DANMAP 2011

DANMAP 2011 - 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

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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 of antimicrobial use and antimicrobial resistance in food animals, food and humans in 2011. 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 Science, Innovation and Higher Education; the Ministry of Food, Agriculture and Fisheries; and the Ministry of Health.

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STATENS SERUM INSTITUT

DTU Vet
National Veterinary Institute

DTU Food
National Food Institute

1. Introduction

1.1 About DANMAP

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, was established in 1995 on the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries, as a coordinated national surveillance and research programme for antimicrobial consumption and antimicrobial resistance in bacteria from animals, food and humans. The programme is coordinated by Statens Serum Institut and the National Food Institute at the Technical University of Denmark (DTU). The Danish Health and Medicines Authority, the Danish Food Administration and the National Veterinary Institute at the DTU also provide data. The DANMAP programme is funded jointly by the Ministry of Science, Innovation and Higher Education; the Ministry of Food, Agriculture and Fisheries; and the Ministry of Health.

The objectives of DANMAP are:

- to monitor the consumption of antimicrobial agents for food animals and humans;
- to monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans;
- to study associations between antimicrobial consumption and antimicrobial resistance;
- 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, zoonotic bacteria, and indicator bacteria.

Human and animal pathogens are included because these cause infections and they reflect primarily resistance caused by use of antimicrobial agents in the respective reservoirs.

Zoonotic bacteria are included because they can develop resistance in the animal reservoir, which may subsequently compromise treatment effect when causing infection in humans.

Indicator bacteria are included due to their ubiquitous nature in animals, food and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in both reservoirs.

This report, DANMAP 2011, describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs in Denmark in 2011. Results from the monitoring programme as well as from selected research projects are presented in overview tables and figures. The report also include a list of abbreviations, explanations of terminology and description of materials and methods.

Please note that in this year's report, the specific MIC distributions as well as some detailed tables of antimicrobial consumption in animals and humans are presented in a web annex located at www.danmap.org. Pdf versions of this and previous DANMAP reports are also available at the DANMAP website.

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- the Department of Medication Statistics and Research Support at SSI (formerly the Danish Medicines Agency) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies;
- the staff of the Regional Veterinary and Food Control Authorities for collection of food samples and isolation of bacteria;
- the staff of the Zoonosislab at the National Food Institute at DTU;
- the staff of the research group of Antimicrobial resistance and molecular typing at the National Food Institute at DTU.

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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.

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2. Summary

2.1 Sammendrag

DANMAP har siden 1995 beskrevet det årlige forbrug af antibiotika og forekomsten af antibiotikaresistens hos dyr og mennesker i Danmark.

Antibiotikaforbrug til dyr

Siden 2001 er al anvendelse af receptordineret medicin til dyr registreret i det offentlige register VetStat. I VetStat databasen indgår oplysninger om dyreart, aldersgruppe og besætningsniveau samt i et vist omfang oplysninger om den sygdom, der behandles.

I 2011 blev der brugt 107,9 ton antibiotika til dyr i Danmark, hvor størstedelen blev anvendt i svineproduktionen (77 %), og en mindre andel i kvæg (14 %), pelsdyr (4 %), fisk (2 %) og fjerkræproduktionen (1 %). Det totale forbrug i tons til dyr i 2011 var 15 % lavere end i 2010.

Svin: I 2011 faldt det totale antibiotikaforbrug til svin med i alt 19 ton. Opgjort i antal standard doser pr produceret svin, svarer dette til et fald på 30 % i forhold til året før. Antibiotika forbruget til svin har været faldende siden "Gult kort-ordningen" blev bekendtgjort den 1. juli 2010. Ordningen er rettet mod svinebesætninger med højest antibiotikaforbrug per svin med henblik på at reducere forbruget.

I 2011 var faldet i antal standard doser pr svin især tydeligt for pleuromutiliner (60 %), tetracykliner (27 %) og makrolider (26 %), som primært ordineres i forbindelse med mave-tarm lidelser. Desuden nærmede forbruget af 3. og 4. generations cefalosporiner sig nul som følge af, at svinebranchen frivilligt indførte stop for brugen af disse stoffer, som er kritisk vigtige til behandling af mennesker, i juli 2010.

Kvæg: Det totale antibiotikaforbrug til kvæg har ligget relativt stabilt på ca. 14–15 ton om året siden 2005, og i 2011 var forbruget 14,7 ton. Beta-lactamase følsomme penicilliner, som hovedsageligt bruges til behandling af yverbetændelse hos malkekøer, målt i standard doser udgjorde mere end halvdelen (59 %) af forbruget til kvæg. I forhold til kvægholdets størrelse, opgjort som biomasse (ton levende dyr), er forbruget generelt lavt. Det højeste forbrug er til kalve; som er på niveau med forbruget i kalkuner, men kun halvt så højt som forbruget til svin. Forbruget til kalve udgøres hovedsageligt af tetracykliner og makrolider. Forbruget af amfenikoler til behandling af luftvejsinfektioner hos kalve er steget væsentligt over de sidste 10 år. Forbruget af 3. og 4. generations cefalosporiner, både til systemisk og intramammær behandling af malkekvæg, er faldet med henholdsvis 34 % og 68 % siden 2008 målt i kg aktivt stof. Forbruget af fluorkinoloner har ligget tæt på nul siden 2003.

Fjerkræ: Antibiotikaforbruget til fjerkræ faldt med 8 %, fra 879 kg i 2010 til 810 kg i 2011, men niveauet lå stadig højere end i perioden 2001–2008. Dette generelle fald i forbrug til fjerkræ skyldes især et nedsat forbrug til kalkuner, ægglæggende høner og forældredyr i slagtekyllingeproduktionen. Ser man på slagtekyllinger alene, steg forbruget af antibiotika - særlig forbruget af amoxicillin - i 2011. Forbruget af fluorkinoloner har været lavt siden 2006. I 2011 blev fluorkinoloner slet ikke brugt til fjerkræ, ligesom cefalosporiner ikke har været anvendt til fjerkræ i mindst 10 år.

Fisk: Fra 2010 til 2011 faldt total forbruget af antibiotika i fiskeproduktionen til 2.700 kg, svarende til et overordnet fald på 11 %. Målt i standard doser, udgjorde sulfonamid/trimethoprim størstedelen af forbruget (60 %). Kinoloner (oxolinsyre) og florfenicol udgjorde henholdsvis 30 % og 10 %. Under forudsætning af at produktionen af fisk i 2011 var den samme som året før, faldt forbruget af antibiotika til saltvandsfisk med 50 % (til 4,5 ADDkg pr kg fisk produceret), mens forbruget til ferskvandsfisk steg med 12 % (til 2,6 ADDkg pr kg fisk produceret).

Kæledyr og heste: Informationerne i VetStat vedrørende antibiotikaforbruget i kæledyr er mangelfulde sammenlignet med informationerne vedrørende produktionsdyr, men der blev brugt ca. 2 ton antibiotika til kæledyr og ca. 1 ton til heste i 2011. Målt i standard doser er brugen af antibiotika til oral behandling af kæledyr steg med 8 % fra 2010 til 2011, og stigningen siden 2005 er nu på i alt 36 %. Stigningen kan i særlig grad tilskrives et øget forbrug af 'kombinationspenicillinet' amoxicillin i kombination med clavulansyre. Brug af amoxicillin/clavulansyre, som er et af de mest brugte antibiotika til kæledyr, udgjorde 91 % målt i kg aktivt stof af det veterinære forbrug af 'kombinationspeniciliner' i 2011.

For fluorkinoloner blev omkring halvdelen (51 %) af fluorkinoloner, som blev benyttet veterinært, anvendt til behandling af kæledyr. I 2011 var forbruget af 3. og 4. generations cefalosporiner til kæledyr stabilt og udgjorde cirka 3 kg. Brugen af 3. og 4. generations cefalosporiner i udvalgte hestepraksis varierede mellem 0 % og 30 % af det totale antibiotikaforbrug opgjort i standard doser i den enkelte praksis (**Textbox 1**). Det høje forbrug af især kritisk vigtige stoffer for human behandling og forbrug af bredspektrede antibiotika til kæledyr og heste samt den store variation af forbrug imellem hestepraksis kan afspejle mangelfuld behandlingsvejledning i brug af antibiotika til kæledyr og heste.

Det totale antibiotikaforbrug til svin er faldet væsentligt efter indførelsen af "Gult kort" ordningen, ligesom svinebranchens frivillige stop har reduceret forbruget af cefalosporiner til svin til nær nul.

Antibiotikaforbruget til kæledyr er steget siden 2005, hvilket hovedsageligt skyldes en stigning i forbruget af amoxicillin kombineret med clavulansyre.

Antibiotikaforbrug til mennesker

Lægemiddelstyrelsen har, siden begyndelsen af 1990erne, overvåget forbruget af receptordineret medicin på patientniveau.

Totalforbrug: I 2011 lå det totale forbrug af antibiotika til systemisk brug til mennesker (primærsektoren og hospitalssektoren sammenlagt) på samme niveau som i 2010 (18,84 DDD pr. 1000 indbyggere pr. dag (DID) i 2010 sammenlignet med 18,90 DID i 2011). Forbrugsniveauet i 2011 fulgte dog stadig den generelt stigende tendens fra de seneste år. Primærsektoren udgjorde 90 % af forbruget, og forbruget i denne sektor var i 2011 det højeste siden 1995. Fra 2002 til 2011 steg det totale forbrug af antibiotika i Danmark med 28 % (4,13 DID).

Primærsektor: Antibiotikaforbruget i primærsektoren i 2011 var relativt konstant sammenlignet med 2010 (17.06 DID i 2011 og 16,93 DID i 2010). Som i de tidligere år udgjorde beta-laktamase følsomme penicilliner den største gruppe af antibiotika (31 %), og penicilliner udgjorde 63 % af det totale forbrug i 2011. I 2011 var forbruget af bredspektrede antibiotika 6,58 DID (39 %), en stigning på 0,1 DID (2 %) i forhold til 2010. Fra 2010 til 2011 steg forbruget af 'kombinationspenicilliner' med 31 %, mens mindre stigninger blev observeret for beta-laktamase følsomme penicilliner (1,1 %) og makrolider (1,2 %).

Der kan være forskellige forklaringer på det stadigt høje forbrug i 2011: (1) Et øget antal patienter i behandling, (2) et udbrud af *Mycoplasma pneumoniae* (atypisk pneumoni) i oktober–december 2011, som medførte et øget forbrug af dels beta-laktamase følsomme penicilliner (til behandling af nedre luftvejsinfektioner) og dels makrolider (til behandling af bekræftede og mistænkte *M. pneumoniae* infektioner) og (3) en kontinuerlig respons på ændringer i de nationale behandlingsvejledninger (især for 'kombinationspenicilliner').
Sygdomsspecifikke ændringer, som kan medføre et øget antal behandlede patienter, kan ikke vurderes på grund af manglende indikationskoder.

I det seneste årti er forbruget af antibiotika i primærsektoren steget med 29 %, fra 13,26 DID i 2002 til 17,06 DID i 2011. I 2011 udgjorde bredspektrede antibiotika 6,58 DID (39 %) af det samlede antibiotikaforbrug i primærsektoren, hvilket er en stigning på 78 % sammenlignet med 2002 (3,85 DID, 29 %). Denne stigning skyldes sandsynligvis til dels et øget antal DDD per behandlet patient (definerede dagsdoser) og et øget antal DDD per udskrevet medicinpakning. Sidstnævnte kan afspejle ændrede retningslinjer for behandling, med kortere behandlingstider men med højere doser. Andre forklaringer for det stigende antibiotikaforbrug er på nuværende tidspunkt svære at vurdere på grund af manglende indikationskoder. Dette forventes dog at blive bedre fremover, da det i 2012 blev vedtaget at fjerne de brede indikationskoder ('mod betændelse' og 'mod infektion') og erstatte dem med mere specifikke indikationskoder på alle danske antibiotikarecepter.

Analyser af sammenhængen mellem demografiske faktorer og det stigende forbrug af antibiotika fra 2001 til 2010, påviste stigninger i specifikke antibiotika-typer og substanser, især blandt personer over 65 år og blandt yngre voksne. Alders-relaterede stigninger i tetracykliner og pivmecillinam skyldes højst sandsynligt ændringer i behandlingsmønstret for hhv. akne og urinvejsinfektioner. Stigninger i forbruget af roxithromycin, uanset aldersgruppe, hænger sandsynligvis sammen med flere store udbrud af *M. pneumoniae*. Generelt tyder det på, at forbrugsstigningerne mellem 2001 og 2010 skyldes en kombination af sygdomsspecifikke faktorer og ikke mindst en tendens til, at de danske praktiserende læger udskriver mere antibiotika per besøg og i højere doser (**Textbox 3**).

Hospitalssektor: Det samlede forbrug af antibiotika i hele hospitalssektoren (rehabiliteringscentre, hospices, private-, psykiatriske-, special- og somatiske hospitaler) i 2011 faldt fra 1,91 DID i 2010 til 1,84 DID i 2011 (et fald på 3,7 %). Siden 2002 er forbruget steget med 0,38 DID (26 %). I 2011 udgjorde bredspektrede antibiotika 1,22 DID af totalforbruget sammenlignet med 0,75 DID i 2002 (en stigning på 67 %).

Somatiske hospitaler: I 2011 steg det totale antibiotikaforbrug på somatiske hospitaler opgjort i DDD per 100 sengedage (DBD) med 3,6 % (3,12 DBD), fra 87,72 DBD i 2010 til 90,84 DBD i 2011. Ved opgørelse i DDD per 100 indlæggelser (DAD) blev der ikke observeret en ændring fra 2010 til 2011 (282,53 DAD i 2011 og 284,89 DAD i 2010 (0,8 %). Disse tal er baseret på et fald på 0,08 DDD (4,2 %) fra 2010 til 2011, kombineret med færre sengedage og flere indlæggelser i 2011 sammenlignet med 2010.

Fire antibiotikagrupper dominerede forbrugsstigningen fra 2010 til 2011, opgjort som DBD: kombination af sulfonamider/trimethoprim (1,07 DBD, 35 %), 'kombinationspenicilliner (1,38 DBD, 19 %), fluorkinoloner (0,26 DBD, 6,7 %) og makrolider (0,17 DBD, 4,7 %). Fra 2010 til 2011 faldt forbruget af beta-laktamase resistente penicilliner (0,41 DBD, 5,3 %) mens der var mindre nedgang for penicilliner med udvidet spektrum (0,20 DBD, 1,4 %) og beta-laktamase følsomme penicilliner (0,17 DBD, 1,8 %).

I 2011 udgjorde cefalosporiner 19 % af det totale antibiotikaforbrug på somatiske hospitaler, efterfulgt af penicilliner med udvidet spektrum (15 %), fluorkinoloner (12 %) og beta-laktamase følsomme penicilliner (10 %).
I løbet af de seneste ti år er forbruget af antibiotika på somatiske hospitaler steget fra 51,73 DBD i 2002 til 90,84 DBD i 2011 (76 %) eller fra 258,46 DAD i 2002 til 282,53 DAD i 2011 (9,3 %), afhængigt af nævneren. Udover at fremhæve stigninger i det totale antibiotikaforbrug understreger disse tal også den ændrede hospitalisering i Danmark med et stigende antal indlæggelser og faldende antal sengedage siden 2002.

I 2011 lå det totale forbrug af antibiotika til systemisk brug til mennesker (primærsektoren og hospitalssektoren sammenlagt) på samme høje niveau som i 2010. Forbruget i primærsektoren udgjorde 90 %, mens forbruget på hospitalerne udgjorde de resterende 10 %. Fra 2002 til 2011 steg det totale forbrug af antibiotika til mennesker i Danmark med 28 %. Forbruget af bredspektrede antibiotika udgjorde 39 % af det totale forbrug i primærsektoren i 2011, hvilket er en stigning på 78 % sammenlignet med 2002.

Resistens i zoonotiske bakterier

Zoonotiske bakterier som *Salmonella* og *Campylobacter* er sygdomsfremkaldende bakterier, som kan overføres fra dyr til mennesker, enten via direkte kontakt eller via kontaminerede fødevarer.

I DANMAP 2011 præsenteres antibiotika resistensforekomsten for ***Salmonella* Typhimurium** udelukkende fra danske svin og fra dansk svinekød, da der var for få isolater fra andre kilder – fjerkræ og kvæg.

Blandt *S.* Typhimurium isolater fra danske svin blev der i 2011 observeret signifikante stigninger i resistensforekomsten af ampicillin, neomycin, sulfonamid og tetracyklin i forhold til 2010. I modsætning til tidligere blev der i 2011 fundet generelt højere resistensforekomst i dansk svinekød end i danske svin. Ingen af de fundne *S.* Typhimurium isolater var resistente over for cefalosporiner (ceftiofur og cefotaxim), fluorkinoloner (ciprofloxacin) eller kinoloner (nalidixansyre).

Blandt *S.* Typhimurium fra mennesker var forekomsten af fluorkinolon og cefalosporin resistens hyppigere hos de rejserelaterede tilfælde (hhv. 16 % og 12 %) i forhold til de patienter, som havde erhvervet infektionen i Danmark (hhv. 2 % og 1 %).

