterns and any frequent associated *spa* types.

Andreas Petersen, Robert L. Skov
and Anders Rhod Larsen

Table 8.9. Resistance markers in addition to cefoxitin demonstrated in MRSA cases, Denmark

DANMAP 2012

No. of markers	No. of cases	Frequent patterns (no of isolates)	Frequent <i>spa</i> type (no of isolates)
0	495	–	t019(89), t002(57)
1	240	T(85)	t034(28)
		F(50)	t002(18)
		N(43)	t032(18)
		K(43)	t4272(5)
2	269	E,C(78)	t002(17), t024(15)
		C,T(68)	t034(58)
3	370	E,C,N(101)	t032(67)
		E,C,T(69)	t034(33), t011(14)
		E,N,K(59)	t008(37)
		T,F,K(37)	t044(27)
4	149	E,C,T,K(36)	t127(17), t437(8)
		E,C,T,F(30)	t1028(28)
		E,C,N,K(28)	
		E,C,T,N(24)	t034(23)
5	22	E,C,T,N,K(13)	t189(6)
6	6	E,C,T,N,K,S(3)	
7	4	E,C,T,F,N,K,S(2)	t064(2)
		E,C,T,R,N,K,S(2)	
8	1	E,C,T,F,R,N,K,S(1)	t2872(1)

a) T = tetracycline, N = norfloxacin, F = fusidic acid, K = kanamycin, E = erythromycin, C = clindamycin, R = rifampicin

Table 8.8. Resistance (%) in the six most prevalent *spa* types demonstrated in MRSA cases compared with all MRSA cases, Denmark

DANMAP 2012

<i>spa</i> type	t034	t002	t008	t019	t032	t304	All cases
Clonal complex	CC398	CC5	CC8	CC30	CC22	CC8	
	%	%	%	%	%	%	%
Erythromycin	33	31	58	1	79	0	38
Clindamycin	81	26	10	0	78	0	37
Tetracycline	100	6	10	2	0	0	31
Fusidic acid	2	23	9	2	3	1	15
Rifampicin	0	1	0	0	1	0	1
Norfloxacin	32	21	41	2	100	0	26
Kanamycin	1	13	49	1	0	0	23
Linezolid	0	0	0	0	0	0	0
Mupirocin	0	0	2	1	0	0	<1
Trimethoprim-sulfamethoxazole	1	0	1	0	0	0	2
Number of isolates	185	141	130	97	89	74	1556

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)

Background: Since 2003, livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has emerged worldwide. LA-MRSA primarily belong to clonal complex 398 and is especially associated with pigs. In addition, a new type of MRSA carrying a different resistance determinant *mecC* has been found associated to cattle and sheep in Denmark and internationally [García-Álvarez *et al.* 2011. Lancet Infect. Dis.11: 595–603; Petersen *et al.* 2013. Clin. Microbiol. Infect. 19: E16–22]. In humans, MRSA CC398 has especially been found in persons with contact to pigs. *mecC* positive isolates have been detected in bulk milk in the United Kingdom and in swabs from sheep and cows in Denmark [García-Álvarez *et al.* 2011. Lancet Infect. Dis.11: 595–603; Eriksson *et al.* 2013. Vet. Microbiol. 163: 110–5]. Despite that transmission of *mecC* MRSA from colonized cows and sheep have been confirmed by whole genome sequencing, human findings do not appear to have the same close relationship to animals as CC398 [Petersen *et al.* 2013, Clin. Microbiol. Infect. 19: E16–22; Harrison *et al.* 2013. EMBO Mol. Med. 5: 509–15].

The prevalence of MRSA CC398 in Danish pigs at slaughter increased from 13% in 2009 to 44% in 2011 [DANMAP 2011]. The prevalence was also measured in pig herds in 2010 and 2011 and was in both years 16%. The higher prevalence found in pigs at the slaughterhouse is likely due to contamination during transport and handling of pigs [Broens *et al.* 2011. Vet. J. 189: 302–5], but could also be due to an increase in herd prevalence at positive farms. Prevalence studies in cattle at slaughter of MRSA performed in 2010 and 2011 did not detect any MRSA of either *mecA* or *mecC* type in cattle [DANMAP 2011].

In 2012, the prevalence and diversity of LA-MRSA was investigated in pigs at slaughter, in cattle bulk milk samples and in human samples as part of the national surveillance of MRSA.

Investigation of MRSA in pigs at slaughterhouses: Nasal swabs were collected from pigs at slaughter during February through December 2012. Presumptive MRSA was isolated in 555 out of 709 samples, as previously described [DANMAP 2009–2011]. A random subset ($n = 235$) of the presumptive MRSA were tested by PCR for the presence of *mecA*, 232 isolates were PCR positive, whereby the prevalence of MRSA in pigs at slaughter could be estimated to 77%.

The prevalence in 2012 was significantly higher than in 2009 (13%) and in 2011 (44%). The high and increasing prevalence could have several explanations: It could be caused by a combination of truly infected/colonized pigs from positive pig herds and contamination of pigs originating from negative farms due to e.g. MRSA contaminated transport vehicles; MRSA in the surroundings where pigs were kept before slaughter or MRSA in the slaughter chain in general; but it could also be due to an increase of infected pig herds.

A total of 110 MRSA isolates from pigs were *spa* typed. The detected *spa* types all corresponded to CC398 (t034 (61.8%), t011 (34.5%), t1451 (2.7%) and t898 (0.9%)). All *spa* types have previously been found in pigs in Denmark.

Investigation of MRSA in bulk milk: The bulk milk samples were collected from 219 different farms from September 2012 through January 2013. MRSA and *S. aureus* were isolated after freezing of the milk by adding 1 ml of milk to 4 ml of Mueller-Hinton broth supplemented with 6.5% NaCl and incubated for 23–25 hours at 37°C. 50 µL of culture were spread on Brilliance MRSA 2 agar plates and Brilliance Staph 24 agar from Oxoid to isolate presumptive MRSA and *S. aureus*, respectively. MRSA was verified by multiplex PCR and *spa* typed.