Forekomsten af resistens i *S.* Typhimurium isolater fra svin, dansk svinekød og humane tilfælde var i 2011 stærkt påvirket af den klonale spredning af de monofasiske varianter af *S.* Typhimurium, som er resistente overfor ampicillin (A), streptomycin (S), sulfonamide (Su) og tetracyklin (T), også kaldt ASSuT resistensprofilen. Denne tendens ses gennem hele "jord til bord" kæden, hvor andelen af multiresistente isolater steg markant, samtidig med at andelen af fuldt følsomme isolater faldt.

Blandt ***S.* Enteritidis** isolaterne fra humane infektioner i 2011, blev kinolon (ciprofloxacin og nalidixansyre) resistens fundet i 18 % at de sporadiske humane tilfælde erhvervet i Danmark. Dette svarer til en stigning på 10 % i forhold til året før. Resistensforekomsten blandt de hjemligt erhvervede infektioner nærmer sig derved niveauet blandt de rejserelaterede tilfælde (24 %) og blandt tilfælde med ukendt oprindelse (27 %).

Resistensforekomsten i *Salmonella Typhimurium* isolater fra dansk svinekød steg voldsomt i 2011, mens niveauet i isolater fra svin blot fortsatte den stigning der er set gennem de seneste år. Forekomsten af tetracyklinresistens blandt *S.* Typhimurium isolater fra svin og svinekød steg signifikant til trods for, at tetracyklin forbruget til svin er faldet de to foregående år. Dette understøtter teorien om at stigningen, i hvert fald delvist, skyldes spredningen af resistente kloner.

Resistensforekomsten i ***Campylobacter jejuni*** isolater fra danske kyllinger og kvæg og i ***Campylobacter coli*** isolater fra danske svin var på samme niveau som i 2010. Generelt er tetracyklinresistens forekomsten steget siden 2005, hvilket især er tydelig for *C. coli* isolater fra svin i perioden 2008 til 2011. I denne periode var tetracyklin et af de hyppigst anvendte antibiotika til svin.

Som i foregående år var fluorkinolon (ciprofloxacin) resistensforekomsten i *C. jejuni* signifikant højere blandt isolater fra importeret kyllingekød (57 %) end fra dansk produceret kyllingekød (11 %). Denne forskel er øget over de seneste år.

Forekomsten af tetracyklin- og fluorkinolonresistens steg i *C. jejuni* isolater fra human tilfælde i forhold til 2010 - både bland de hjemlig erhvervede og rejse relaterede tilfælde. Som i de foregående år var fluorkinolon resistensforekomsten i *C. jejuni* isolater fra rejse relaterede humane tilfælde (84 %) signifikant højere end i isolater fra personer, hvor infektionen var erhvervet i Danmark (33 %).

Resistensforekomsten blandt *Campylobacter jejuni* isolater fra dansk kyllingekød var signifikant lavere end i isolater fra udenlandsk kyllingekød. Det samme gjorde sig gældende for isolater fra humane tilfælde, hvor der blandt de tilfælde der var opstået i Danmark blev fundet signifikant lavere resistens end blandt de rejserelaterede tilfælde.

Resistens i indikatorbakterier

Indikator bakterier er inkluderet i DANMAP overvågningen for at kunne give et indblik i de generelle resistensforekomster i raske husdyr og kød.

Blandt ***Enterococcus faecium*** isolater fra svin, steg antibiotika resistensforekomsten for beta-lactamer (penicillin og ampicillin) signifikant fra 2010 til 2011. Derudover er streptomycinresistensen gradvist steget, uden at dette har kunnet knyttes til en tilsvarende stigning i forbrug. Stigningen kan dog hænge sammen med forekomsten af tetracyklinresistens, som kan medføre co-selektion for streptomycinresistens. Forekomsten af erythromycinresistens faldt både blandt isolater fra svin og blandt isolater fra slagtekyllinger. På trods af, at det er mere end ti år siden, at brugen af vækstfremmerne avoparcin og virginiamycin (quinupristin/dalfopristin) ophørte, blev der i 2011 stadig fundet lav forekomst af resistens mod disse stoffer.

Forekomsten af resistens i ***Enterococcus faecalis*** isolater fra svin steg signifikant for en række antibiotika: chloramphenicol, erythromycin, kanamycin og gentamicin. Blandt disse isolater forekom ofte co-resistens, hvor kombinationen af chloramphenicol-, kanamycin- og gentamicinresistens sammen med tetracyklin og/eller erythromycinresistens var dominerende.

Blandt både *E. faecium* and *E. faecalis* isolater var forekomsten af resistens mod erythromycin, kanamycin and streptomycin signifikant højere i isolater fra importeret kyllingekød end fra dansk produceret kyllingekød. Tilsvarende var tetracyklinresistensen i *E. faecalis* isolater fra importeret svinekød højere end i isolater fra dansk svinekød.

Vancomycin og quinupristin/dalfopristin resistens forekommer stadig på lavt niveau i *E. faecium* fra svin, til trods for at brugen af disse vækstfremmere ophørte for mere end ti år siden.

Resistensforekomsten i **indikator *E. coli*** isolater fra danske kyllinger og kvæg var på samme niveau som i 2010. Forekomsten af streptomycinresistens faldt signifikant i isolater fra svin, og idet streptomycin resistens ofte (99 % i 2010 og 75 % i 2011) forekommer i kombination med tetracyklin- og/eller sulfonamidresistens.

I dyr sås de højeste resistensforekomster blandt indikator *E. coli* fra svin, bortset fra fluorkinolon (ciprofloxacin) hvor resistensforekomsten var højere i isolater fra kyllinger. De seneste ti år har forekomsten af fluorkinolonresistens i isolater fra svin og kvæg været meget lav, mens niveauet i isolater fra kyllinger har varieret mellem 7 % og 13 %. I de seneste ti år er fluorkinolon kun anvendt i meget begrænset omfang i den danske husdyrproduktion, med undtagelse af i kyllingeproduktionen, hvor det var almindeligt brugt indtil 2007.

Resistensforekomsten var signifikant højere i indikator *E. coli* isolater fra importeret kyllingekød end fra dansk produceret kyllingekød (for 11 ud af 16 testede antibiotika), herunder både for cefalosporiner (ceftiofur) og fluorkinolon. Tilsvarende var chloramphenicol- og kinolonresistensen i *E. coli* isolater fra importeret svinekød højere end i isolater fra dansk svinekød. Som de foregående år var resistensforekomsten i *E. coli* fra importeret og dansk oksekød lavt.

ESBL-producerende bakterier er resistente overfor bred-spekterede cefalosporiner, der ofte bruges til behandling af livstruende infektioner humant. Derfor er forekomsten af disse, selv i et lavt niveau, et potentielt alvorligt problem. Ved brug af selektive opformeringsmetoder blev forekomsten af disse bakterier undersøgt i svinebesætninger, hos kvæg og svin på slagterierne samt i kød fra detailforretninger og engroslagre.

Det frivillige forbud mod brug af cefalosporiner i den danske svineproduktion har medført en reduceret forekomst af ESBL-producerende *E. coli* i svin. Hos kvæg forblev forekomsten på samme niveau som i 2010, sandsynligvis fordi 3. og 4. generations cefalosporiner stadig benyttes til intramammær og systemisk behandling.

Forekomsten af ESBL-producerende *E. coli* i dansk kyllingekød steg (fra 8,6 % i 2010 til 44 % i 2011) og er nu på samme niveau som i importeret kyllingekød (48%), på trods af at cefalosporiner ikke har været brugt i den danske kyllingeproduktion i mindst ti år. Forekomsten skyldes sandsynligvis at ESBL producerende *E. coli* introduceres via importerede forældredyr. Derudover er forbruget af bred-spektrede penicilliner i den danske kyllingeproduktion øget, hvilket kan resulterer i co-selektion af ESBL producerende *E. coli*.

Tilstedeværelsen af de forskellige ESBL-gener afhang af dyrearten. Hos svin og kvæg var det hyppigst forekommende ESBL-gen CTX-M-1, men også CTX-M-14 og CTX-M-15 blev påvist. CMY-2 blev ofte påvist i både dansk og importeret kyllingekød. Alle er ESBL-gener der forekommer i ESBL-producerende *E. coli,* som forårsager human sygdom. Dansk såvel som importeret kyllingekød synes at være et vigtigt reservoir for ESBL-producerende *E. coli.*

Det frivillige stop for brugen af cefalosporiner i den danske svineproduktion afspejler sig i et fald i forekomsten af ESBL-producerende *E. coli* i både svinebesætninger og i svin ved slagtning. Selv i lande som Danmark, hvor cefalosporiner ikke anvendes i slagtekyllingeproduktionen er kyllingekød tilsyneladende også en vigtig kilde til ESBL-producerende *E. coli*.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Rapporteringen af antibiotikaresistens i bakterier fra diagnostiske 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 anmeldepligtige. Data vedr. disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt ***Escherichia coli* isolater fra blod** var 3. generations cefalosporin resistensforekomsten 8 % i 2011, hvilket er det samme niveau som i 2010, men højere end i de andre nordiske lande i 2010. Ciprofloxacin resistens var 14 % i 2011 (min. 12 %, max. 31 % for de individuelle KMAer), dette var det samme niveau som i 2010. I løbet af de seneste 10 år er resistens overfor cefuroxim, ciprofloxacin og gentamicin steget signifikant. Resistens overfor 3. generations cefalosporiner er rapporteret siden 2008; i denne periode er resistensforekomsten steget. Carbapenem (meropenem) resistens blev ikke observeret i *E. coli* isolater fra blod i 2011.

Blandt ***E. coli* isolater fra urin fra hospitaler** var 3. generations cefalosporin resistensforekomsten 5 % i 2011, hvilket er det samme niveau som i 2010. Fluorkinolon resistensforekomsten steg signifikant fra 2010 til 2011 (i 2011: ciprofloxacin 13 %, nalidixansyre 17 %). I løbet af de seneste 10 år er resistens overfor fluorkinoloner steget signifikant (i 2002: ciprofloxacin 2 %, nalidixansyre 6 %). Aminoglykosid (gentamicin) resistensforekomsten steg signifikant fra 3,7 % i 2010 til 4,4 % i 2011.

Blandt ***E. coli* isolater fra urin fra praksis** var 3. generations cefalosporin resistensforekomsten 3 % i 2011, hvilket er det samme niveau som i 2010. Sulfonamid resistensforekomsten faldt signifikant fra 37 % i 2010 til 35 % i 2011.

Carbapenem (meropenem) resistens blev observeret i *E. coli* isolater fra urin fra både hospitaler og praksis. Forekomsten af carbapenem resistens er ikke anmeldepligtig; derfor kunne frekvensen af carbapenem resistens i *E. coli* ikke beregnes.

Blandt ***Klebsiella pneumoniae* isolater fra blod** var resistensforekomsten for 3. generations cefalosporiner (10 %), fluorkinoloner (ciprofloxacin 12 %, nalidixansyre 18 %) og aminoglykosid (gentamicin 6 %) på samme niveau som i 2010. Niveauet var højere end hvad der blev rapporteret for de andre nordiske lande i 2010 og på samme niveau som i flere andre europæiske lande. Carbapenem (meropenem) resistens blev observeret i ét *K. pneumoniae* isolat fra blod i 2011.

Blandt ***K. pneumoniae* isolater fra urin** faldt resistensforekomsten for 3. generations cefalosporiner signifikant i isolater fra hospitaler (fra 12 % i 2010 til 10 % i 2011) og fra praksis (fra 7 % i 2010 til 5 % i 2011). Cefuroxim resistensforekomsten faldt signifikant fra 13 % i 2010 til 11 % i 2011 i isolater fra hospitaler. Fluorkinolon resistensforekomsten faldt signifikant fra 2010 til 2011 blandt urin-isolater fra hospitaler (ciprofloxacin fra 14 % i 2010 til 11 % i 2011) og praksis (ciprofloxacin fra 12 % i 2010 til 9 % i 2011, nalidixansyre fra 20 % i 2010 til 14 % i 2011). Der sås også et signifikant fald i forekomsten af mecillinamresistens fra 2010 til 2011 i isolater fra hospitaler (i 2011: 12 %) og praksis (i 2011: 12 %). Blandt *K. pneumoniae* isolater fra hospitaler steg sulfonamid resistensforekomsten signifikant fra 29 % i 2010 til 33 % i 2011.

Carbapenem (meropenem) resistens blev observeret i *K. pneumoniae* isolater fra urin fra både hospitaler og praksis. En af de carbapenemresistente isolater var KPC-2 positiv (**Textbox 11**). Forekomsten af carbapenemresistens er ikke anmeldepligtig; derfor kunne frekvensen af carbapenemresistens i *K. pneumoniae* ikke beregnes.

I *E. coli* isolater fra blod (2008–2010) blev der observeret en stigende resistensforekomst af gentamicin, ciprofloxacin og 3. generations cefalosporin og multi-resistens (gentamicin, ciprofloxacin og 3. generations cefalosporin) parallelt med et stigende forbrug af bredspektrede antibiotika (2007–2010) (**Textbox 8**).

I oktober 2011 screenede 12 af 13 KMAer alle *E. coli* og *K. pneumoniae* urin- og blod-isolater for ESBL-produktion. ESBL-prævalensen fra 2011 blev sammenlignet med data fra tilsvarende studier udført i 2007 og 2009. Fra oktober 2009 til oktober 2011 steg prævalensen af ESBL-producerende *E. coli* signifikant i urinprøver fra både praksis (fra 2,3 % i 2009 til 3,2 % i 2011) og hospitaler (fra 3,8 % i 2009 til 4,7 % i 2011). Som i tidligere år dominerede CTX-M-15 blandt ESBL-producerende *E. coli* og *K. pneumoniae* isolater fra urinvejs- og blod-infektioner fra patienter, mens CTX-M-1, CTX-M-14 og CMY-2 kun var til stede i et begrænset antal isolater (**Textbox 9**).

Resistensforekomsten for 3. generations cefalosporiner, ciprofloxacin og gentamicin i *K. pneumoniae* fra urinvejs- og blod-infektioner fra patienter på hospital var signifikant højere i den østlige del af Danmark (Sjælland) end i den vestlige del (Fyn og Jylland). Den samme tendens sås blandt *K. pneumoniae* urin-isolater fra patienter i praksis, hvor resistensforekomsten for 3. generations cefalosporiner og fluorkinoloner (ciprofloxacin og nalidixansyre) var signifikant højere i isolater fra den østlige del af Danmark (Sjælland) end i isolater fra den vestlige del (Fyn og Jylland) (**Textbox 10**).

I perioden 2008–2011 blev der påvist 15 carbapenemase-producerende enterobakterier (CPE) i Danmark. Disse omfattede 2 VIM-, 3 KPC-, 3 NDM-1- og 7 OXA-48-producerende isolater. Af de 15 observerede tilfælde blev 10 påvist i 2011 og de fleste af disse var importeret fra Libyen fra patienter sendt til Danmark for at modtage medicinsk assistance for skader pådraget under konflikten i Libyen (**Textbox 11**). Der er ikke anmeldepligt af CPE i Danmark, og der kan muligvis have været flere cases i 2011 end dem, der er beskrevet i Textbox 11.

Resistensforekomsten i ***Pseudomonas aeruginosa* isolater fra blod** var lav for alle de testede antibiotika.
I 2011 var resistensforekomsten for penicillin og erythromycin stadig lav blandt ***Streptococcus pneumoniae*** og **gruppe A, B, C** og **G streptokokker**.

Forekomsten af ampicillinresistens i ***Enterococcus faecium*** isolater fra blod var 93 % i 2011, dette var det samme niveau som i 2010. Resistensforekomsten for vancomycin var 1,3 % i *E. faecium* mens ingen ***Enterococcus faecalis*** blod-isolater var vancomycinresistente. Kun én KMA testede enterokokker fra blod-infektioner for høj-niveau gentamicinresistens (HLGR). Her var 31 % af de testede *E. faecalis* isolater og 74 % af de testede *E. faecium* isolater HLGR.

I 2011 blev der indrapporteret 1.525 tilfælde af ***Staphylococcus aureus*** bakteriæmi svarende til en incidens på 24,6 tilfælde per 100.000 indbyggere. Antallet af methicillinresistente *S. aureus* (MRSA) var 21 (1,4 %), hvilket er på samme niveau som tidligere år og blandt de laveste incidenser observeret i Europa. Den højeste resistensforekomst udover penicillinresistens var resistens for fusidinsyre (13,3 %), erythromycin (6,6 %), clindamycin (5,8 %) og norfloxacin (3,6 %). Niveauet af resistens overfor de testede antibiotika var det samme som i 2010.

Antallet af nye MRSA tilfælde (både koloniserede og inficerede personer) steg i 2011 til 1292 sammenlignet med 1097 i 2010. Den stigning, der blev observeret i 2010, fortsatte således i 2011. Stigningen blev primært set i tilfælde kategoriseret som samfundserhvervede, 578 i 2010 vs. 751 i 2011. Andelen af patienter med infektion var lavere i 2011 end i 2010 (53 % vs. 59 %). Antallet af hospitalserhvervede tilfælde var fortsat lavt og udgjorde kun 5 % af det totale antal MRSA tilfælde i 2011.

Antallet af MRSA tilhørende det klonale kompleks CC398, som er associeret med svin, steg fra 111 i 2010 til 164 i 2011. I 24 af disse tilfælde var der ikke rapporteret nogen kendt kontakt til svin eller personer med kontakt til svin. Tolv af disse tilfælde var kategoriseret som samfundserhvervede uden kendt eksponering, men størstedelen af tilfældene boede i områder med rapporteret CC398 i mennesker og/eller svin. Den mest almindelige spa type relateret til CC398 var type t034 (n = 130), hvoraf 49 af t034-tilfældene havde en egentlig MRSA infektion. MRSA isolater med den nye *mecA* homolog *mecC* blev fundet i 37 tilfælde (9 i 2009, 21 i 2010).