MRSA were detected in 1.8% (4/219) of the bulk milk samples and *S. aureus* in 70% of the samples (153/219). This corresponded to a MRSA prevalence among *S. aureus* of 2.6% (4/153). A follow-up sampling found MRSA in bulk milk samples from two out of four of the farms previously tested positive for MRSA. This could be due to these farms being transiently contaminated with MRSA or that MRSA is present at a concentration close to the detection level. This is the first finding of MRSA from bulk milk in Denmark. None of the bulk milk MRSA isolates were positive for *mecC*. All MRSA carried the *mecA* gene and belonged to CC398 (t011, t034) and CC1(t127), which have previously been detected in pigs. This might indicate transmission of MRSA from pig production to dairy cattle [Agersø *et al.*, 2012. Vet. Microbiol. 157: 246–50].

Livestock associated MRSA in humans: MRSA CC398 was found in 232 human cases in 2012 (42 in 2009, 111 in 2010 and 164 in 2011). It should be noted that there was no targeted screening for CC398 in 2012. The most frequent *spa* type related to CC398 was type t034 ($n = 185$). The majority of CC398 cases (183, 79%) were in persons with documented close contact to pigs or being a household member. Ninety-two CC398 cases (40%) presented with infections. There were still no signs of significant spread of CC398 to urban areas. Thirteen (0.9%) bacteraemia ($N = 1,528$) cases in 2012 belonged to CC398 (two MRSA and 11 methicillin sensitive) and had no known contact to pig farming. The corresponding numbers were five in 2007, six in 2008, 10 in 2009, 11 in 2010 and 11 in 2011.

MRSA isolates carrying the new *mecA* homologue *mecC*, were demonstrated in 24 cases in 2012 (9 in 2009, 21 in 2010 and 37 in 2011). Sixteen of the cases (67%) had infections, including one bacteraemia at the time of diagnosis. No livestock contact was registered for any of the 24 *mecC* cases.

Conclusions: The prevalence of MRSA in pigs at slaughter has increased to 77%, but whether more herds are positive compared to previous years is unknown. Pigs still seem to be the most important reservoir for MRSA CC398, but detection of LA-MRSA CC398 in bulk milk depicts a spread possibly from the pig production. In 2012, CC398 was the second most common CC group among human MRSA cases. The number of MRSA isolates carrying the *mecC* gene seems to have stabilized.

Yvonne Agersø, Anne Mette Seyfarth, Andreas Petersen, Robert L. Skov and Anders Rhod Larsen

For further information on the veterinary studies: Yvonne Agersø, E-mail: yvoa@food.dtu.dk

For further information on the human cases: Robert L. Skov, E-mail: rsk@ssi.dk



9. Resistance in diagnostic submissions from animals

The DANMAP programme monitors antimicrobial susceptibility in *Escherichia coli* O149 via diagnostic submissions from pigs. Due to the low number of samples, *Staphylococcus hyicus* via diagnostic submissions from pigs as well as *E. coli* F5 (K99) via diagnostic submissions from cattle were not included in the analyses this year.

Most isolates from diagnostic submissions originated from animals in antimicrobial therapy. A higher frequency of resistance is therefore expected in the bacteria from these diagnostic submissions compared with the bacteria originating from healthy animals sampled at slaughter.

E. coli isolates resistant to three or more of the ten different antimicrobial classes included in the test panel were considered multi-resistant (see the definition of multi-resistance and the included antimicrobial classes in Section 10.5).

The distribution of MICs among *E. coli* O149 from pigs is presented in the web annex (Table A9.1). Data from the figure are also presented in the web annex.

9.1 *Escherichia coli*

Pigs

The available *E. coli* O149 isolates (n=36) from pigs originated from diagnostic submissions of faecal samples mainly from weaners with diarrhea (about 7-30 kg bodyweight). The detection of *E. coli* O149 in piglets with neonatal diarrhea has decreased significantly during the last ten years, partly due to the general use of vaccination. Further, the prevalence of diarrhea caused by *E. coli* O149 is decreasing in all age groups apparently due to a breeding programme successively eliminating the F4 (K88) receptors in the Danish pig population (*E. coli* O149 possesses the fimbrial type F4).

Trends in resistance to the selected antimicrobial agents in *E. coli* O149 isolates from pigs are presented in Figure 9.1. Although the resistance pattern of individual isolates is variable, the average level of resistance to different antimicrobial agents has been quite stable during the surveillance period. Most (78%) of the isolates were multi-resistant; however the level decreased compared with 2011. Only one isolate (3%) was fully susceptible.

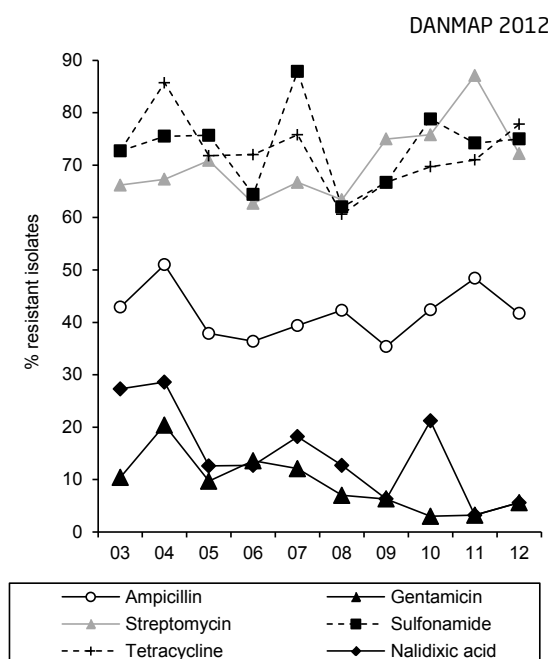
As in previous years, high levels of resistance were found to tetracycline (78%), sulfonamide (75%) and streptomycin (72%, Figure 9.1). Sulfonamide and streptomycin are not used for weaning pig diarrhea, and the consumption in weaners has been stable at a low level (Figure 4.4). The resistance to these agents may be co-selected with tetracycline resistance since tetracyclines are the most common antimicrobial agents used for weaned pigs (Figure 4.4).