Hel-genom sekventering af 90 CC398 isolater viste en fylogenetisk adskillelse mellem isolater fra svin, kalkuner og mennesker. Fylogenien understøtter, at CC398 i svin er efterkommere af et menneske-til-dyr værts-skifte. Efter at være blevet introduceret i svin undergik CC398 en række ændringer, der bl.a. indebar tab af flere gener af betydning for den medfødte immunitet i mennesker (f.eks. *scn*) og erhvervelse af tetracyklin og methicillinresistens (MRSA)-gener. Tabet af flere funktioner associeret med human adaptation kan forklare, hvorfor MRSA CC398 har en nedsat evne til at kolonisere og smitte mennesker. På den anden side viser resultaterne, at MRSA CC398 er i stand til at generhverve de samme gener, hvilket kan styrke dens evne til at kolonisere og inficere mennesker **(Textbox 12).**

Forekomsten af MRSA i danske svinebesætninger (16 %) var på samme niveau som i 2010, mens forekomsten i svin ved slagtning var steget markant (44 %) i forhold til 2009 (13 %). Dette kunne indikere en højere forekomst i de positive besætninger end tidligere, hvilket kan føre til øget transmission mellem svinene under transport og før slagtning. Dette er yderligere understøttet af en øget trend i forekomsten i dansk svinekød, så muligvis er slagtekæden mere forurenet end tidligere år. I kød var MRSA forekomsten signifikant højere i importeret kyllingekød sammenlignet med 2010, men indtil videre regnes kød ikke for en kilde til human infektion. MRSA blev ikke fundet i kvæg ved slagtning. Svin anses derfor stadig for det vigtigste reservoir for MRSA CC398.

I 2011 steg antallet af nye MRSA tilfælde til 1292. Stigningen sås primært i tilfælde kategoriseret som samfundserhvervede. Antallet af hospitalserhvervede tilfælde var fortsat lavt og udgjorde kun 5 % af det totale antal MRSA tilfælde i 2011. Antallet af MRSA af typen CC398, som er associeret med svin, steg til 164 i 2011. Svin er stadig det vigtigste reservoir for MRSA CC398 og forekomsten i de positive besætninger er muligvis steget.

Fra oktober 2009 til oktober 2011 steg prævalensen af ESBL-producerende *E. coli* signifikant i urinprøver fra både praksis og hospital.

Resistensforekomsten for 3. generations cefalosporiner og ciprofloxacin i *K. pneumoniae* fra urinvejs- og blodinfektioner fra hospitalspatienter var signifikant højere i den østlige del af Danmark (Sjælland) end i den vestlige del (Fyn og Jylland).

I perioden 2008–2011 blev der påvist 15 tilfælde med carbapenemase producerende enterobakterier i Danmark, hvoraf de fleste var importerede. Ti af disse tilfælde blev påvist i 2011.

2.2 Summary

Resistance Monitoring and Research Programme, DANMAP, has monitored antimicrobial resistance and consumption of antimicrobial agents in food animals and in humans.

Antimicrobial consumption in animals

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

In 2011, the total veterinary consumption of antimicrobial agents amounted to 107.9 tonnes. This represents a 15% decrease compared to 2010, mainly attributed to a decreased consumption in pigs. The antimicrobial consumption in pigs, cattle, fur animals, aquaculture, and poultry accounted for 77%, 14%, 4%, 2% and 0.7% of the total veterinary consumption, respectively.

Pigs: The total consumption of veterinary antimicrobial agents in Danish pig production decreased by 19 tonnes from 2010 to 2011, corresponding to a 30% decrease in number of defined doses per pig produced. This continued the trend observed since measures to reduce consumption were announced by the veterinary authorities on July 1st 2010 (the 'yellow card' initiative). The 'yellow card' control of imposes preventive measures in the pig herds with highest consumption per pig. In 2011, the decrease in number of defined doses consumed could mainly be attributed to a reduction in the use of the main classes of pleuromutilins (60%), tetracyclines (27%) and macrolides (26%) prescribed for gastrointestinal disease. The use of 3rd and 4th generation cephalosporins was almost zero in the pig production, due to a voluntary ban on cephalosporins enforced by the Danish pig industry from July 2010.

Cattle: Overall, the antimicrobial consumption in cattle was similar to 2010. In 2011, approximately 14.7 tonnes of antimicrobial agents were prescribed for cattle. Estimated in number of defined doses per animal, the use of beta-lactamase sensitive penicillins accounted for more than half (59%) of the consumption in dairy cattle and was mainly prescribed to treat mastitis. In general, the consumption measured by biomass is low in cattle, with calves accounting for the highest consumption per biomass (at the level of turkeys and half the level of pigs). In calves, the main antimicrobials used were tetracyclines, followed by macrolides. The use of amfenicols for treatment af respiratory disease in calves has increased significantly over the last decade. In contrast, use of 3rd and 4th generation cephalosporins has decreased since 2008 both for systemic (34%) and intramammary treatments (68%) when measured in kg active compound, and the use of fluoroquinolones has been close to zero since 2003.

Poultry: The consumption of antimicrobial agents in poultry decreased by 8% to approximately 810 kg in 2011 compared to 2010, but was higher than the levels observed during 2001–2008. The decrease in the total consumption in poultry can be explained by reduced consumption in turkeys, layers and parent flocks in the broiler production. In contrast, the consumption in broilers continued to increase in 2011. The use of fluoroquinolones has been low since 2006 and they were not used for poultry in 2011. Cephalosporins have not been used in Danish poultry production for at least a decade.

Aquaculture: from 2010 to 2011 the antimicrobial consumption in aquaculture decreased by 11% to 2,700 kg. Measured in defined doses, the major class of antimicrobial was sulfonamide/trimethoprim, constituting 60% of the consumption in aquaculture. The consumption of quinolones (oxolinic acid) and florfenicol comprised 30% and 10%, respectively. Assuming an unchanged production volume, the consumption in salt water fish decreased by 50% (to 4.5 ADDkg/kg fish produced), while the consumption in fresh water fish increased by 12% in 2011 (to 2.6 ADDkg/kg fish produced).

Companion animals: The available information on antimicrobial consumption in companion animals is not as detailed as for production animals. The total consumption in companion animals was estimated to 3 tonnes with pet animals accounting for approximately 2 tonnes. For oral treatment, the consumption in daily doses increased by 8% from 2010 to 2011, continuing the trend since 2005. This trend is mainly caused by an increase in the use of combination penicillins (amoxycillin with clavulanic acid), which is combination is the drug of choice in pet animals. Measured in kg active compound, most (91%) of the combination penicillins and approximately half (51%) of the fluoroquinolones used for animals in Denmark during 2011 was used for pets. The consumption of 3rd and 4th generation cephalosporins in pet animals was stable and accounted for 5% (approximately 3 kg) of the total veterinary consumption.

Use of 3rd and 4th generation cephalosporins in horse practice was highly variable between veterinary practitioners, ranging from 0% to 30% of the total practice prescription of antimicrobials (**Textbox 1**).

The extended use of antimicrobial agents critical for treatment of human infections and the use of broad-spectrum antibiotics for pets and horse, as well as the large variation in antimicrobial use in different horse practices can reflect inadequate guidelines for the use of antimicrobial agents in pets and horses.

The total consumption in pigs has decreased significantly due to the yellow card initiative and the voluntary ban of use of cephalosporins for pigs. Use of antimicrobial agents in pet animals has increased since 2005, mainly due to use of amoxicillin with clavulanic acid.

Antimicrobial consumption in humans

In humans, the Danish Medicines Agency has monitored the use of prescription medicines at the level of the individual patient since the early 1990s.

Total consumption: The overall consumption of antimicrobial agents for systemic use (primary health care and hospital care) remained at the same level as in 2010 (18.84 DIDs in 2010 compared to 18.90 DIDs in 2011), although still following the trend towards a general increase. Primary health care contributed with 90% of the overall consumption. The level of consumption in primary health care in 2011 was the highest observed since 1995. Since 2002, the total consumption of antimicrobial agents in Denmark has increased by 4.13 DID (28%).

Primary health care: In primary health care, the consumption of antimicrobial agents for systemic use was 17.06 DID, compared to 16.93 DID in 2010. Beta-lactamase sensitive penicillins still represented the largest therapeutic group of antimicrobial agents consumed (31%), and penicillins overall accounted for 63% of all antimicrobial agents consumed. Broad-spectrum agents represented 6.58 DID (39%); 0.1 DID (2%) higher than 2010. From 2010 to 2011, the consumption of combination penicillins increased by 31%. Additionally, smaller upward changes were observed for beta-lactamase sensitive penicillins (1.1%) and macrolides (1.2%).

There were several possible explanations for the high level of consumption in 2011: (1) an increased number of patients treated, (2) a *Mycoplasma pneumoniae* outbreak in October–December 2011 correlating with increased consumption of beta-lactamase sensitive penicillins (treatment for lower respiratory tract infection) and macrolides (for confirmed or suspected *M. pneumoniae* infection), and (3) a continuous response to changes in treatment guidelines (particularly for 'combination penicillins'). Disease-specific changes (causing an increased number of treated patients) are difficult to assess due to lack of specific codes of indication.

During the past decade, antimicrobial consumption in primary health care has increased by 29%, from 13.26 DID in 2002 to 17.06 DID in 2011. In 2011, broad-spectrum agents accounted for 39% (6.58 DID) of the total antimicrobial consumption in primary health care which, compared to 2002 (3.85 DID, 29%), represented an increase of 78%. This increase is most likely partially driven by rises in DDDs per treated patient and number of DDDs per prescribed package. The latter may reflect changed guidelines advising shorter treatment regimens at higher dosages. Possible other underlying reasons for the increased consumption are not possible to assess with certainty because of the lack of indication codes. However, following the decision to implement mandatory codes of indication for all prescribed antimicrobial agents in primary care in Denmark from 2012, improved data will become available.

An analysis of the demographic factors associated with the increased antimicrobial consumption from 2001 to 2010 showed that antimicrobial-type and substance specific increases were driven primarily by people older than 65 and adolescents. Observed age-related increases in tetracyclines and pivmecillinam most likely reflect changes in the treatment of acne and urinary tract infections, respectively. Population-wide increases in the consumption of roxithromycin, irrespective of age, may partly be explained by the occurrence of several large outbreaks of *M. pneumoniae.* Generally, evidence suggests that increases observed from 2001 to 2010 are a combination of disease-specific factors and not least an inclination of general practitioners to prescribe more antimicrobial agents per visit and in larger doses (**Textbox 3)**.

Hospital care: For all hospital types (i.e. rehabilitation centres, hospices, private-, psychiatric-, specialised- and somatic hospitals), the total consumption of antimicrobial agents decreased from 1.91 DID in 2010 to 1.84 DID in 2011 (a decrease of 3.7%). Since 2002, the total hospital consumption has increased by 0.38 DID (26%). In 2011, broad-spectrum agents comprised 1.22 DID of the total consumption, compared to 0.75 DID in 2002 (an increase of 67%).

Somatic hospitals: In somatic hospitals, the consumption of antimicrobial agents (J01) expressed in DDDs per 100 occupied bed-days (DBD) increased by 3.12 DBD (3.6%) from 87.72 DBD in 2010 to 90.84 DBD in 2011. When expressed as the number of DDDs per 100 admissions (DAD), it remained relatively constant (284.89 DAD in 2010 compared to 282.53 DAD in 2011, a change of 0.8%). These figures reflect a 0.08 DDD decrease (4.2%) from 2010 to 2011, combined with less occupied bed-days and more hospitals admissions in 2011 than 2010.

When expressed as DBDs, four therapeutic groups accounted for most of the increase: combinations of sulfonamides and trimethoprim (1.07 DBD, 35%), 'combination penicillins' (1.38 DBD, 19%), fluoroquinolones (0.26 DBD, 6.7%) and macrolides (0.17 DBD, 4.7%). From 2010 to 2011, consumption decreased for beta-lactamase resistant penicillins (0.41 DBD, 5.3%), while smaller decreases were observed for penicillins with extended spectrum (0.20 DBD, 1.4%) and beta-lactamase sensitive penicillins (0.17 DBD, 1.8%).
In 2011, cephalosporins accounted for 19% of the total consumption of antimicrobial agents in somatic hospitals, followed by penicillins with extended spectrum (15%), fluoroquinolones (12%) and beta-lactamase sensitive penicillins (10%).

Over the past decade, the consumption of antimicrobial agents in somatic hospitals has increased from 51.73 DBD in 2002 to 90.84 DBD in 2011 (76%) or from 258.46 DAD in 2002 to 282.53 DAD in 2011 (9.3%), the increase depending on the choice of denominator. In addition to highlighting increases in antimicrobial consumption, these numbers also reflect changing hospitalization patterns by showing increased numbers of hospital admissions and a decreasing number of bed-days since 2002.

In 2011, the overall consumption of antimicrobial agents for systemic use (primary health care and hospital care) remained at the same high level as in 2010. Antimicrobial consumption in the primary sector represented 90% of the total consumption and the hospital sector accounted for the remaining 10%. From 2002 to 2011, the total consumption of antimicrobial agents in Denmark increased by 28%. In the primary sector, the consumption of broad spectrum antimicrobial agents accounted for 39% of the total antimicrobial consumption in 2011, representing a 78% increase since 2002.

Resistance in zoonotic bacteria

In 2011, data on antimicrobial resistance in zoonotic bacteria were available for *Salmonella* Typhimurium and *Campylobacter*. Due to insufficient number of isolates from other sources, only *S.* Typhimurium isolates from Danish pigs and Danish pork and humans were included in the report.

Among ***Salmonella* Typhimurium** isolates from Danish pigs, a significant increase in resistance to ampicillin, neomycin, sulfonamide and tetracycline was observed from 2010 to 2011. In contrast to previous years, the levels of resistance were generally higher in isolates from Danish pork than from Danish pigs. In 2011, no isolates of *S.* Typhimurium from pigs were found resistant to cephalosporins (ceftiofur and cefotaxime) or quinolones (ciprofloxacin or nalidixic acid).

Among isolates from human cases, the level of resistance to fluoroquinolones (ciprofloxacin) and cephalosporins was higher in cases associated with travel (16% and 12%, respectively) compared to resistance levels in cases acquired domestically (2% and 1%, respectively).

In 2011, the occurrence of resistance the *Salmonella* isolates from pigs, Danish pork and humans were markedly influenced by the clonal spread of the monophasic variants of *S.* Typhimurium which are resistant to ampicillin (A), streptomycin (S), sulfonamide (Su) and tetracycline (T), often referred to as the ASSuT-profile. This tendency is observed throughout the farm to fork pathway, where the proportion of multi-resistance increased dramatically, while the number of fully sensitive isolates decreased.

Among the ***S.* Enteritidis** isolates from human infections in 2011, 18% of isolates from domestic sporadic human cases were resistant to quinolones, a 10% increase compared with 2010. The level of resistance among the domestic sporadic isolates thereby approaches the level of resistance in isolates from cases with a known history of travel (24%) or unknown origin (27%).

No significant changes in resistance were observed for ***Campylobacter jejuni*** isolates from Danish broilers and cattle or for *Campylobacter coli* from Danish pigs from 2010 to 2011. However, the levels of tetracycline resistance have increased since 2005, and this trend has been particularly clear in *C. coli* isolates in pigs from 2008 to 2011. During this period, tetracyclines have been the most, or second most frequently used antimicrobial agents in pigs.

As in previous years, the level of fluoroquinolones (ciprofloxacin) resistance in *C. jejuni* was significantly higher among isolates from imported broiler meat (57%) compared with isolates from Danish broiler meat (11%), and the difference between domestic and imported broiler meat is increasing.

From 2010 to 2011, the level of tetracycline and fluoroquinolone resistance increased in *C. jejuni* isolates from cases of human campylobacteriosis, from both domestically acquired cases and from cases associated with travel. This is an indication of the impact of resistance in imported food stuffs on human disease. Furthermore, as has been observed in previous years, *C. jejuni* isolates from cases associated with a history of travel continue to have a significantly higher level of fluoroquinolone resistance (84%) compared to domestically acquired cases (33%).

Use of tetracycline in Danish pig production has decreased over the last two years, while the occurrence of tetracycline resistance continues to increase significantly. This indicates that the increase is, at least in part, explained by the spread of resistant Salmonella clones, especially the monophasic *S.* Typhimurium like clones, which are the predominant clones in isolates from Danish pork.

While the level of resistance in *S.* Typhimurium isolated from Danish pigs continued the gradual increase observed over the last years, the resistance level in *S.* Typhimurium from Danish pork increased much more dramatically from 2010 to 2011.

The level of resistance in *Campylobacter jejuni* from Danish broiler meat was significantly lower than in isolates from imported meat. A similar pattern was observed among the human *C. jejuni* cases, where cases associated with a history of travel had significantly higher levels of resistance compared to domestically acquired cases.

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.

For ***Enterococcus faecium*** isolates from pigs there was a significant increase in antimicrobial resistance to beta-lactams (penicillin and ampicillin) from 2010 to 2011. Furthermore, resistance to streptomycin has gradually increased, even though there have been no indications of increased use. This increase could be linked to the occurrence of tetracycline resistance, which may lead to co-selection for streptomycin resistance. In *E. faecium* from broilers resistance to avoparcin and quinupristin/dalfopristin remains at low levels, more than a decade after the use of these growth promoters was discontinued.

Among ***Enterococcus faecalis*** isolates from pigs, a significant increase in resistance in antimicrobial resistance to the aminoglycosides kanamycin and gentamicin was observed, together with an increase in resistance to chloramphenicol, erythromycin, kanamycin and gentamicin. In these isolates, co-resistance to tetracycline and erythromycin was seen for chloramphenicol, kanamycin and gentamicin was frequent.

For both *E. faecium* and *E. faecalis* isolates, resistance to erythromycin, kanamycin and streptomycin was significantly higher in imported broiler meat compared to Danish broiler meat. Similarly, significantly higher levels of tetracycline resistance were found in *E. faecalis* isolates from imported pork, compared to isolates from Danish pork.

Resistance to vancomycin and quinupristin/dalfopristin persists at low levels among *E. faecium* isolated from pigs even though avoparcin and virginiamycin have not been used for more than ten years.

No significant changes in resistance were observed for **indicator *E. coli*** isolates from Danish broilers and cattle from 2010 to 2011, where as the level of streptomycin resistance decreased significantly in isolates from pigs. In isolates from pigs, resistance to streptomycin very often occur in combination with tetracycline and/or sulfonamide (99% in 2010 and 75% in 2011).

In general, the highest resistance levels were found in indicator *E. coli* isolates from pigs, except for fluoroquinolone (ciprofloxacin) resistance which was higher in isolates from broilers. The fluoroquinolone resistance in isolates from pigs and cattle has remained at a low level over the past decade, while the level in isolates from broilers varied between 7% and 13%. In this context, it is noted that fluoroquinolones have not been used in production animals in Denmark to any significant extent for a decade, except for the production of broilers where it was widely used until 2007.

In 2011, *E. coli* isolates from imported broiler meat, the resistance levels were significantly higher compared to isolates from Danish broiler meat (for 11 of the 16 tested antimicrobial agents), including cephalosporin (ceftiofur) and fluoroquinolone resistance. Correspondingly, resistance to chloramphenicol and quinolones (ciprofloxacin and nalidixic acid) was significantly higher in isolates from imported pork compared with those from Danish pork. As previous years, the occurrence of resistance in indicator *E. coli* from imported and Danish beef was low.

ESBL-producing bacteria are resistant to extended-spectrum cephalosporins, which are often essential for treatment of infections in humans. Consequently, even a low occurrence of these bacteria can potentially be hazardous. Therefore, the occurrence of ESBL-producing *E. coli* in pig at the farms, cattle and pigs at slaughter and in meat at retail was investigated using selective enrichment.

The voluntary ban of cephalosporin usage in the Danish pig production has reduced the level of ESBL-producing *E. coli* in pigs. In contrast, the prevalence of ESBL-producing *E. coli* in cattle remained unchanged compared to 2010. Probably because 3rd and 4th generation cephalosporins are still used for intramammary and systemic treatment in cattle, even though the usage is decreasing.

The occurrence of ESBL-producing *E. coli* in Danish Broiler meat increased from 8.6% in 2010 to 44% in 2011; a level similar to what is observed in the imported broiler meat (48%), even though cephalosporins have not been used in the Danish broiler production for a decade. A possible explanation is the increased use of extended spectrum penicillins in the Danish broiler production, which may co-select for ceftriaxone resistant *E. coli* being introduced via imported breeding animals.

The presence of ESBL-genes differed depending on animal reservoir. CTX-M-1 was the most common ESBL-gene in pigs and cattle, but also CTX-M-14 and CTX-M-15 was found. All are ESBL-genes that are often found in ESBL-producing *E. coli* causing human infections. Broiler meat, both Danish and imported, seemed to be the most important meat sources and CMY-2 was the most common type in broiler meat. CMY-2 was also found in ESBL-producing *E. coli* causing human infections.

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.

Among ***Escherichia coli* blood isolates**, resistance to 3rd generation cephalosporins was 8% in 2011, the same level as reported in 2010, but above the 2010 level in the other Nordic countries. In 2011, ciprofloxacin resistance was 14% (min. 12%, max. 31% at the individual DCM), the same level as in 2010. Over the last decade, resistance to cefuroxime, ciprofloxacin and gentamicin has increased significantly. Resistance to 3rd generation cephalosporins has only been reported since 2008; in this period the resistance has increased. In 2011, carbapenem (meropenem) resistance was not observed in *E. coli* blood isolates.

In ***E. coli* urine isolates obtained from hospitals**, resistance to 3rd generation cephalosporins was 5% in 2011, the same level as in 2010. A significant increase in fluoroquinolone resistance (in 2011: ciprofloxacin 13%, nalidixic acid 17%) was observed from 2010 to 2011. Over the last decade, an increase in fluoroquinolone resistance has been observed (in 2002: ciprofloxacin 2%, nalidixic acid 6%). Aminoglycoside (gentamicin) resistance increased significantly from 3.7% in 2010 to 4.4% in 2011.

In ***E. coli* urine isolates obtained from primary health care**, resistance to 3rd generation cephalosporins was 3% in 2011, the same level as in 2010. Sulfonamide resistance decreased significantly from 37% in 2010 to 35% in 2011.

Carbapenem (meropenem) resistance was observed in *E. coli* urine isolates from both hospitals (n = 5) and primary health care (n = 3). The occurrence of carbapenem resistance is not mandatory reportable and no calculation of the frequency of carbapenem resistance could be made for *E. coli*.

In ***Klebsiella pneumoniae* blood isolates**, resistance to 3rd generation cephalosporin (10%), fluoroquinolones (ciprofloxacin 12%, nalidixic acid 18%) and aminoglycoside (gentamicin 6%) was the same level as reported in 2010. The level was above the level reported by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2010. In 2011, carbapenem (meropenem) resistance was observed in one *K. pneumoniae* blood isolate.

In ***K. pneumoniae* urine isolates**, 3rd generation cephalosporin resistance decreased significantly in isolates obtained from hospitals (from 12% in 2010 to 10% in 2011) and from primary health care (from 7% in 2010 to 5% in 2011). Cefuroxime resistance decreased significantly from 13% in 2010 to 11% in 2011 in isolates obtained from hospitals. A significant decrease in fluoroquinolone resistance was observed from 2010 to 2011 among urine isolates from hospitals (ciprofloxacin from 14% in 2010 to 11% in 2011) and primary health care (ciprofloxacin from 12% in 2010 to 9% in 2011, nalidixic acid from 20% in 2010 to 14% in 2011). Also, mecillinam resistance decreased significantly from 2010 to 2011 in isolates from hospitals (in 2011: 12%) and primary health care (in 2011: 12%). In *K. pneumoniae* isolates obtained from hospitals, sulfonamide resistance increased significantly from 29% in 2010 to 33% in 2011.

Carbapenem (meropenem) resistance was observed in *K. pneumoniae* urine isolates from both hospitals (n = 5) and primary health care (n = 1). One of the carbapenem resistant isolates was KPC-2 positive (**Textbox 11**). It is not mandatory to report the occurrence of carbapenem resistance and calculation of the frequency of carbapenem resistance could not be made for *K. pneumoniae*.

An increased frequency of gentamicin, ciprofloxacin, 3rd generation cephalosporin resistance and multi-resistance (gentamicin, ciprofloxacin, 3rd generation cephalosporins) in *E. coli* blood isolates (2008–2010) has been found to parallel an increased consumption of broad spectrum antimicrobial agents (2007–2010) (**Textbox 8**).

In October 2011, 12 of the 13 DCM screened all *E. coli* and *K. pneumoniae* urine and blood isolates for ESBL-production. The ESBL-prevalence obtained in 2011 was compared to data from similar studies performed in 2007 and 2009. From October 2009 to October 2011, the prevalence of ESBL-producing *E. coli* increased significantly in urine samples from both community (from 2.3% in 2009 to 3.2% in 2011) and hospitals (from 3.8% in 2009 to 4.7% in 2011). As in previous years, CTX-M-15 dominated among ESBL-producing *E. coli* and *K. pneumoniae* isolates from urinary tract and bloodstream infections from patients, while CTX-M-1, CTX-M-14 and CMY-2 were present in a limited number of the isolates only (**Textbox 9**).

In the Eastern part of Denmark (Zealand), the occurrence of resistance to 3rd generation cephalosporin, ciprofloxacin and gentamicin in K. pneumoniae from urinary tract and bloodstream infections from hospitalised patients was significantly higher than in the western part of the country (Funen and Jutland). The same tendency was observed in *K. pneumoniae* urine isolates from primary health care patients, where resistance to 3rd generation cephalosporins and fluoroquinolones (ciprofloxacin and nalidixic acid) was significantly higher in isolates from the eastern part of Denmark (Zealand) than the western part (Funen and Jutland) (**Textbox 10**).

In the period 2008–2011, 15 carbapenemase producing enterobacteria (CPE) were detected in Denmark. These included 2 VIM-, 3 KPC-, 3 NDM-1- and 7 OXA-48-producing isolates. Of the 15 CPE cases observed, 10 were detected in 2011 and most of these were recovered from Libyan patients sent to Denmark to receive medical

assistance for injuries during the Libyan conflict (**Textbox 11**). CPE are not mandatory reportable in Denmark, and it is possible that more cases could have been present than those described in Textbox 11.

Antimicrobial resistance in ***Pseudomonas aeruginosa* blood isolates** was low for all the tested antimicrobial agents.

Resistance to penicillins and erythromycin in ***Streptococcus pneumoniae*** and in **Group A, B, C and G streptococci** remained low in 2011.

In 2011, resistance to ampicillin was 93% in ***Enterococcus faecium* isolates from blood**, the same level as in 2010. Vancomycin resistance was 1.3% in *E. faecium*, whereas none of *Enterococcus faecalis* blood isolates were vancomycin resistant. Only one DCM tested all enterococci from bloodstream infections for High-level gentamicin resistance (HLGR). Here, 31% of the ***E. faecalis*** isolates tested and 74% of *E. faecium* isolates were HLGR.

In 2011, 1,525 cases of ***Staphylococcus aureus*** bacteraemia were reported, corresponding to 24.6 cases per 100,000 citizens. The number of methicillin resistant S. aureus (MRSA) was 21 (1.4%), 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 (13.3%), erythromycin (6.6%), clindamycin (5.8%) and norfloxacin (3.6%). Susceptibility to the tested antimicrobial agents was at the same level as in 2010.

The number of new cases of MRSA (both infected and colonized persons) increased in 2011 to 1,292 compared to 1,097 in 2010. Thus the increase observed in 2010 continued in 2011. The increase was primarily seen in cases categorised as community-acquired (CA), 578 in 2010 vs. 751 in 2011. The proportion of cases presenting with infection was lower in 2011 than in 2010 (53% vs. 59%). The number of hospital-acquired (HA) cases continued to be low and constituted only 5% of the total number of MRSA cases in 2011.

The number of MRSA belonging to clonal complex CC398, which is associated with pigs, increased from 111 in 2010 to 164 in 2011. In 24 of these cases, no known contact to pigs or people with contact to pigs was reported. Twelve of these cases were categorised as CA with no known exposure but the majority of the cases lived in areas with reported CC398 in humans and/or pigs. The most frequent spa type related to CC398 was type t034 (n = 130). Forty-nine t034 cases represented infections. MRSA isolates carrying the new *mecA* homologue *mecC* were demonstrated in 37 cases (9 in 2009, 21 in 2010).

Whole genome sequencing of 90 *S. aureus* isolates belonging to the pig related CC398 clone revealed a phylogenetic distinction between isolates obtained from pigs, turkeys and humans. The phylogeny indicates that CC398 in pigs are descendants of a human-to-animal host jump. After being introduced in pigs, CC398 underwent rapid diversification, accompanied by loss of several genes that encode modulators of human innate immunity (e.g., scn) and acquired the tetracycline and methicillin resistance (MRSA) genes. The loss of several functions associated with human adaptation may explain why MRSA CC398 has lost part of its colonization and transmission ability in humans. On the other hand, the findings show that MRSA CC398 is able to reacquire the same genes, which may enhance its ability to colonize and infect human (**Textbox 12**).

The prevalence of MRSA in Danish pig farms (16%) were at the same level as in 2010, while the level in pigs at slaughter increased dramatically from 2010 to 2011. This may reflect higher within-herd prevalence, increasing the transmission between pigs during transport and before slaughter. Also, the prevalence in Danish pork increased, suggesting that the pork production chain is more highly contaminated compared to previous years. In meat, MRSA was found in a significantly higher prevalence compared to 2010 in imported broiler meat, but so far meat is not considered a source for human infection. The relatively frequent occurrence of MRSA in meat combined with very few cases in humans in urban areas indicates that there is very little risk of MRSA being food borne hazard.

In 2011, the total number of new MRSA cases increased to 1292. This increase was primarily apparent in cases defined as having community-acquired infection. The number of hospital-acquired cases remained low and accounted for only 5% of the total number of MRSA cases in 2011. The number of MRSA type CC398, which is associated with pigs, increased to 164 in 2011. Pigs are still the most important reservoir for MRSA CC398 and the occurrence in positive herds have possibly increased.

From October 2009 to October 2011, the prevalence of ESBL-producing *E. coli* increased significantly in urine samples, from both primary health care and the hospital sector.

The prevalence of resistance to 3rd generation cephalosporins and ciprofloxacin in *K. pneumoniae* from urinary tract and bloodstream infections in hospital patients was significantly higher in the eastern part of Denmark (Zealand) compared to the western part (Funen and Jutland).

During 2008–2010, a total of 15 cases of carbapenemase-producing enterobacteriaceae were detected of which the majority was imported. Ten of these cases were detected in 2011.


General information
DATA

3. General information

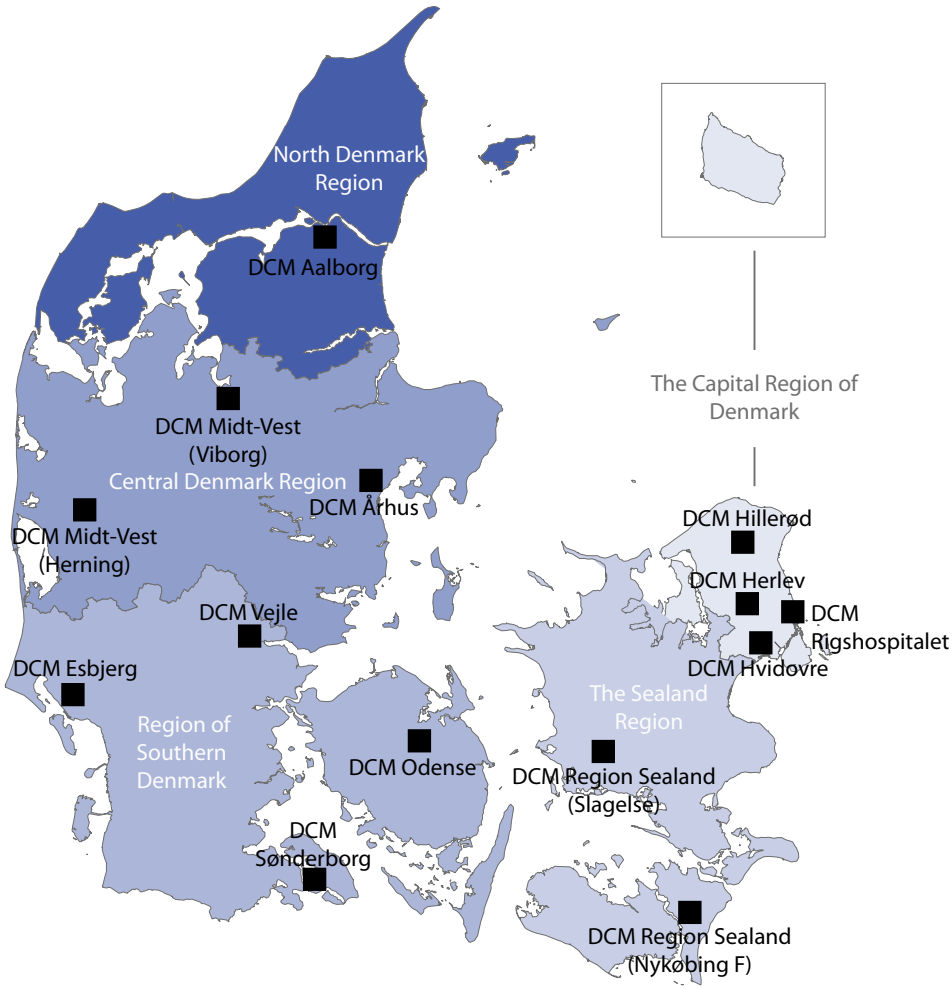
The distribution of the Danish population in which antimicrobial agents were used in 2011 is displayed in Figure 3.1, together with the five health care regions and the 14 Departments of Clinical Microbiology (DCMs).

In 2011, the number of slaughtered cattle increased by 6% compared to 2010 (Table 3.1). The number of pigs produced (slaughtered or exported) increased by 3%, mainly due to an 8% increase in production of weaning pigs (around 30 kg). Hence, the production in kg pork produced (including export) only increased, by 2% (Table 4.1). The export of fattening pigs (15–50 kg) has increased almost fivefold since 2004 and at export; these pigs have received a large amount of antimicrobial agents relative to their bodyweight.

From 2010 to 2011, the production of turkeys and turkey meat decreased by 19% and 33%, respectively. 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 availeble in Denmark are listed as imported.