The consumption of quinolones has been at a very low level in the pig production since 2003 due to legislative restrictions of the use for food-producing animals (web annex, Table A4.2) and the corresponding resistance to nalidixic acid has decreased during this period. A decrease in the proportion of isolates resistant to trimethoprim (77% to 50%) was observed from 2011 to 2012 (data not presented).

In 2012, one of the 36 *E. coli* O149 isolates was resistant to 3rd generation cephalosporins (cefotaxime and ceftiofur) even though a voluntary ban on use of cephalosporins has been implemented in the pig production since 2010.

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Figure 9.1. Resistance (%) in *Escherichia coli* O149 from diagnostic submissions from pigs, Denmark



Note: The number of isolates varies between years (pigs: n = 31-118)



10. Materials and methods

10.1 General information

For the DANMAP 2012 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 as the individual farm, meaning that only one isolate per bacterial species per farm was included in the report. The same epidemiological unit was applied for broilers, except for *Salmonella* where the individual flock of broilers was defined as the epidemiological unit. 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 patient per year was included.

Unless stated specifically, all differences and temporal trends noted in the text are statistically significant ($p < 0.05$) using either Chi-square tests or linear logistic regression (see Section 10.6.4).

10.2 Data on antimicrobial consumption

Antimicrobial agents used for humans and animals in Denmark are presented in Table 3.3.

10.2.1 Data on antimicrobial consumption in animals

Since 2001, consumption data presented in this report have been obtained from the national monitoring programme VetStat, which is a database hosted by the Danish Veterinary and Food Administration. Prior to 2001, data were based on national sales figures from the pharmaceutical industry.

Data registration

In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals, except in a few instances when medicines are prescribed on special license (i.e. medicines not approved for marketing in Denmark). In addition, data on consumption of coccidiostats as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) are collected by VetStat.

Until 2007, antimicrobial agents could only be purchased at the pharmacy or in medicated feed from the feed mills. From April 2007, the monopoly was suspended and private companies (two in 2011) were permitted - on certain conditions (identical conditions as for pharmacies) - to sell prescribed veterinary medical products for animals. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration costs related to sale of large quantities to the veterinarians.

The pharmacy or company either sells the medicines to veterinarians for own use in practice or for re-sale to farmers, or sells the medicines directly to the animal holder on presentation of a prescription. By law, the profit that veterinarians may make on the sale of medicine is very small (5%), thereby limiting the economic incentive to sell medicine.

In 2012, the animal owners and veterinarians purchased the antimicrobial agents almost equally between the pharmacies and the veterinary drug trading companies, while only 2% was purchased from the feed mills. Sales from feed mills additionally comprised zinc chloride for the pig production on veterinary prescription, and non-prescription sales of coccidiostatic agents for domestic fowl (*Gallus gallus*).

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to VetStat. Veterinarians are required by law to report to VetStat all use and prescriptions for production animals (monthly submissions). For most veterinarians, the registration of data is linked to the writing of invoices. However, errors in the veterinarians invoice system sometimes cause errors in amounts reported, and these data are not validated at entry to Vetstat. 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 identity of drugs.

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” - a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no]. See further description of the ADD system in the DANMAP 2009 report [www.danmap.org].

Methods

In DANMAP we want to compare consumption of antimicrobials between different animal populations and between veterinary and human sectors. In order to do this we need to take into account the quantity of antimicrobials used, their potency, their formulation, the route of administration and - sometimes - the age of the animals in which they are used. We also need to know the size of the populations to which the antimicrobials are administered.

The animal daily doses (ADDs) used in previous DANMAP reports, are an integrated part of the VetStat database and have been described elsewhere [DANMAP 2009; Jensen et al., Prev Vet Med. vol: 64, 201-215, 2004]. In principle, the ADD should be identical for all products within medicinal groups, and defined by the active compound, route of administration and the formulation. However, in VetStat, the ADDs are defined by the dosage level that was part of the product registration, and over time doses for products within the same medicinal group sometimes have changed. The greatest variation occurs when the ADD for some products have been defined solely based on the approved dosage. For example, the approved dosage of a product registered in 2011 may differ from a similar product, registered in 2001.

In DANMAP 2012, we therefore introduce two new metrics to follow trends in antimicrobial consumption to ensure robustness of the analyses over time. The new metrics, DADD and DAPD, are defined below.

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 DADDs used in DANMAP 2012 are presented in the web annex.

The basic principles for the DADD are similar to the principles previously described for the ADD. The designation of the DADD is based on the VetStat ADDs, but re-defined for each group of antimicrobial agents, i.e. for each combination of active compound, administration route, formulation, considering the following principles:

1. Minor inconsistencies, e.g. due to rounding of numbers, have been corrected;
2. Approved dosage for the most widely used antimicrobial products were given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group are maintained as the common DADD even if a new product is approved with a higher dosage;
4. In determining the dosage for a group with large variation between approved dosages of the products, the dosages in accordance with the dose given in “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 group/indication or formulation.

When principle 2 and 3 are conflicting, principle 4 is applied.

Denominator

Trends in veterinary consumption, both within and across species, 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 body-weight and the average life-span of the species.

DAPD - DADD per 1,000 animals per day

The number of DADDs administered to a specific animal species during a year (in thousands) divided by the number of standard animals at risk per day. The number of standard animals at risk per day takes into account species differences in average body-mass and life-span. 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, providing a rough estimate of the proportion (in thousands) 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.

In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, Defined daily dose per 1,000 inhabitants per day (DID), see Section 10.2.3.