The amount of meat available for consumption in Denmark during 2007–2011 is 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. Overall, 2.7 mill tonnes pork, 2.0 mill tonnes beef and 1.3 mill tonnes broiler meat was available for consumption in Denmark during 2011. Approximately 64% of the pork and broiler meat, and 50% of the beef was of Danish origin. Compared to 2010, the volume of imported pork and broiler meat increased by 7% and 4%, respectively, where as the volume of imported beef and turkey meat decreased by 2% and 16% respectively.

Figure 3.1 The five health care regions and 14 Departments of Clinical Microbiology (DCM) of Denmark



DANMAP 2011

North Denmark Region

No. of inhabitants	580,293
No. of inhabitants/km²	73
No. of inhabitants/GP	1692

Central Denmark Region

No. of inhabitants	1,265,601
No. of inhabitants/km²	96
No. of inhabitants/GP	1519

The Capital Region of Denmark

No. of inhabitants	1,713,624
No. of inhabitants/km²	669
No. of inhabitants/GP	1568

The Sealand Region

No. of inhabitants	818,321
No. of inhabitants/km²	113
No. of inhabitants/GP	1583

Region of Southern Denmark

No. of inhabitants	1,201,365
No. of inhabitants/km²	98
No. of inhabitants/GP	1485

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

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

DANMAP 2011

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish[a]	
												Fresh water	Salt water
	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. Kg milk	1,000 heads[b]	Export 1,000 heads[c]	mill. kg	mill. kg	mill. kg
2002	136350	190	1073	12.8	668	169	611	4455	24203	-	1892	32	8
2003	129861	181	777	11.2	625	161	596	4540	24434	-	1898	34	8
2004	130674	181	1086	19.6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	180	1237	17.4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	163	785	11.3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	178	1009	14.4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	186	1068	12.3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	181	1175	11.1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	199	1184	14.0	519	142	574	4830	28505	7074	1974	28	10
2011	115454	201	960	9.4	551	145	575	4801	29399	7632	2008	-	-
Increase (%)[d]	-2	1	-19	-33	6	2	0	-1	3	8	2	-	-

Source: Statistics Denmark (2012), the Danish Directorate for Fisheries and Danish Agriculture and Food Council. All data include export of live animals for slaughter.
a) The production of farmed fish includes fish transferred from one production facility to another. In 2010, this included 4 tonnes transferred between freshwater facilities (9.4% of the freshwater production), and 2.7 tonnes transferred from freshwater to salt water facilities (18% of the saltwater production)
b) Including export of all age groups (not only for slaughter)
c) Export of 15-50 kg pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside Denmark
d) Increase from 2010 to 2011

Table 3.2. Danish and imported chilled and frozen fresh meat available for consumption (in 100 tonnes)[a], Denmark

DANMAP 2011

Year	Pork		Beef		Broiler meat[b]		Turkey meat
	Danish	Import	Danish	Import	Danish	Import	Import
2007	1847	402	730	803	626	304	84
2008	2158	831	831	814	478	325	83
2009	1868	833	845	888	513	303	70
2010	1851	896	968	1005	555	437	87
2011	1759	961	1004	984	829	453	74
Increase (%)[c]	-5	7	4	-2	49	4	-16

Source: Statistics Denmark (2012)
a) The volumes of Danish meat is estimated as production minus export
b) Natural-marinated broiler meat included
c) Increase from 2010 to 2011

Table 3.3 shows the antimicrobial agents that are registered for treatment of bacterial infections in animals and humans. 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 agents used in non-human sources to treat diseases that may be transmitted to humans, or human diseases caused by bacteria that may acquire resistance genes from non-human sources. 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 the growth promoters.

Table 3.3. Antimicrobial agents marketed [a] for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2011

DANMAP 2011

ATC / ATCvet codes [b]	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
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	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, olaquindox)	
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) Animal growth promoters used before 1999 are listed in parentheses, but not marketed in 2011
b) ATCvet codes starts with a Q
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 antimicrobial consumption in humans has gradually increased since the end of the 1990'ies. In contrast, changes in the antimicrobial use for animals, during the same period, have been more notable. Figure 4.1 shows the total antimicrobial consumption in animals and humans since 1990 and 1997, respectively.

Since the early 1990'ies there has been both political and public focus on the use of antimicrobial agents in the Danish animal production. This resulted in the ban of avoparcin for growth promotion in 1994 and voluntary phasing out of the remaining antimicrobial agents for growth promotion from 1996 to1999. The antimicrobial growth promoters comprised mainly tylosin (macrolide) and other antimicrobial agents acting on mainly the Gram-positive flora.

Use of coccidiostatic agents as feed additives for poultry is legal without prescription. These data are not presented in this report, but are published on the DANMAP web page (web annex, Table A4.5).
Regarding veterinary prescription medicines, a number of interventions affect the present antimicrobial consumption pattern in Denmark [DANMAP 2010].

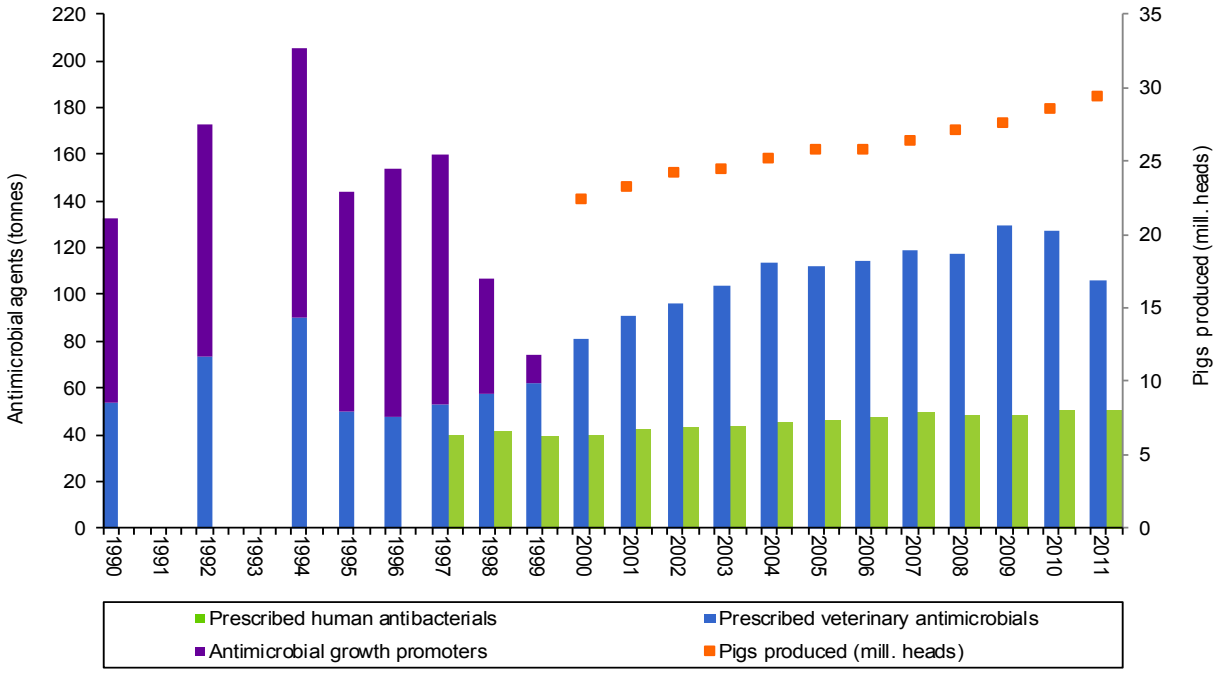
Some of the implemented legislation has clearly influenced the prescription pattern. For example, a steep decrease in antimicrobial consumption from 1994 to 1995 was likely the result of 1) Limitation of veterinarians profit from sales of medicine [Directive (DK) 60/1995], and 2) Enforcement of the so called "cascade rule" [Order (DK) 142/1993], which dramatically 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. Effects of other parts of the legislation may be less obvious, but are important to keep in mind, when interpreting the veterinary prescription patterns.

During the period 2001–2009, the antimicrobial consumption in production animals increased gradually by 36% (Figure 4.1), where the number of pig produced increased by 19% during the same period (Table 3.1).

However, from 2010 to 2011 consumption decreased by 17%, following the introduction of the "yellow card"

Figure 4.1. Consumption of antimicrobial agents and growth promoters in animal production, number of pigs produced and prescribed antibacterials in humans, Denmark

DANMAP 2011



Sources: Human therapeutics: The Danish Medicines Agency. Veterinary consumption: 1990–2000 data based on reports from the pharmaceutical industry of total annual sales; 1990–1994 data from 'Use of antibiotics in the pig production' [Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.)]; 1996–2000 data from Danish Medicines Agency and Danish Plant Directorate; 2001–2011 data from VetStat

legislation, which enforces legal actions on pig farmers with high antimicrobial use per pig [DANMAP 2010]. Furthermore, in July 2010, the pig industry imposed a voluntary ban on use of cephalosporins, due to concerns regarding extended beta-lactamase resistance (ESBL).

Despite an increase of 17% in the total meat production since 1994 (Table 3.1), the overall consumption was 51% lower in 2011 than in 1994, before the aforementioned interventions were implemented.

Official guidelines for the choice of antimicrobial agents in pigs and cattle have been available for veterinarians 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, the National Veterinary Institute). Since 2005, the guidelines have been updated by the Danish Veterinary and Food Administration (DVFA) in collaboration with the National Veterinary Institute, the National Food Institute, the Practicing Veterinarians Organization, University experts, 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].

4.1.1 Data sources

Data on antimicrobial use have been collected in Denmark since 1996, including historical data back to 1990. Until 2001, data were available on product level, based on reports from the pharmaceutical industry of the total annual sales. In addition, sales of antimicrobial growth promoters and coccidiostatic agents approved as feed additives were reported by the feed mills to the Plant Directorate before 2001. Since 2001, the consumption data presented in the DANMAP report have been obtained from the national monitoring programme, VetStat (web annex, Table A4.1).

In Denmark, all therapeutic medicine is prescription-only, and VetStat collects data on all medicines prescribed by veterinarians for use in animals. Data on consumption of antimicrobial feed additives, including coccidiostatic agents (non-prescription) and antimicrobial growth promoters (no longer in use), are also collected by VetStat. The VetStat database contains detailed data on all prescription medicines for animals, including item ID (Nordic Item Number) and amount, date of sale, recipient (farm-ID or practice ID), prescribing veterinarian (ID), species, age group, and disease group (see Section 10.2 for further description).

4.1.2 Methods

The measures of antimicrobial consumption are numerous, each of which has advantages and limitations. Therefore, the chosen measure must depend on the purpose and the available information.

Numerators

The consumption may be measured in grams of active compound or in number of doses. Doses are species specific and defined as "Defined Animal Daily Doses" (ADD), and these can vary importantly, particularly between mammals and poultry. Consequently, grams of active compound are only used as the measure for the overall consumption in DANMAP.

ADDkg is defined as the assumed average maintenance dose per day, for treatment of one kg animal for the main indication in a specified species. Since this value is independent of the weight of the animals treated, ADDkg is used for measuring consumption across age groups. Correspondingly, the ADDx, is the species and/or age-group specific assumed average maintenance dose for a standard body weight of 'x' kg. The standard body weight is the assumed average body weight at treatment; e.g. for weaning pigs (7–30 kg) it is 15 kg. Thus, the antimicrobial consumption for weaning pigs can be measured in ADD15 (for more details, see DANMAP 2009).

Denominators

In DANMAP, the animal production is used as the preferred denominator the description of trends in consumption within species. Animal production is measured either in kg-meat-produced (including export of live animals) or in number of animals produced, including slaughtered and exported animals.

For comparison of selection pressure between species, the population at risk (biomass-year-at risk) is a more suitable denominator. The biomass-year-at risk takes into account both species differences in body-mass and life-span (Figure 4.2). Measuring the population at risk as animal-year-at-risk (or biomass-year-at risk) is similar to the denominator used in human pharmaco epidemiology (inhabitant days, as in DID, see Chapter 5), except for timespan (year vs. 1000 days).

For the evaluation of trends over time, data should be adjusted for changes in production structure, as discussed in DANMAP 2009. For example, important changes have occurred in the pig production, with the 5-fold increase in number of pigs exported around 30 kg bodyweight, involving 26% of pigs produced in 2011. Pigs produced only to 30 kg contribute relatively little to the total production (by weight), while the amount of antimicrobials used for this part of the production (birth to 30 kg, i.e. including sows, piglets and weaning pigs) comprise two thirds of the total antimicrobial consumption for pigs. Thus, the consumption would be underestimated if it was merely divided by number of pigs produced(see also Figure 4.3). Conversely, the consumption would be overestimated if the pigs exported at 30 kg were included in the production by bodyweight produced.

In this report, trends in antimicrobial consumption in pigs are measured using number of pigs produced as the denominator, adjusted for changes in production. The adjustment is based on the assumption that pigs exported at 30 kg, compared to those not exported, on average received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg. This calculation provides a more robust measure of production (see formulas in Section 10.2.).

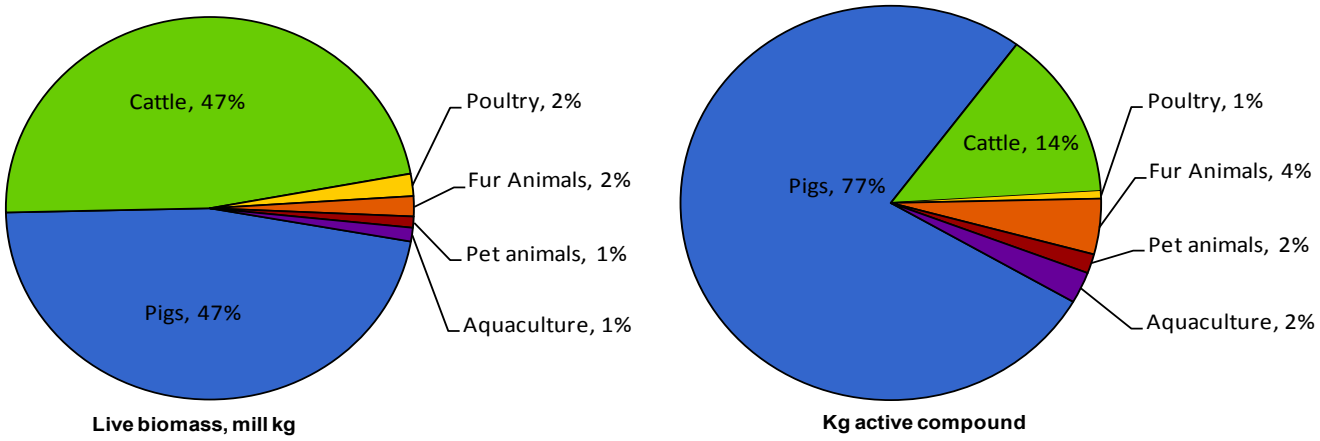
4.2 Total antimicrobial consumption

In 2011, the total veterinary consumption of antimicrobial agents, including agents used for companion animals amounted to 107.9 tonnes (Table 4.1), representing a 15% decrease compared to 2010. The decrease was mainly attributed to a 19% decrease in consumption in pigs. The distribution of total consumption of antimicrobial agents between the three major animal species has not changed importantly from previous years. In 2011, the antimicrobial consumption in pigs, cattle and poultry comprised 77%, 14%, and 0.7% of the total veterinary consumption, respectively (Figure 4.2).

The two major species, cattle and pigs, comprise equal proportions of the standing live biomass. However, the vast majority of cattle biomass is comprised by dairy cows, which have a very low consumption of antimicrobial agents compared to growing animals. Antimicrobial consumption in cattle comprises only a minor part of the total veterinary consumption.