Due to a relative high number of pigs exported around 30 kg (30% of pigs produced in 2012, Table 3.1), an adjusted measure of consumption per pig was calculated. The adjustment is based on the assumption that pigs exported at 30 kg, on average, received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg.

Antimicrobial use per pig produced (adjusted) =
$$[\text{DADDs} + \text{DADDw} + (1+Q) \cdot \text{DADDf}] / (\text{biomass-days-total} + \text{Nw} \cdot 5800(\text{kg} \cdot \text{days})),$$

where DADDs = amount of antimicrobial agents used in sows; DADDw = amount of antimicrobial agents used in weaners; DADDf = amount of antimicrobial agents used in finishers; Q is the proportion of weaning pigs exported around 30 kg. Nw = number of pigs exported at 30 kg bodyweight, and Nw*5800 is the number of biomass days the exported pigs would have contributed to the live biomass if not exported.

10.2.2 Estimation of live biomass of animals

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. The estimated live biomass for the animal species are presented in the web annex (table A4.1).

Broiler and layer production (*Gallus gallus*). The live biomass is estimated based on number of broilers produced (Table 3.1), and an average live weight at slaughter of 1.97 kg [Statistics

Denmark, 2013] 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. In addition, the biomass of the parental animals (rearing and breeding) for the broiler production was estimated for 2011 based on number of hens per year (2.9 mill) annual rotations, length of the empty periods, percentage of cocks, and average weight of the cocks and hens in rearing and in breeding [DANHATCH, 2013; S. Kabell, Danish Agriculture and Food Council, personal communication]. For the other years, the biomass of rearing and breeders was assumed to be proportionately to the broiler production as in 2011.

In the layer production chain, the biomass of the parent flocks is estimated separately for each production type, based on number of eggs produced, eggs per hen, average production length [Danish Poultry Producers, Statistics Denmark]. We have assumed an average weight of 2.3 kg per hen, based on slaughter weight [Statistics Denmark, 2013].

Turkey production. The live biomass is estimated based on number of turkeys produced (Table 3.1) 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.

Pig production. The estimation was based on number of pigs produced, including exports at different ages [Statistics Denmark; Danish Agriculture and Food Council], productivity data for each year [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) were estimated from the productivity number. 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, 2013] 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.

Fur animals. The live biomass of mink is estimated from production data [Statistics Denmark, 2012; Copenhagen Fur, 2013] and the average weight at pelting was 2.45 kg [Kopenhagen Fur, 2013]. The progeny live for approximately 7 months. The biomass for the breeding animals (female) was estimated based on census data and an assumed average live weight of 2 kg.

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 2011 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 (NaturErhvervstyrelsen) on produced amounts in each subtype of production, and information on the typical lifespan and entrance and exit body weights, and were calculated in cooperation with Danish Aquaculture [NH Henriksen, Danish Aquaculture]. Data from 2012 were not available at the time of publication.

10.2.3 Data on antimicrobial consumption in humans

Data on consumption of antibacterial agents in humans were obtained from Statens Serum Institut (SSI), National Register of Medicinal Products Statistics. SSI has the legal responsibility for monitoring the consumption of all human medicinal products. This is performed by monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies, to SSI. Data from the primary health care sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

Certain categories of hospitals were excluded when the consumption was measured by occupied bed-days and admissions. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centers and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

In Denmark, all antimicrobial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDDs), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and place (pharmacy, hospital pharmacy, institution) of the transaction, and information regarding reimbursement of cost, if applicable. The data are transferred monthly to SSI in an electronic format.

For the first time since 1995, the consumption of certain infusion substances, such as cephalosporins, carbapenems and trimethoprim, has been directly reported by the hospital pharmacies to SSI. In previous DANMAP reports, the consumption of these substances was corrected by direct data collection from all Danish hospital pharmacies; however, in 2012 all data were delivered only by SSI.

The present report includes data on the consumption of antibacterial agents for systemic use, or group J01, of the 2012 update of the ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitants-day). Consumption in primary health care is also reported as a number of packages per 1,000 inhabitants. Consumption of antibacterial agents in hospitals is expressed as DIDs, for comparison with primary health care, and DBDs, the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-day).

Since antimicrobial consumption expressed as DDD/100 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DAD (the number of DDD/100 admitted patients).

The number of occupied bed-days is calculated as the date of discharge minus the date of admission (minimum one day), and the number of admissions is calculated as one admission whenever a patient is admitted to one specific ward (one patient can be registered as admitted multiple times if transferred between wards during one hospital stay). Data on the number of occupied bed-days (or patient-days) and number of admissions in each hospital were obtained from the National Patient Registry at the National Board of Health [www.sst.dk].

10.3. Collection of bacterial isolates

10.3.1 Animals

Samples from animals are collected from healthy production animals randomly selected at slaughter. From pigs, isolates of *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis*, *Campylobacter coli*, *Campylobacter jejuni* and *Salmonella* spp. were collected. From cattle, isolates of *E. coli*, *C. coli* and *C. jejuni* were collected, and from broilers isolates of *E. coli*, *C. coli*, *C. jejuni*, *E. faecalis* and *E. faecium* were collected. In addition, isolates of *E. coli* O149, *E. coli* F5 (K99) and *Staphylococcus hyicus* were collected from diagnostic submissions.

***Campylobacter*, indicator *E. coli* and enterococci.** Samples from healthy pigs, cattle and broilers were collected for the DANMAP programme at slaughter by meat inspection staff or company personnel and sent for examination at DTU National Food Institute. For broilers, cloacal swabs were collected weekly from May through October and the sampling programme represented 86% of all broiler farms in Denmark. A Danish broiler farm is typically comprised of more than one unit each generating several flocks per year, but even though a farm was sampled more than once through the sampling period, only one isolate per farm of each bacterial species was included.

For pigs and cattle, the slaughter plants included in the DANMAP programme accounted for 98% and 94% of the total number of animals slaughtered in Denmark during 2012, respectively. The number of pigs and cattle samples from each slaughter plant was proportional to the annual number of animals slaughtered at the plant. Samples were collected once a month from January through November as caecum samples from pigs and rectum samples from cattle. Only one isolate per farm of each bacterial species was included.

Accordingly, the bacterial isolates from the Danish production animals may be regarded as representing a stratified random sample of the respective populations, and the observed prevalence of resistant isolates provides an estimate of the true occurrence in the population.

An overview of the number of samples analysed, isolates obtained and MIC determinations performed for pigs, cattle and broilers is presented in Table 10.1. The isolation rates of *C. jejuni* from pigs and *C. coli* from cattle and broilers were low and therefore MIC-analyses were not performed.

***Salmonella*.** DTU National Food Institute is the national reference laboratory for *Salmonella* in animals and food and receives all isolates for typing. Only one isolate per serotype per farm was selected for the DANMAP report, except for isolates from broilers, where one isolate per flock was included. Isolates of *S. Typhimurium* include the monophasic variants with antigenic formulas S. 4,5,12:i:- and S. 4,12:i:-.

The *Salmonella* isolates from pigs originated both from the random sampling of healthy animals at slaughter for DANMAP and from the national *Salmonella* surveillance programme where the results of a serological surveillance at the slaughterhouses and in all breeding herds appoint risk herds to be further examined by microbiological analysis of pen-faecal samples.

No *Salmonella* data from layers, broilers and cattle are presented in DANMAP 2012 due to the low findings of serotype *S. Enteritidis* and *S. Typhimurium*. *Salmonella* isolates from diagnostic submissions were not included in the DANMAP 2012.

Further details on the sampling procedures and the findings of the Danish *Salmonella* surveillance programs are presented in Textbox 6, and in the Annual Report on Zoonoses in Denmark, 2012 [www.food.dtu.dk].

Isolates from diagnostic submissions were specifically collected for the DANMAP programme at the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup (*E. coli* O149 from diarrheic pigs and *S. hyicus* from skin infections) and at DTU National Veterinary Institute (*E. coli* F5 (K99) from diarrheic cattle). Only one isolate per farm was included. Due to low annual numbers of isolates, only results for *E. coli* O149 from diarrheic pigs are included in DANMAP 2012.

10.3.2 Meat

***Campylobacter*, indicator *E. coli* and enterococci.** The meat isolates originated from meat samples collected at wholesale and retail outlets by the Regional Veterinary and Food Control Authorities (RFCA) in all regions of Denmark. The samples were collected during the course of routine inspection by the authorities or on specific request from the Danish Veterinary and Food Administration (DVFA) for the DANMAP program. The sampling includes both Danish and imported meat. Only one isolate per bacterial species per meat sample was selected for DANMAP.

***Salmonella*.** The *Salmonella* isolates from Danish pork originated from the national *Salmonella* surveillance programme (swab samples from pork and beef carcasses taken at the slaughterhouse after cooling). *Salmonella* isolates from imported poultry meat and other imported fresh meats derived from a case-by-case risk assessment control programme (DFVA), are not presented due to the low number of isolates (<15). Further details on sampling and findings are presented in Textbox 6. Only one isolate per positive swab sample or batch of meat was included for DANMAP. Isolates of *S. Typhimurium* include the monophasic variants, antigenic formula S. 4,5,12:i:- and S. 4,12:i:-.

10.3.3 Humans

S. Typhimurium, S. Enteritidis and C. jejuni. Antimicrobial susceptibility was performed on human faecal isolates submitted to Statens Serum Institut (SSI). *Campylobacter* isolates were submitted from Departments of Clinical Microbiology (DCM) covering three geographical regions: Northern Jutland, Funen and Roskilde/Køge. Information on travel history was obtained for the patients. *Salmonella* isolates were submitted from all DCM in Denmark. Exact figures of the proportion tested and the sampling strategy for the different species can be found in Sections 6.1 and 6.2.

Staphylococcus aureus. All blood isolates were referred to the Staphylococcus reference laboratory at SSI on a voluntary basis. In November 2006, methicillin-resistant *S. aureus* (MRSA) became a notifiable disease in Denmark and it became mandatory to submit all MRSA isolates to the reference laboratory.

Invasive Streptococcus pneumoniae, Streptococcus pyogenes (group A streptococci), group B, C and G streptococci. Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all blood and spinal fluid isolates nationwide are sent to SSI for identification or confirmation as well as susceptibility testing and typing. Group A, B, C and G streptococcal isolates are referred to SSI on a voluntary basis.

E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, invasive E. faecium and invasive E. faecalis. Data were provided on all isolates recorded from either blood samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecium* and *E. faecalis*) or urine samples (*E. coli* and *K. pneumoniae*) submitted for susceptibility testing to the DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev, Hillerød, Region Zealand, Odense, Esbjerg, Vejle, Herning/Viborg, Aarhus and Aalborg.

No samples were collected from healthy humans.

10.4 Isolation and identification of bacteria

10.4.1 Animals

Salmonella. Examination of samples at DTU National Food Institute was performed by non-selective pre-enrichment of 25 g material in a 1:10 dilution with buffered peptone water (BPW) incubated 16-20 hours at 37°C. A Modified Semi-solid Rappaport-Vassiliadis (MSRV) plate was inoculated with 0.1 ml deposited as 3 drops. After incubation o/n at 41.5°C, material from swarming zones was inoculated onto Brilliant Green Agar (BGA). Incubation o/n at 37°C was followed by serotyping of suspect colonies by slide agglutination according to the White-Kauffmann-Le Minor Scheme. *Salmonella* isolates received at DTU National Food Institute for typing were isolated according to the standard methods at the submitting laboratory, and upon reception, inoculated onto BGA followed by serotyping. All isolates received for typing are stored at -80°C.