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

DANMAP 2011



Note: The live biomass is estimated from census data (pigs, cattle and pet animals), production (poultry, fur animals, aquaculture). The estimation procedures are described in Section 10.2. For poultry: the figures comprise only the biomass and antimicrobial consumption for the main production types (turkey and broiler production)

Table 4.1. Antimicrobial agents sold (kg active compound)[a] by animal species and age group, Denmark DANMAP 2011

Therapeutic group[a]	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macro	Pleuro	Pen-β-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATCvet groups[c]	QJ01 B	QJ01 G	QJ01 DA	QJ01 MA	QJ01 MB	QJ01 FF	QJ01 FA	QJ01 XX	QJ01 CE	QJ01 CA	QJ01 E	QJ01 AA	QJ01 X	
Pigs, total	*226*	*4409*	*1*	*5*	*0*	*2257*	*9894*	*7531*	*16158*	*7316*	*7135*	*26129*	*382*	*81443*
- Sows and piglets	181	1942	<1	5	0	495	502	683	8396	3815	5895	1887	56	23859
- Weaners	23	2195	<1	<1	0	686	6010	2805	1565	2440	1061	14603	324	31713
- Finishers	22	270	<1	<1	0	1076	3378	4040	6196	1058	174	9635	3	25853
- Age not given	<1	<1	<1	<1	0	<1	3	3	<1	2	5	3	<1	18
Cattle, total [c]	*504*	*755*	*117*	*1*	*0*	*24*	*227*	*4*	*7933*	*1197*	*2092*	*1813*	*10*	*14678*
- Intramammaries	0	19	69	0	0	3	0	0	193	150	6	<1	2	442
- Cows and bulls	24	330	42	<1	0	7	138	<1	6902	689	1042	1300	<1	10475
- Calves<12 months	459	280	2	<1	0	2	52	0	353	185	419	422	7	2181
- Heifers, Steers	20	24	<1	0	0	<1	9	0	158	23	58	53	<1	345
- Age group unknown [d]	1	102	3	1	0	12	27	4	328	150	567	38	<1	1235
Poultry, total[e]	*1*	*1*	*0*	*<1*	*0*	*<1*	*148*	*5*	*50*	*292*	*43*	*227*	*<1*	*769*
- Broilers	0	0	0	0	0	0	27	0	26	254	0	86	0	394
- Breeding and rearing, broilers	0	0	0	0	0	0	0	0	8	10	0	3	0	21
- Layers incl. rearing	0	0	0	0	0	0	4	4	<1	6	3	<1	<1	18
- Turkeys	<1	0	0	0	0	0	108	0	11	5	4	122	0	251
- Geese and ducks	0	<1	0	0	0	0	<1	0	<1	0	4	3	<1	7
- Gamebirds	<1	1	0	0	0	<1	6	1	4	13	25	10	<1	61
- Species unknown	<1	<1	0	<1	0	<1	3	<1	0	3	8	3	0	17
Other production animal species	*188*	*377*	*<1*	*1*	*357*	*185*	*707*	*0*	*7*	*1886*	*2857*	*1017*	*<1*	*7582*
- Small ruminants	<1	2	<1	0	0	<1	4	0	3	4	3	11	<1	29
- Fur animals	<1	373	<1	1	0	185	703	0	1	1877	684	994	<1	4820
- Aquaculture	188	<1	0	0	357	0	0	0	0	<1	2160	6	0	2711
- Other production animals	<1	<1	<1	<1	0	<1	0	0	2	2	11	<1	<1	17
species unknown[f]	0	<1	<1	<1	0	0	-1	0	<1	2	<1	4	<1	6
Companion animals, total	*<1*	*33*	*317*	*14*	*0*	*68*	*16*	*2*	*390*	*677*	*1097*	*90*	*42*	*2748*
- Pet animals	<1	22	313	12	0	68	15	<1	106	670	349	64	41	1663
- Horses or pets	<1	2	<1	<1	0	<1	<1	0	25	4	50	3	<1	84
- Horses	<1	10	3	2	0	<1	<1	2	259	4	698	23	<1	1002
Species unknown[g]														
- Systemic use	2	88	2	1	0	6	-10	7	241	71	128	145	0	680
- Topical drugs	<1	2	0	<1	0	0	0	0	0	0	<1	25	3	30
- Intramammaries	0	1	1	0	0	<1	0	0	4	<1	3	0	<1	9
Total	922	5665	438	24	357	2541	10981	7549	24783	11440	13356	29445	438	107940

a) Amcol = amphenicols; Amglc = aminoglycosides; Ceph = cephalosporins; FQ = fluoroquinolones; Quinol = other quinolones; Linco = lincosamides; Macro = macrolides; Pleuro = Pleuromutilins; Pen-β-sens = beta-lactamase sensitive penicillins; Pen-other = penicillins with extended spectrum, cloxacillin and amoxicillin/clavulanic acid; Sulfa-TMP = sulfonamides+trimethoprim; Tet = tetracyclines. Sulfaclozin (a prescription coccidiostat) is included in the sulfonamide/trimethoprim group

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

c) In 2011, half of the prescribed antimicrobials for cattle was purchased at pharmacies; half was either administered or handed out by veterinary practitioners. Reporting from large animal practice on medicines for cattle is validated against data on medicines sold from pharmacies to cattle practice (not mixed practice), and the proportion in accordance is included under the respective age groups. Medicines sold to cattle practice, but usage not reported by a veterinarian are included under "age group unknown"

d) About 10% of the pharmacy sales for use in cattle practice is not reported (possibly due to factor errors at reporting), when used or sold in practice, amounting to 1.2 tonnes in 2011. In addition, part of pharmacy sales to farms are lacking age group ID, amounting to 33 kg in 2011

e) In 2011, sulfamethoxazole was used in poultry on special permission, due to problems with resistance in E.coli infections. A total of approximately 40-50 kg was used, species/age group not known, and the data are not part of the VetStat database, presumably mostly in the broiler production. These data are not included in this table, as the precise target species/age group not known, and the data are not part of the VetStat database

f) Sales to farmers (valid farm ID codes) given, but animal species not identifiable. Negative numbers are due to redrawel of sales to specific species (not picked up by the farm owner)

g) Negative numbers are due to over-reporting by veterinarians of antimicrobial medicines used in practice to specific species

Patterns of antimicrobial consumption in Danish horse practices

Background: In general, national monitoring programs have focused on surveillance of antimicrobial consumption rates as well as antimicrobial resistance in food animals, food and humans, while antimicrobial consumption in companion animals has received little attention. The aim of this study was to investigate the prescription patterns and trends in antimicrobial consumption in 11 Danish horse practices.

Materials and methods: Data on prescription of antimicrobial agents in 11 horse practices during 2007 through 2010 were extracted from the pharmacy data at Statens Serum Institute (SSI; formerly the Danish Medicine Agency). These data only included antimicrobial agents prescribed by veterinarians currently employed in those practices, excluding prescription for use in other practices. Thus historical data were increasingly incomplete further back in time, when other veterinarians were employed in these practices. Only antimicrobial agents prescribed for horses or for use in practice were included. The consumption was measured in kg active compound by antimicrobial class as well as in ADDs, defined as the highest approved dose for 400 kg horse according to the registered summary of product characteristics (SPC).

For validation purposes, data for the 11 practices were also extracted from VetStat, including additional prescriptions by formerly employed veterinarians for use in the 11 practices. Differences in proportions of antimicrobial classes and differences in prescription patterns between different practice types were tested using an analysis of variance (Tukey-Kramer) and multiple comparison test (LS-means and Tukey).

Results: In the 11 practices, the consumption of beta-lactamase sensitive penicillin (Penicillin-G) and sulfonamide-trimethoprim (S-TMP) was significantly higher than consumption of the other antimicrobial classes, increasing from 62% to 81% of the total use in 2007 and 2010, respectively (Table 1).

Yearly consumption of S-TMP was relatively constant, accounting for approximately one third of all antimicrobial agents consumed. The majority of S-TMP given was as oral paste, as the only approved oral antimicrobial preparation for horses. This might explain the high consumption due to high owner compliance. Penicillin-G consumption increased from 32% in 2007 to 48% in 2010 (Table 1). Also, the proportional use of cephalosporins and tetracyclines increased during the four year, coinciding with decreasing use of aminoglycosides, macrolides and lincosamides. In 2007, aminoglycosides (mainly gentamicin) was the third most used class, accounting for 28%; however the usage was reduced to only 2% in 2010. From 2008-2010, cephalosporins were the third most used class after penicillins and S-TMP.

Comparison with the more complete VetStat data (Figure 1), indicated that the pharmacy data from SSI for the 11 practices underestimated the usage; particularly of cephalosporins by 21% across the study period 2007-2010. Especially 2007 data were underestimated, while 2010 data were most complete. In 2010, the consumption of cephalosporin accounted 0.32% of the total consumption (in kg active compound) in the 11 practices. At the National level (Table 4.1), 0.34% of the antimicrobial agents prescribed for horses (or horse practice) in 2011 were cephalosporins, suggesting that the 2010 data for the 11 practices were relatively representative.

Between practice variation
Over the four year period, the proportion of penicillin-G used varied significantly between practice type, with the highest use in horse hospitals and mobile practices (Figure 1). The use of other antimicrobial classes did not differ significantly between practice types, but large variations were seen in the use of S-TMP (between 13% and 74%) and 3rd generation cephalosporins (between 2% and 31%) between individual practices.

Fluoroquinolones only accounted for 1% of the overall usage (Figure 1), and as for the cephalosporins, there were large variations in usage. In 2010, one of the hospitals (HSP1) used 80% of the total amount of fluoroquinolones and one practice (CL2) used 61% of all cephalosporins used in the 11 practices that year.

The proportion of 3rd and 4th generation cephalosporins used in horse hospitals was lower (2% and 6%) than in the other practice types (clinics and mobile practices; between 18% and 22%) (Figure 1). This was somewhat surprising because the hospitals were expected to treat more severe and complicated cases. As expected, the proportion of fluoroquinolones was higher in the hospitals (2%) as compared to the other practice types (between 0.1% and 2%) (Figure 1).

The decreasing use of aminoglycosides (gentamicin) was probably related to the fact that it is not approved for horses, combined with increasing attention from the authorities towards non-complience with the cascade rule [Order (DK) 142/1993]. However, a questionnaire study conducted in the 11 practices shows that cephalosporins are now used for indications that were treated previously with gentamicin. Thus, the increasing use of cephalosporin might be

related to the decreasing use of gentamicin. However, this does not explain the large differences in cephalosporin use between practices. The large variation in prescription practices, and in particular variation in use of critically important antimicrobial agents, stresses the need for guidelines for antimicrobial use in horse practice, and probably also more frequent use of resistance testing in some practices, particular before using critically important agents.

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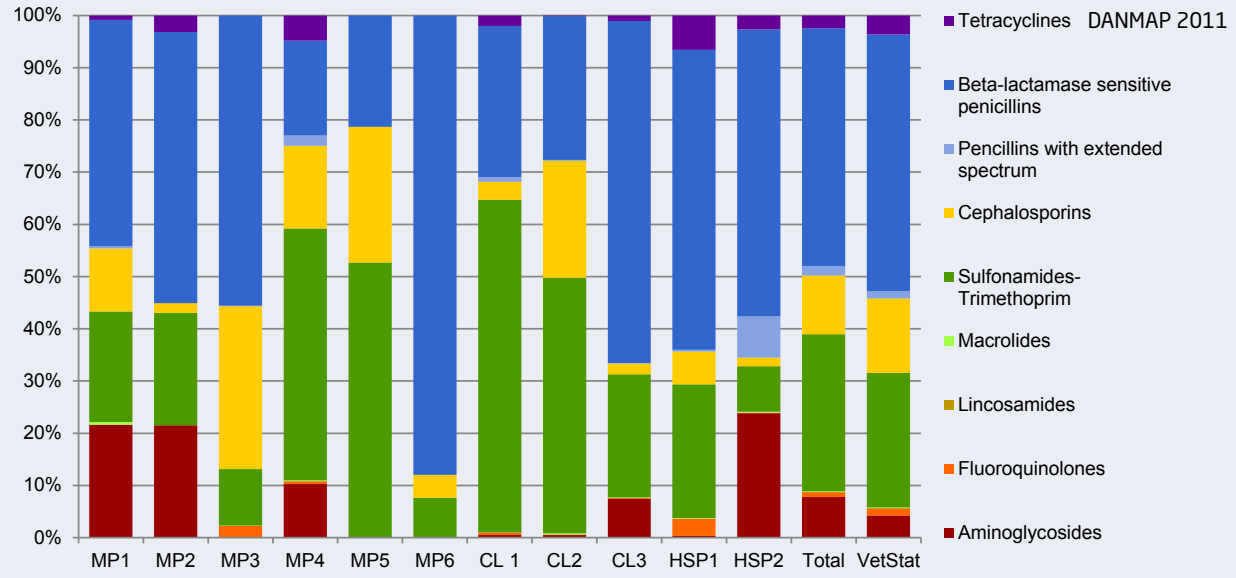
Table 1. Proportional consumption[a] (ADD400)[b] of antimicrobial agents in 11 Danish horse practices, Denmark 2007–2010

DANMAP 2011

Antimicrobial agent	Year			
	2007	2008	2009	2010
Beta-lactamase sensitive penicillin	32	51	47	48
Aminoglycosides	28	6	3	2
Broad spectrum penicillin[c]	1	2	2	2
Sulfonamide-Trimethoprim	32	27	28	34
Tetracyclines	<1	2	3	3
Cephalosporins	6	10	15	11
Macrolides	<1	<1	<1	<1
Lincosamides	<1	<1	<1	<1
Fluoroquinolones	<1	1	1	1

Source: C. Hensgen, Master Thesis, LIFE Faculty, Copenhagen University, 2012
a) National consumption in Danish horse practices estimated from data from the Statens Serum Institute (formerly Danish medicine Agency). Note that the historical data become increasingly incomplete (see text)
b) ADD400 is defined as the maximum approved dose for a 400 kg horse according to the SPC
c) Includes amoxicillin combined with clavulanic acid

Figure 1. Antimicrobial consumption (ADD400) pattern in 11 Danish horse practices, Denmark 2007–2010



Note: MP = mobile Practice; CL = Clinic; HSP = Hospital; Total = sales from pharmacies to the 11 practices or to horse owners, by request of vets, presently employed in the practices; Vetstat = sales from pharmacies to the 11 practices (disregarding identity of the prescribing vet), or prescribed for horses by a veterinarian presently employed in the practice

4.3 Antimicrobial consumption by animal species

A comparison of trends in consumption of antimicrobial agent, by species, is shown in Figure 4.3. Note that different units are used in the three diagrams within the figure, and that the trend lines differ depending on the unit used. For example, for turkeys, the highest peak was in 2009 when measured by mg but in 2002, when measured by ADD. This difference is explained by a shift in consumption from amoxicillin towards tetracyclines. Correspondingly, the consumption in aquaculture is extremely high when measured in mg, but more similar to the consumption in other species (pigs) when measured in ADDkg. This is becaue the dominant drug (sulfonamide-trimethoprim) in aquaculture is used in a very high dosage (in all species). Finally, trends may also be affected by shifts in the production, as described for pigs in section 4.2.

For comparison between species, kg meat produced has been used in Figure 4.3 A and B; this measure overestimates the selection pressure in species with long lives (e.g. cattle) relative to species slaughtered at an early age (e.g. poultry). Alternatively, live biomass should be used for comparison of selection pressure between species. Using this method, the difference between pig and poultry production becomes less pronounced (Figure 4.3, C). The consumption in dairy cattle is very low, measured in ADDkg per kg live biomass, while the antimicrobial consumption in young growing cattle (half of which is for slaughter) is at the level seen in the turkey production. Because cattle in Denmark are almost entirely dairy cattle, living many years after reaching the slaughter weight, the consumption cannot be directly compared with other species using 'kg meat produced' as the denominator.

In 2011, antimicrobial sales for use in pet animals amounted to more than 5.6 ADDkg/ kg-live- biomass (app. 10-15% underestimation, see section 4.3.4). Thus, the consumption in pet animals was higher than for cattle and poultry, but lower than for pigs.

Figure 4.3. Antimicrobial consumption per kg meat produced from pigs, broilers, turkey and aquaculture measured against different denominators, Denmark

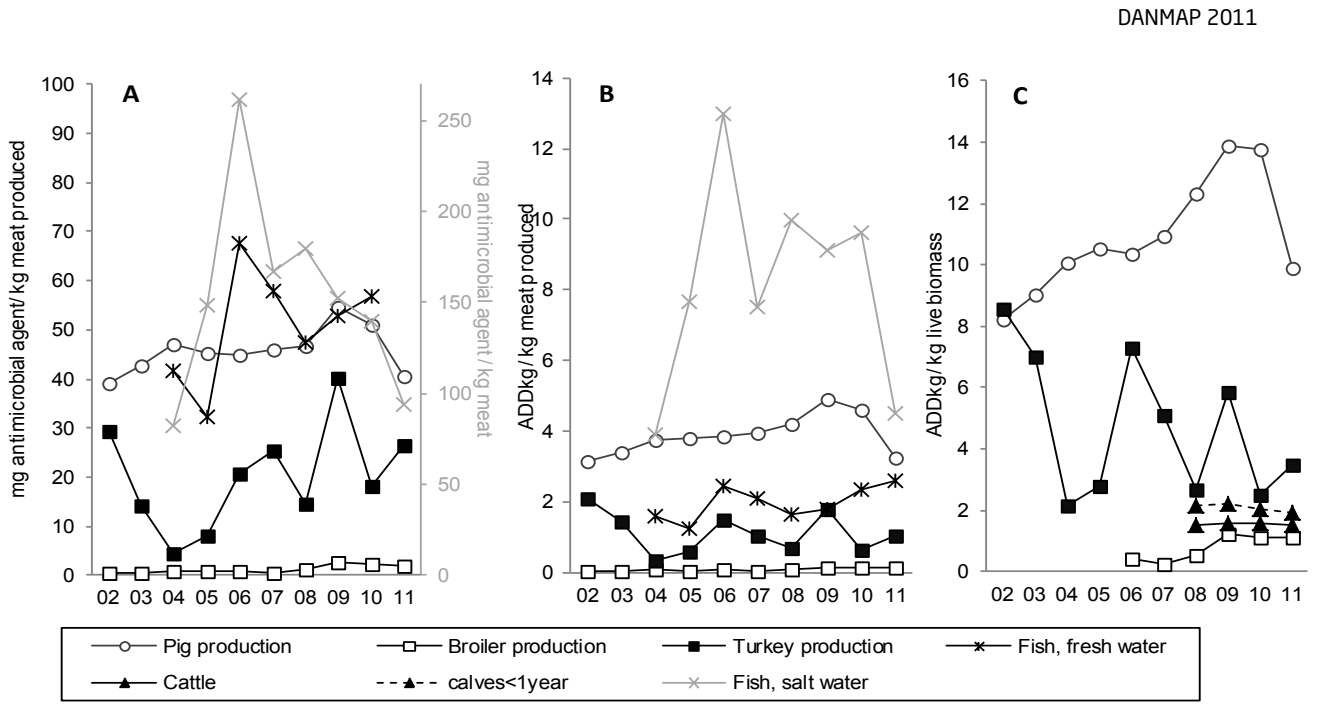


Figure A: Export of animals for rearing or slaughter is included. However, the data for pig production are not adjusted for the increasing export of pigs at 30 kg body weight, although the body mass at export is included in the production (see text). Thus, the figures overestimate the increasing consumption in the pig production, particularly during the last three years
In 2006, the consumption reached 262 mg/kg fish produced in salt water, related to unusually warm summer months. The doses for fish are defined as 30 mg/kg for sulfonmide-trimethoprim, 10 mg/kg for oxolinic acid and 15 mg for florphenicol (source: Danish Aquaculture)
Figure B: The doses for fish are defined as 30 mg/kg for sulfonamide-trimethoprim, 10 mg/kg for oxolinic acid and 15 mg for florphenicol (source: Danish Aquaculture)
Figure C: The biomass is estimated from census data (pig and cattle) og from production data (poultry). See Section 10.2