Campylobacter. The samples from broilers was inoculated directly onto mCCD agar (Oxoid, Denmark) and incubated in microaerophilic atmosphere for 2-3 days at 41.5°C. For samples from cattle, selective enrichment in Preston broth at a ratio of 1:10 incubated in microaerophilic atmosphere for 24 h at 41.5°C was performed followed by inoculation of 10 µl of the enrichment broth to mCCD agar. *Campylobacter*-like colonies were verified by microscopy and identification was performed by a real-time PCR assay [Mayr et al. 2010, J Food Prot. 73(2):241-50]. All isolates of *C. jejuni* and *C. coli* were stored at -80°C.

Indicator E. coli. The material was inoculated directly onto Drigalski agar (SSI Diagnostica, Denmark) and incubated o/n at 37°C. Yellow colonies were inoculated onto BBL CHROM agar Orientation Medium (Becton Dickinson, Germany) and red colonies were collected as *E. coli* after o/n incubation at 37°C. All isolates were stored at -80°C.

Table 10.1. Number of DANMAP samples, number of isolates and MIC-tests from healthy production animals at slaughter, Denmark
DANMAP 2012

		<i>E. coli</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>C. jejuni</i>	<i>C. coli</i>
Pigs ^(a)	No. of samples analysed (1 per farm)	300	807	807	300	300
	No. of isolates obtained	266	216	191	19	141
	No. of isolates MIC-tested/reported	152	112	119	0	103
Cattle	No. of samples analysed (1 per farm)	149	0	0	172	172
	No. of isolates obtained	134	0	0	110	3
	No. of isolates MIC-tested/reported	98	0	0	89	0
Broilers	No. of samples analysed (no. of flocks)	164	177	177	202	202
	No. of farms represented	125	125	125	125	125
	No. of isolates MIC-tested/reported	115	107	100	41	0

Note: Data in this table should not be used for reporting of prevalences of the bacterial species

a) From 2011, the DANMAP samples from pigs were also part of the surveillance programme for *Salmonella*

Table 10.2. Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values (blue fields) and the corresponding EUCAST clinical breakpoints (white fields)

DANMAP 2012

Antimicrobial agent	<i>Salmonella</i>		<i>E. coli</i>		<i>E. faecium</i>		<i>E. faecalis</i>		<i>C. jejuni</i>		<i>C. coli</i>	
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8*	>8*	>8*	>8*	>4*	>8*	>4*	>8*				
Apramycin	>16		>16									
Cefotaxime	>0.5*	>2*	>0.25*	>2*								
Cefoxitin												
Ceftiofur	>2*		>1*									
Chloram-phenicol	>16*	>8*	>16*	>8*	>32*		>32*		>16*		>16*	
Ciprofloxacin	>0.06 *	>1*	>0.06*	>1*	>16 ^(b)		>8 ^(b)		>0.5*	>0.5*	>0.5*	>0.5*
Colistin	>2*/ >8 ^(c)	>2*	>2*	>2*								
Erythromycin					>4*		>4*		>4*	>4*	>8*	>8*
Florfenicol	>16*		>16*									
Gentamicin	>2*	>4*	>2*	>4*	>32*		>32*		>2*		>2*	
Kanamycin					>1,024		>1,024					
Linezolid					>4*	>4*	>4*	>4*				
Nalidixic acid	>16*		>16*						>16*		>16*	
Neomycin	>4*		>8*									
Penicillin					>16*		>16*					
Quinupristin/dalfopristin					>4 ^(a)	>4*						
Salinomycin					>4		>4					
Spectinomycin	>64		>64*									
Streptomycin	>16*		>16*		>128*		>512*		>4*		>4*	
Sulfonamide	>256		>64*									
Teicoplanin					>2*	>2*	>2*	>2*				
Tetracycline	>8*		>8*		>4*		>4*		>1*	>2*	>2*	>2*
Tiamulin												
Tigecycline					>0.25*	>0.5*	>0.25*	>0.5*				
Trimethoprim	>2*	>4*	>2*	>4*								
Vancomycin					>4*	>4*	>4*	>4*				

* EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints. Changes in ECOFF values since DANMAP 2011 are highlighted by orange

a) The EUCAST ECOFF (>1) was not applied for quinopristin/dalfopristin (tradename synergid) according to investigations presented in DANMAP 2006

b) The EUCAST ECOFF (>4) was not applied for ciprofloxacin. The aim was to look for high level ciprofloxacin-resistance as described by Werner et al, 2010 (Int J Antimicrob Agents;35:119-125)

c) 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 as recommended by Agerø et al., 2011 [DANMAP 2011, Textbox 6]

Indicator enterococci. One drop of material suspended in 2 ml of sodium chloride (0.9%) was spread on Slanetz Bartley agar and incubated two days at 42°C. Three colonies typically of *E. faecalis* and *E. faecium* were sub-cultivated on blood agar. Colonies were identified by colour, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose and raffinose. All isolates of *E. faecium* and *E. faecalis* were stored at -80°C.

Veterinary pathogens. Diagnostic submissions were examined according to the standard methods at the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup (*E. coli* O149 and *S. hyicus*) and at DTU National Veterinary Institute (*E. coli* F5 (K99)).

10.4.2 Meat

Salmonella was isolated by the regional laboratories at the DVFA according to the open reference methods issued by the NMKL (NMKL No. 187, 2007 or NMKL No. 71, 1999), the ISO 6579:2002 or alternative methods validated against the reference method according to ISO 16140:2001. Sero- and phage-typing was performed at DTU National Food Institute.

Campylobacter was isolated according to the guidelines for microbiological examination of food (NMKL No. 119, 2007). Identification was performed by microscopy or test kit DRO150M (Oxoid), and by oxidase activity, catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. Isolation and identification was performed by the regional laboratories at the DVFA. All isolates of *C. jejuni*, *C. coli* and *C. lari* were sent to DTU National Food Institute for MIC-testing and storage at -80°C.

Indicator E. coli was isolated by the regional laboratories at the DVFA by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulphate-broth, which was incubated o/n at 44°C, subsequently streaked onto violet red bile agar and incubated for 24 h at 44°C. Presumptive *E. coli* was identified by CHROMagar Orientation Medium or by indole- and lactose testing in laurylsulphate-broth incubated o/n at 44°C. *E. coli* isolates were sent to DTU National Food Institute for MIC-testing and storage at -80°C.

Indicator enterococci were isolated by the regional laboratories at the DVFA by adding 5 g of the sample to 45 ml azide dextrose broth, incubated o/n at 44°C and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 h, colonies typically for *E. faecium* and *E. faecalis* were identified by a real-time PCR assay, and finally sent to DTU National Food Institute for MIC-testing and storage at -80°C.

10.4.3 Humans

Salmonella isolates were serotyped by slide agglutination according to the Kauffman-White Scheme.

Campylobacter. Species identification was performed using a species-specific PCR assay [Klena *et al.* 2004. J Clin Microbiol. 42: 5549–5557].

Staphylococcus aureus. Sequencing of the *S. aureus* specific *spa* gene was performed for species confirmation and typing. *Spa*-negative isolates were confirmed as *S. aureus* by MALDI-

TOF. The *spa*-typing [Harmsen *et al.* 2003. J Clin Microbiol. 41: 5442–5448] and additional typing by multi locus sequence typing (MLST) [Enright *et al.* 2000. J Clin Microbiol. 38: 1008–1015] was annotated using eBURST v.3 software (www.mlst.net). Based on the *spa* and MLST typing, each isolate was assigned to a clonal complex (CC). For MRSA isolates, presence of the *mecA* or *mecC* methicillin resistance genes was confirmed by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611–614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395–400]. For all isolates, presence of *lukF-PV* gene (PVL) was demonstrated by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611–614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395–400].

10.5 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter*, indicator *E. coli*, *Enterococcus* and the veterinary pathogens was performed as microbroth dilution MIC with Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK). 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 quality control strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560 were used.

Isolates from animals and meat were tested at DTU National Food Institute, and the *Salmonella* and *Campylobacter* isolates of human origin were tested at SSI. MIC-testing at DTU National Food Institute is accredited by DANAK (the national body for accreditation).

One isolate per bacterial species per farm, per meat sample or per patient was tested for antimicrobial susceptibility. For *Salmonella* isolates from poultry, one isolate per serotype per flock was tested. For isolates in excess numbers (e.g. isolates from healthy animals), a random selection was appointed to MIC.

Table 10.2 presents the interpretation of MIC-values used for any combination of bacteria and antimicrobial agent. Since 2007, data were interpreted by EUCAST epidemiological cut-off values (ECOFFs) with a few exceptions described in Table 10.2. The corresponding clinical breakpoints validated by EUCAST are presented both in Table 10.2 and in the MIC-distribution tables to visualize the impact of the use of ECOFFs contra clinical breakpoints. In general, if ECOFFs were re-validated and changed by EUCAST during the past year, all data presented from previous years were interpreted using the changed ECOFFs. All MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals.

Multi-resistance was defined as resistance to three or more of the antimicrobial classes listed in Table 10.3. Isolates were considered fully sensitive if susceptible to all the antimicrobial agents included in the test panel.

***Staphylococcus aureus* from humans.** Susceptibility testing was performed by disc diffusion according to EUCAST methodology using discs from Oxoid (Ballerup, Denmark) on Mueller-Hinton Agar (SSI, Copenhagen, Denmark). The following antimicrobial agents were tested: Erythromycin, clindamycin, kanamycin, rifampicin, penicillin, cefoxitin, fusidic acid, norfloxacin, linezolid, tetracycline, trimethoprim-sulfamethoxazole and mupirocin. In addition, MRSA isolates were screened for resistance towards glycopeptides by spot test on Brain-Heart infusion (BHI) agar (Becton Dickinson, Germany) with teicoplanin (5 mg/L) and confirmed by Etest® (AB Biodisk, Solna, Sweden) on BHI with inoculum of McFarland 2.0. In case of MIC \geq 8 mg/L for vancomycin or MIC \geq 12 mg/L for teicoplanin, population analysis profile against vancomycin was performed [Wootton et al. 2001. J Antimicrob Chemother. 47: 399–403].

Invasive *Streptococcus pneumoniae* from humans. 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, Hillerød, Denmark). Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK) as recommended by the manufacturer. All breakpoints used were defined by the EUCAST. Both fully and intermediary resistant isolates were defined as resistant.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans. Screening for penicillin- and erythromycin-resistant streptococci was performed with 1 unit penicillin G 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, Hillerød, Denmark). Erythromycin-resistant streptococci were tested with a 15 µg erythromycin disc and a 2 µg clindamycin disc (Oxoid) on Müller-Hinton agar. Erythromycin MICs were determined using the Etest® (AB Biodisk, Solna, Sweden) on Müller-Hinton agar incubated at 36°C in 5% CO₂. The breakpoints used were defined by the EUCAST. Both fully and intermediary resistant isolates were defined as resistant.

***E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *E. faecium* and *E. faecalis* from humans.** The DCM performed either disk (Oxoid, Basingstoke, UK) or tablet (Neo-Sensitabs®, A/S Rosco) diffusion susceptibility testing on a number of media. As per September 2012, all DCM except Rigshospitalet used breakpoints defined by EUCAST.