4.3.1 Antimicrobial consumption in pigs

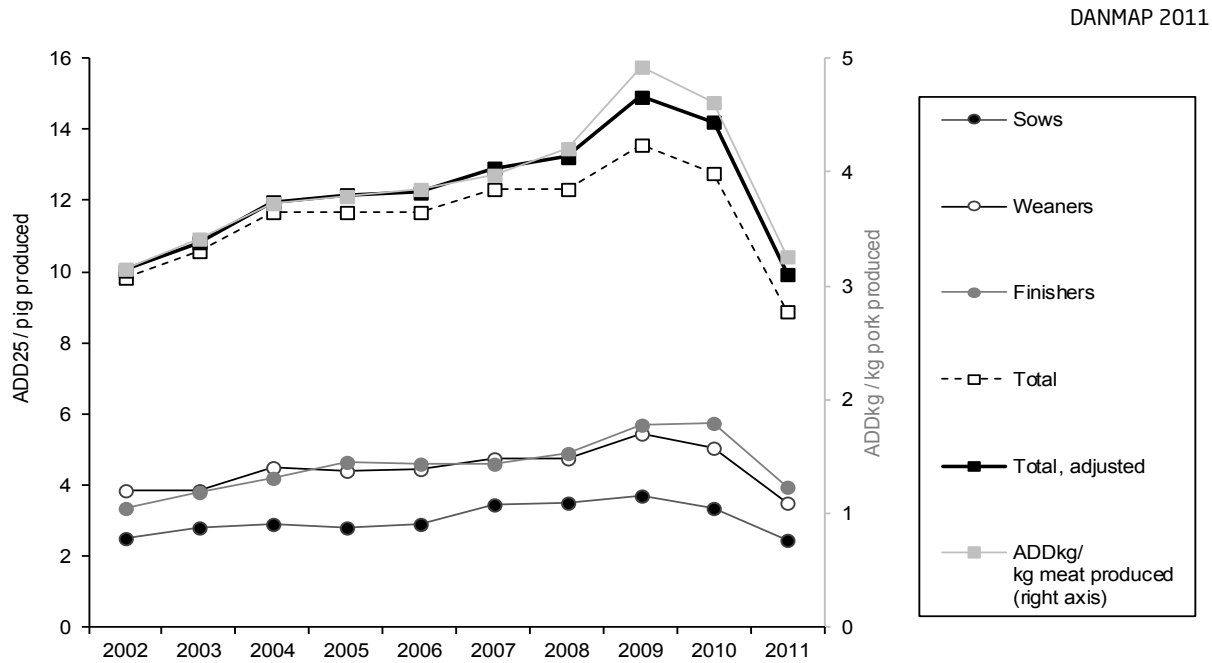
In 2011, the total antimicrobial consumption in pigs was 81.4 tonnes active substance (Table 4.1), representing a decrease of 19 tonnes compared to 2010, while the production of pigs continued to increase. The decrease in 2011 was mainly attributed to decreasing use of agents with high potency; therefore, the decrease was larger when measured in ADDs.

Relative to the meat production, including live export, the consumption decreased by 29% from 4.6 ADDkg per kg to 3.3 ADDkg per kg pork produced (Figures 4.3 and 4.4). Due to evolving demographics in the pig production, the trends should be measured with adjustment for the increasing export of pigs at 30 kg; the consumption decreased by 30% ADDkg per pig produced (adjusted) from 2010 to 2011.

The consumption of antimicrobial agents for systemic use in pigs (2002–2011), given as Animal Daily Doses (ADDs) to the different age groups, is presented in web annex A4.2.

Since 2002, the antimicrobial consumption in pigs increased gradually, reaching a maximum in 2009, with a total increase of 47% in ADDkg per pig produced (adjusted for increase in live export, Figure 4.4). The increase continued into first half of 2010. However, in July 2010, the consumption decreased abruptly following the announcement of the yellow card initiative July 1st, and the decreasing trend continued into first half of 2011. In 2011, the antimicrobial consumption per pig had returned to 2001-2002 levels, i.e. just after the full growth promoter ban was enforced. The decrease in antimicrobial consumption involved all age groups (Figure 4.4). Looking at the entire decrease since 2009, the antimicrobial consumption decreased by 34% in sow herds, 35% in weaning pigs, and 31% in finishers.

Figure 4.4. Consumption of antimicrobial agents in sows, weaners[a], finishers[b] and total pig production[c], given as Animal Daily Doses (ADDs), Denmark



a) Consumption in sows and weaners is given as ADD25 divided by number of weaning pigs produced
b) Consumption in finishers is given as ADD25 divided by number of finisher pigs produced
c) The" adjusted total" is given with the same units as the total, but adjusted for the increasing export of pigs at 30 kg (see text). Consumption per kg meat produced is given as ADDkg divided by meat production including live export

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 gastrointestinal disease in weaning pigs and finishers. The overall decrease seen in 2011 was mainly related to a 60% decrease in consumption of pleuromutilins, a 27% decrease in use of tetracyclines and a 26% decrease in macrolides, as compared to 2010. Furthermore, the use of broad spectrum penicillins was reduced by 21%, while minor decreases was seen in other antimicrobial classes. A large relative reduction (98%) was also observed for 3rd and 4th generation cephalosporins in 2011, approximating zero following a voluntary ban on cephalosporins, enforced by the by the pig industry in July 2010 (Figure 4.7).

Over the past decade, the consumption of macrolides has fluctuated between 2.2–2.6 ADD25 per pig produced, with an overall slightly increasing clear trends, reaching 2.8 ADD25 per pig in 2009. However, from 2010 to 2011, the consumption decreased importantly from 2.7 ADD25 per pig produced to 2 ADD25 per pig produced. The consumption of pleuromutilins was high during 2008-2010, following a 53% increase since 2007, where the prices for tiamulin were reduced making it price competitive with macrolides and tetracyclines. It is not clear if economic incentives have assisted the large decrease in pleuromutilins in 2011.

According to the Danish Agriculture and Meat Council, the reductions in the antimicrobial consumption have not affected the position of the Danish pork industry on the world trade market, and only very limited effects on productivity and economy have been reported [Danish veterinary Bulletin no.6, 2012]. While the yellow card has led to increasing use of vaccines, and a slight decrease in productivity in some herds, disease outbreaks have not increased.

Whereas the decrease in consumption in 2010 was confined to gastrointestinal disease, other indications were also involved in the 2011 decrease (Figure 4.6). In weaning pigs, gastrointestinal infections are the major indications for prescription (70% in 2010), and decreased by 31% from 2010 to 4.1 ADD15 per pig produced in 2011, which is below the level of 2002. Respiratory disease is the target disease for 21% of the antimicrobial use in weaning pigs, and decreased by 24% in 2011 compared with 2010. The prescription for respiratory disease is still at the same level as in 2008, and 140% higher than in 2002, suggesting that the level of respiratory disease has increase significantly from 2002 to 2011.

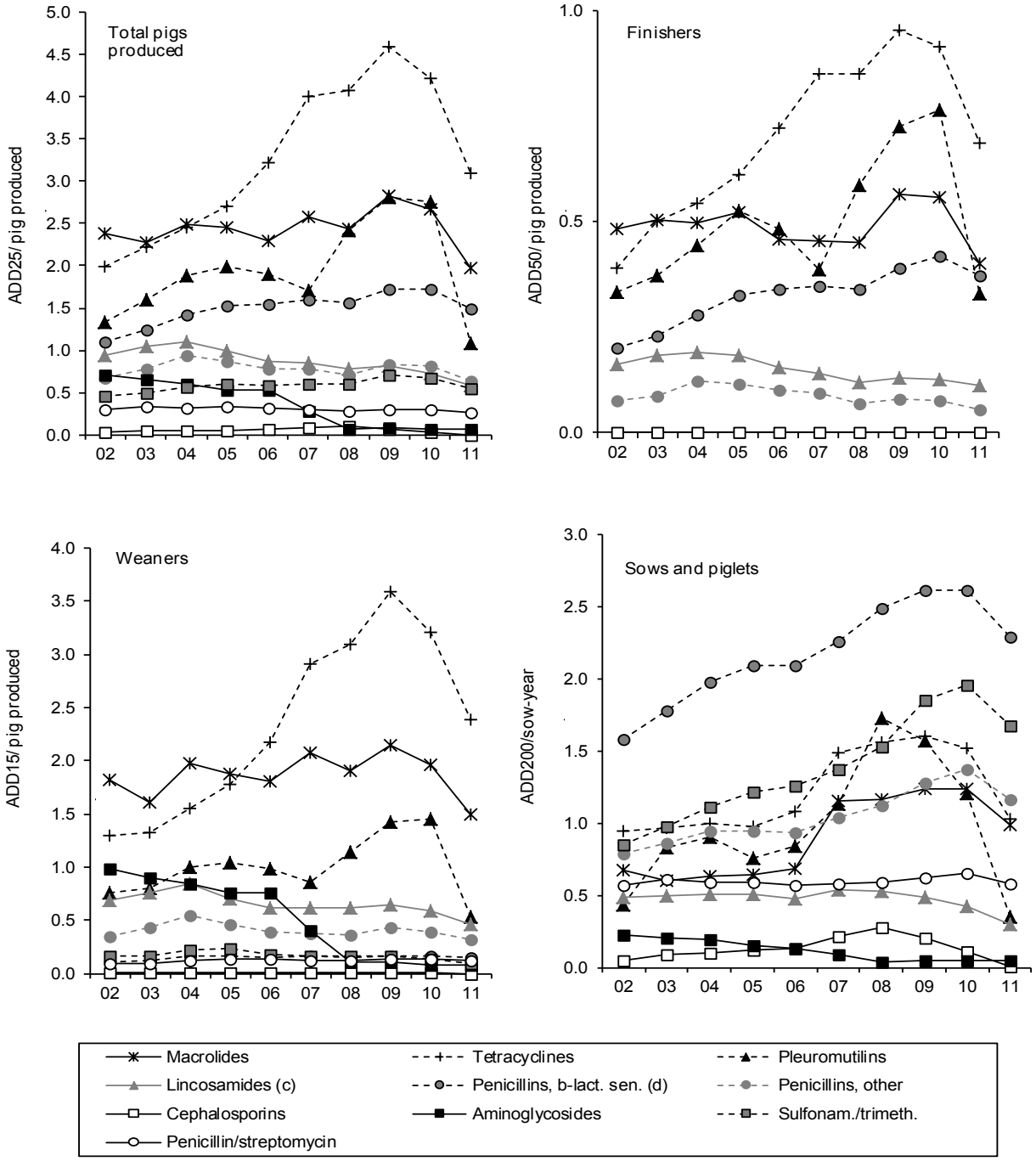
The decrease in prescription for gastrointestinal disease could be due to better disease prevention (changes in management incl. vaccination) and to some degree also abolishment of metaphylactic treatments. Sales of vaccines for GI diseases in pigs increased dramatically in 2010. In particular, vaccines against *E.coli* diarrhoea increased by 350%, from 0.35 million doses in 2009 to 1.56 million doses in 2011. Use of PCV2 vaccines (porcine corona virus, associated with the PMWS syndrome, see DANMAP 2005) has increased gradually since 2006, and tripled from 3.5 million in 2009 to 9.5 million in 2011. Previously, increase in systematic metaphylactic antimicrobial treatment, mainly against gastrointestinal disease, has been suggested as the cause of increasing antimicrobial use in weaning pigs during the past decade [Focus Area 2, DANMAP 2009].

In sow herds, the prescription for gastrointestinal disease decreased by 41% from 2010 to 2011, while the prescription for other diseases (except mastitis) decreased between 18-26%. In finishers, the prescription for gastrointestinal disease decreased by 34% in 2011 compared to 2010, while the prescription for respiratory disease decreased by 32%. For respiratory disease the prescription was above the 2002 level, while for gastrointestinal disease it was similar to the 2002 level. In all age groups, the prescription for respiratory disease has been increasing throughout the last decade until 2010 (Figure 4.6).

Regarding target disease, it should be noted that these statistics are derived from the information on indication (disease group) in the VetStat database. The indication is entered by the veterinarian and refers to current or expected disease within a 30 day period (based on his/her knowledge of the herd). However, in some cases, the prescribed antimicrobial agents may be used for other diseases than originally indicated on the prescription, in which case the antimicrobial agent is re-prescribed by the veterinarian at the farm. Such re-prescriptions are not registered in VetStat.

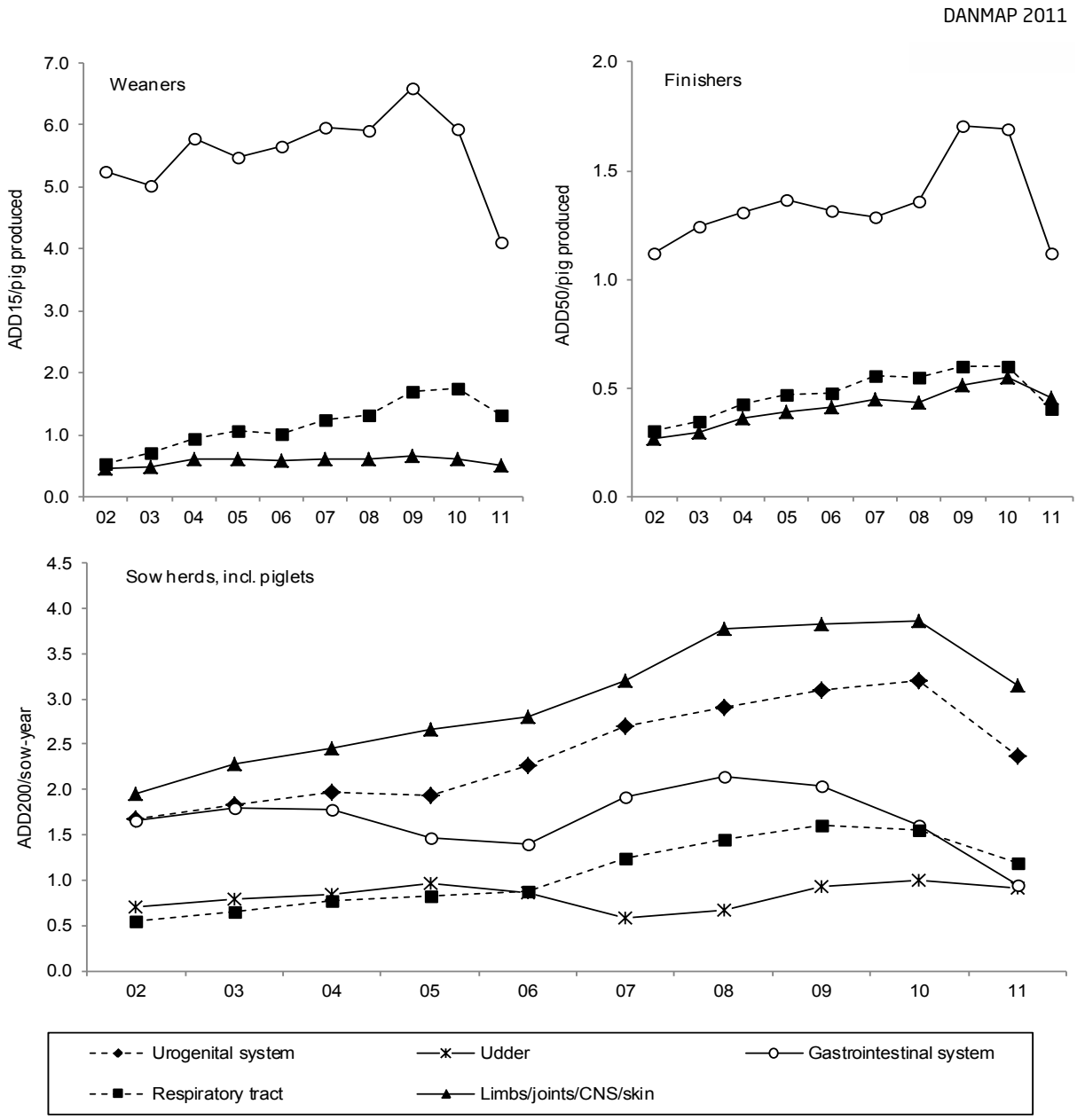
Figure 4.5. Antimicrobial consumption[a] in the total pig production[b], and in finishers, weaners, sows and piglets, Denmark

DANMAP 2011



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) ADD25: doses for treatment of 25 kg pigs, to compare treatment across age groups. ADD15: Doses for treatment of 15 kg pigs, the assumed average dose for treatment of weaners (7.5–30 kg). ADD50: Doses for treatment of 50 kg pigs, the assumed average dose for treatment of finishers (30–110 kg), estimate based on the number of pigs produced excluding those pigs exported at 15–50 kg. ADD200: Doses for treatment of 200 kg pigs: the medicines are used either in sows (bodyweight>200 kg) or in piglets (<2 kg–7.5 kg)
b) Total pigs produced includes pigs exported at 30 kg, which has increased in numbers from 1.7 million in 2004 to 7.6 million in 2011, comprising an 26%, although consumption in these pigs is included only from birth to 30 kg body weight. See discussion in the text
c) Lincosamide/spectinomycin combinations comprise 65% of this group
d) Beta-lactamase sensitive penicillins

Figure 4.6. Antimicrobial consumption by indication[a] for sows/piglets, weaner and finisher pigs, Denmark



a) The indication given on the prescription. At prescription, VetStat codes are used, representing the target organ system. In this figure, indications representing <0.1ADD (per sow year or pig produced) is not shown

4.3.2 Antimicrobial consumption in cattle

In 2011, an estimated 14.7 tonnes of antimicrobial substance were prescribed for cattle, similar to 2010 (Table 4.1). Data on antimicrobial consumption in cattle are not as accurate as data in the other major species, because a large proportion is sold via veterinary practice.