Data on antimicrobial resistance from private hospitals and clinics and from psychiatric hospitals were excluded. All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

Table 10. 3. Definitions of antimicrobial classes for calculation of multi-resistance (MR) in zoonotic and indicator bacteria, DANMAP

DANMAP 2012

Antimicrobial classes	<i>Salmonella</i> and <i>E. coli</i> ^(a)	<i>Campylobacter</i> ^(b)	<i>Enterococcus</i> ^(a)
Tetracyclines	Tetracycline	Tetracycline	Tetracycline
Phenicoles	Chloramphenicol and/or florfenicol	Chloramphenicol	Chloramphenicol
Penicillins	Ampicillin		Ampicillin and/or penicillin
Cephalosporins	Ceftiofur and/or cefotaxime		
Sulfonamides	Sulfonamides		
Trimethoprim	Trimethoprim		
Aminoglycosides I	Gentamicin	Gentamicin	Gentamicin and/or kanamycin and/or streptomycin
Aminoglycosides II	Streptomycin	Streptomycin	
Quinolones	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin and/or nalidixic acid
Polymyxins	Colistin		
Macrolides		Erythromycin	Erythromycin
Glycopeptids			Vancomycin and/or teicoplanin
Ionophores			Salinomycin
Oxazolidinones			Linezolid
Glycylcyclines			Tigecycline

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the panel for the selected bacterial species

a) An isolate is considered multi-resistant if resistant to three or more of the ten antimicrobial classes

b) An isolate is considered multi-resistant if resistant to three or more of the six antimicrobial classes

10.6 Data handling

10.6.1 Animal

The results from the analysis of all animal samples - positive as well as negative findings - and of the bacteria isolated and the susceptibility testing were stored in an Oracle Database 9i Enterprise Edition® at DTU National Food Institute. The susceptibility data were stored as continuous values as well as categorised as susceptible or resistant as defined by the relevant ECOFF. Each isolate was identified by the bacterial species, the subtype as applicable and by the date of sampling and species of animal. Information on the farm of origin was also recorded. All handling and evaluation of results were carried out using SAS®Software, SAS Enterprise Guide 4.3.

10.6.2 Meat

Results from the analysis of food samples were reported via the database administrated by the DVFA, except for the data on *Salmonella*, which were reported to and extracted from the laboratory database at DTU National Food Institute. For each bacterial isolate, information was available on food type, bacterial species, date and place of sampling, date of examination, country of slaughter, the Regional Veterinary and Food Control Authorities collecting and processing the sample, and an identification number, which makes it possible to obtain further information about the isolate from the relevant authorities. Furthermore, information about the country of origin was recorded whenever possible.

10.6.3 Human

***Salmonella* and *Campylobacter*.** 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.

***Staphylococcus aureus*.** 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 whether it was colonisation or 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 colonization/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonization), 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 12 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalizations or stay in long-term care facilities within 12 months prior to MRSA isolation and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts.

Streptococcus pneumoniae, *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. Data on susceptibility testing of isolates were stored as MICs in a Microsoft® Access database placed on a SQL server at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

E. coli, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *E. faecium* and invasive *E. faecalis*. Twelve out of thirteen DCM in Denmark provided data on resistance levels in *E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *E. faecium* and invasive *E. faecalis* isolates. Data were extracted from the following laboratory information systems:

- ADBakt (Autonik AB, Skoldinge, Sweden) for the DCM at Hvidovre, Herlev and Aalborg Hospitals.
- MADS (DCM Skejby Hospital, Aarhus, Denmark) for the DCM at Rigshospitalet and Slagelse/Region Sealand, Odense, Esbjerg, Vejle, Herning/Viborg, and Aarhus (Skejby) Hospitals.
- SafirLIS Microbiology (Profdoc Lab AB, Borlänge, Sweden) for the DCM at Hillerød Hospital.

Resistance data on the first isolate per patient per year were included. Generally, resistance data were excluded if susceptibility to a certain antimicrobial agent was tested on only a selected number of isolates.

10.6.4 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 4.3 or StatCalc in EpiInfo™ v. 6. Difference in par-wise comparisons were tested using Chi-square, or Fisher's Exact Test when the number of samples is low (<25). When appropriate, significance of temporal trends is tested using linear logistic regression using Proc LOGISTIC procedure in SAS (Likelihood ratio test).

In the text, commented differences imply statistically significant differences where $p < 0.05$.

When comparing proportions between years, the EUCAST epidemiological cut-off values for 2012 were also used for interpretation of previous years MICs.

Anne Mette Seyfarth, Vibeke Frøkjær Jensen
and Line Skjøt-Rasmussen



List of abbreviations

DADD	Defined animal daily dose
DAPD	Defined animal daily dose per 1,000 animals per day
AGP	Antimicrobial growth promoter
ATC	Anatomical Therapeutic Chemical Classification System
ATC _{vet}	Anatomical Therapeutic Chemical Classification System for veterinary medicines
CC	Clonal complex
CHR	Central Husbandry Register
CI	Confidence interval
CNS	Central nervous system
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
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
GI	Gastrointestinal
GP	General practitioner
HLGR	High-level gentamicin resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
RFCA	Regional Veterinary and Food Control Authorities
SSI	Statens Serum Institut
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin resistant enterococci
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification. 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.whocc.no/atcddd/indexdatabase/).

The ATC classification for veterinary medicinal products, ATC_{vet}, 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.whocc.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]. Antimycobacterial agents are not included. In the section of 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 usually 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 section of human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system).

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.66 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 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). In contrast to the ADD previously used in DANMAP, the DADD has not been defined for each antimicrobial agent, administration route and animal species but at product level. In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which has been used since DANMAP 2003. For more details, see Chapter 10, Materials and Methods. The DADDs used in DANMAP 2012 are presented 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, allowing for comparison between sectors and adjusting 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 life-span. 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 (Section 4.3 and Chapter 10, Materials and Methods).

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.whocc.no/atcddd/indexdatabase/).

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 account of hospital activity (discharges). 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 indicates that 1% of the population on average gets a certain treatment daily. In figure 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.

Intramammaria. 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.

Multi-resistant. A *Salmonella*, *Campylobacter*, *Enterococcus* or *E. coli* isolate is assumed multi-resistant if it is resistant to three or more of the antimicrobial classes. The number of antimicrobial classes and antimicrobial agent included therein depend on the test panel for each bacterium (See Table 10.3, Materials and methods).

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 cat.

Piglet. The newborn 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.



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