However, pharmacy data have a very high quality, and an increasing proportion of the medicine for cattle has being purchased through the pharmacies, although 53% of the antimicrobial agents for cattle are still sold via the veterinary practices. Pharmacy data, including sales to cattle practice, indicate that the overall consumption in cattle has been stable over time, around 14 tonnes, during 2005–2011. During this period, the veal and beef production has fluctuated around the same level (145,000 tonnes in 2005 and 2011) and the milk production has increased by 8% (Table 3.1).

Consumption of antimicrobial agents for systemic use in cattle (2005–2011), given as Animal Daily Doses (ADDs) to different age groups, are presented in the web annex (Table A4.3). For cattle, data are only shown for the period 2005–2011, because data quality was not acceptable prior to 2005 due to reporting errors from the veterinary practices. In 2011, underreporting from veterinary practice corresponded to an overall 8% underreporting of total use in cattle.

Based on the type of technical errors, it is assumed that the missing data are random, and thus that the data from the veterinarians are representative for the relative choice of drugs over time. Data validation against pharmacy data on sales to cattle practice supports this assumption (Figures 4.8 and 4.9).

In 2011, the choice of antimicrobial agents for systemic treatment of cows was almost identical to the distribution in 2010. Beta-lactam sensitive penicillins for systemic use in cows accounted for 59%, followed by tetracyclines (20%). In cows and bulls, the proportional use of beta-lactamase sensitive penicillins has increased from 48% in 2005 to 59% of the overall consumption in 2011, while the use of macrolides decreased from 11% to 3% of the consumption for systemic use in cows. This trend is in accordance with the official guidelines. The major indication for treatment of cows was mastitis (66% of the systemic treatment in grown cattle).

Also for intramammary treatment (including drying off), penicillins, mainly narrow spectrum (beta-lactamase sensitive or beta-lactmase resistant), was the major classes used, comprising 46% of the treatments (Table 4.2b). Combinations of benzylpenicillins (mainly with dihydrostreptomycin) comprised an additional 27%. A decrease of 8% in use of intramammary treatment per cow-year was observed from 2010 to 2011. Some intramammary formulas are entirely used for drying off treatment, and the use of these has increased by 6%, while the use of intramammary application for therapeutic purposes has decreased by 16%.

The use of antimicrobial agnets for mastitis in cattle has been regulated since 2006 [Order (DK) 1045/2006 and 785/2010], however these trends are probably also a result of a "milk quality campaign" run by the cattle association (Dansk Kvæg) 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 microbiological testing (cell counts) for discriminating the necessity to treat. Some increase in drying-off treatment is accepted, due to refraining from treating during lactation. Also the use of narrow spectrum penicillins in case of Gram-positive infections, unless sensitivity testing shows resistance, has been emphasized [Dansk Kvæg, 2012].

From 2005 to 2011, the overall use of intramammary treatments per cow-year decreased by 12%, while the proportion of narrow spectrum penicillins increased from 23% to 46% of the intramammary treatments (Table 4.4).

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

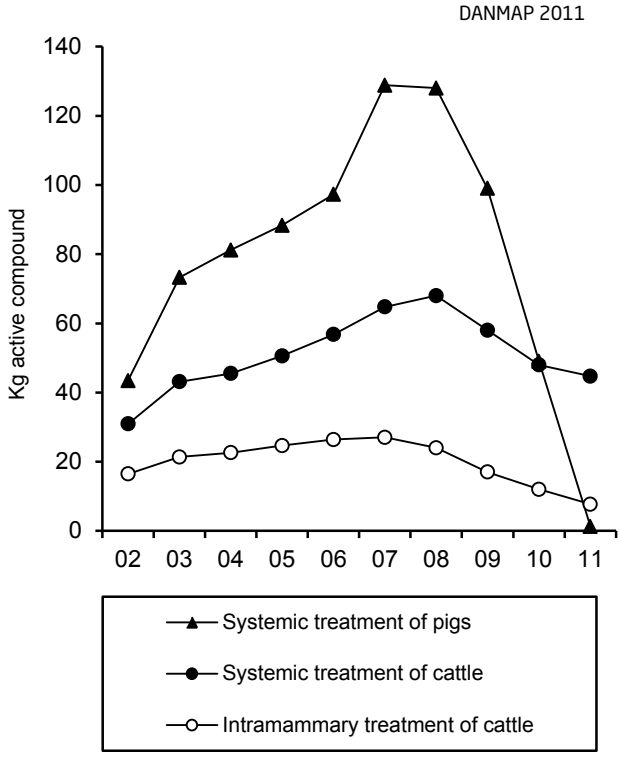


Table 4.2a. Total treatments with antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2011

Total treatments per indication [a]	2005	2006	2007	2008	2009	2010	2011
	ADDs (1000's)						
Drying off treatment (4 teats)	73	75	78	86	96	104	110
Therapeutic treatment (2 teats)	420	408	388	377	378	350	293

Table 4.2b. Total treatments with antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2011

Use per antimicrobial class[a]	2005	2006	2007	2008	2009	2010	2011
	ADDs (1000's)						
Penicillins[b]	127	132	156	185	230	246	235
Aminoglycoside-benzylpenicillin combinations[c]	204	184	155	152	162	161	140
Cephalosporins, 1st generation	103	98	89	85	89	89	96
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34
Others[d]	21	20	16	15	14	12	9
Total	566	558	544	549	570	559	514
Total ADD/cow-year	1.01	1.00	1.00	0.98	1.00	0.97	0.89

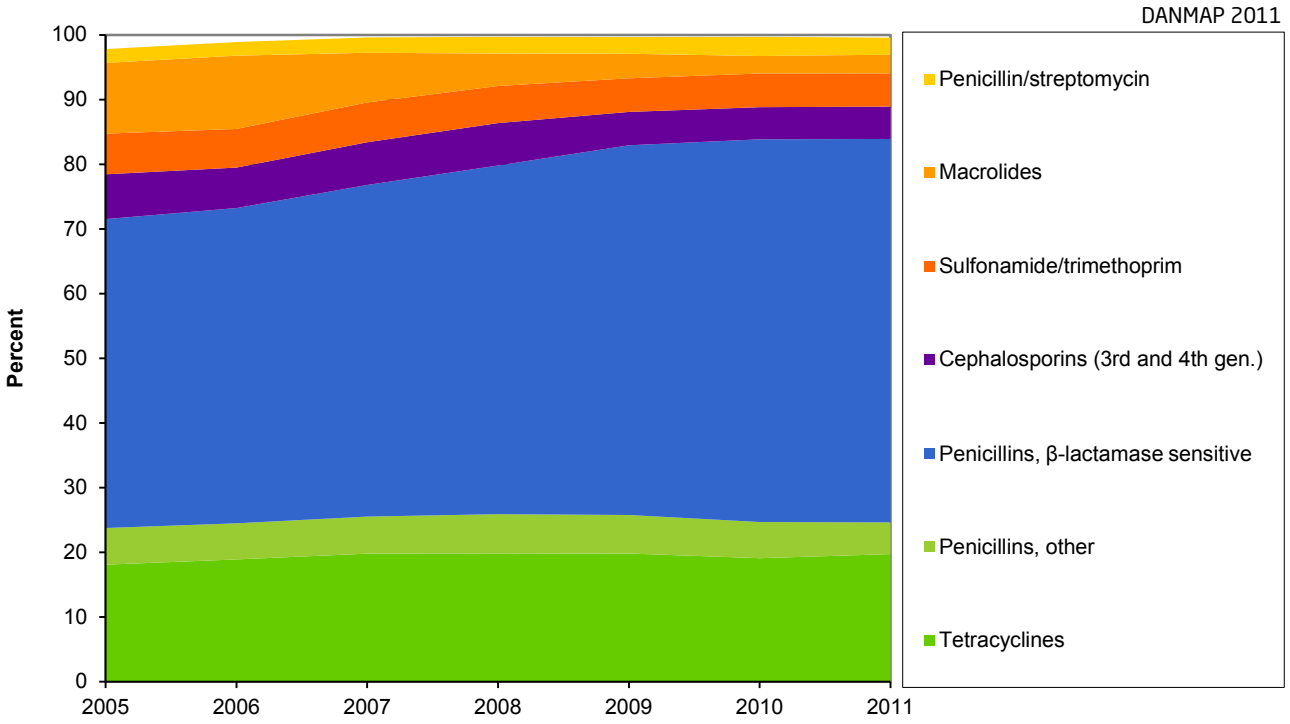
a) For intramammary therapeutic treatment, 1 ADD is defined as the dose to treat two teats for 24 hours. For drying off treatment, 1 ADD is defined as the dose to treat 4 teats
b) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations (QJ51CE, QJ51CF, QJ51RC)
c) Mainly dihydrostreptomycin-benzyl benicillin combinations; includes also combinations of penicillin/aminoglycoside with bacitracin or nafcillin (QJ51RC)
d) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

The major indication in calves was respiratory disease, accounting for 67% of systemic use in calves in 2011, followed by gastrointestinal disease (17%). In calves, tetracyclines (mainly oxytetracyclines) remained the major drug of choice in 2011, although the proportion decreased from 30% of total systemic use in 2010 to 27% in 2011 (Figure 4.9). From 2006 to 2009, macrolides were the most frequently used class, but the use was reduced from 35% in 2009 to 24% in 2010-2011. In 2011, the use of amfenicols increased from 9% to 12% of the medicines for systemic use, and was almost entirely (96%) prescribed for respiratory disease. The use of amfenicols (florfenicol) has increased gradually since 2005.

The use of fluoroquinolones in cattle was only 1 kg active compound, and has remained at a low level since 2003.

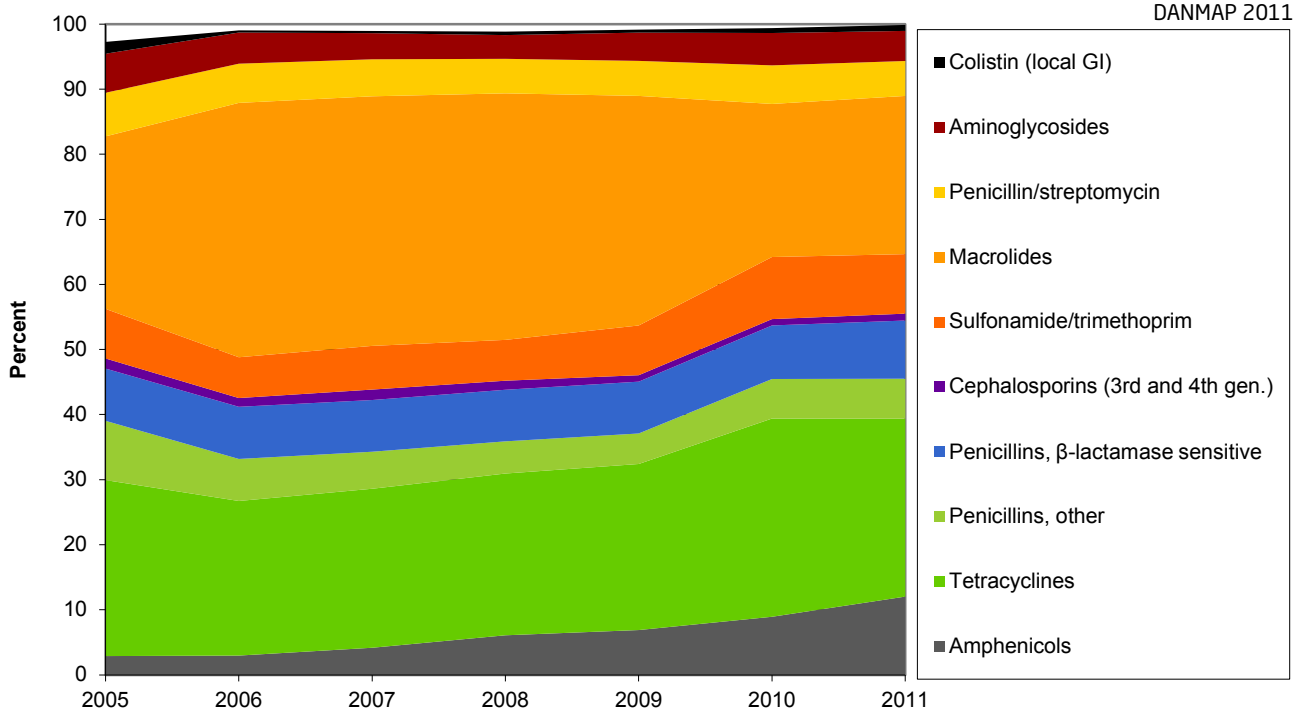
Systemic use of 3rd and 4th generation cephalosporins in cattle decreased by 7% and intramammary use decreased by 34% measured in kg active compound in 2011 compared to 2010. This was part of a continuously decreasing trend, and since 2008 the consumption of 3rd and 4th generation cephalosporins for systemic and intramammary use has decreased by 34% and 68%, respectively (Figure 4.7). These trends probably reflect a response to debate, guidelines and information in recent years about the consequences regarding development of ESBL. The more recent trend may be a result of regulations implemented in 2010, requiring testing for antimicrobial resistance, in cases where antimicrobial agents, other than simple penicillins, are prescribed for mastitis. The trend has also been supported by the above mentioned campaign by the Danish cattle Association.

Figure 4.8. Proportional consumption (in ADD) of antimicrobial agents[a] for systemic treatment in cows and bulls, Denmark



a) The antimicrobials not shown (amphenicols, fluoroquionolones, lincosamides, aminoglycosides, colistin and pleuromutilins), each account for less than one percent of the consumption

Figure 4.9. Proportional consumption (in ADD) of antimicrobial agents[a] for systemic treatment in calves, Denmark



a) The antimicrobial classes not shown (fluoroquionolones, lincosamides, and pleuromutilins), each account for less than one percent of the consumption

4.3.3 Antimicrobial consumption in poultry

In Denmark, the poultry production comprises mainly the broiler production (*Gallus gallus*), followed by egg layers (*Gallus gallus*) and turkey production. In addition, there is a minor production of ducks, geese, and game birds, while pigeons are kept for sports. Consumption of antimicrobial agents for systemic use in poultry (2002–2011), given as Animal Daily Doses (ADDs) to the different species, are presented in the web annex (Table A4.4).

In 2011, the total antimicrobial consumption in poultry was 769 kg active substance plus an additional 40-50 kg sulfamethoxazole used on special license (Table 4.1), representing an 8% decrease compared to 2010. In general, increasing disease problems caused a steep increase in antimicrobial consumption for poultry in 2009 (see DANMAP 2009); these disease problems seems to be under control in 2010 and 2011, as indicated by the decrease in antimicrobial consumption, both for the layers, broiler rearing, and the turkey production. However, regarding the broilers the consumption has continued to increase in 2011. Consequently, the overall consumption in poultry in 2011 (769 kg) was still higher than the levels in previous years (2002–2008), when the annual consumption fluctuated between 400-600 kg.

Note: the use of sulfamethoxazole is not included in the data mentioned below, because the target species is unknown and an ADD has not been defined. However, according to poultry practitioners it has been used for multi-resistant *E. coli* infections in the broiler production.

In Denmark, the antimicrobial consumption in domestic fowl (*Gallus gallus*) is generally low (Figure 4.3. and Figure 4.10). Therefore, a few disease outbreaks in some farms will affect the national consumption, causing considerable fluctuations in annual consumption.

The total consumption in broilers in 2011 was 414 kg including breeding and rearing (Table 4.1). For broilers, the additional increase in consumption was mainly in the prescription of amoxicillin, which has been the major drug of choice for at least a decade (Figure 4.8). Among 202 professional broiler producing farms, antimicrobial consumption was prescribed for 60 farms, corresponding to 29.7% of the farms. However, these farms received a total of only 182 prescription; as only the broilers present at time of prescription were likely to be treated, this means that only a small fraction (estimated 3%) of the flocks have been treated. Due to the decrease in consumption in the parent and grandparent flocks (breeding and rearing), the overall consumption (0.013 ADDkg per kg meat produced) in the broiler production was at the same level as in 2009–2010, but twice to five times higher than in previous years (Figure 4.10). In 2011, only 5% of the antimicrobial agents used in the broiler production were used in the parents and grandparent flocks.

The antimicrobial consumption in the layer production (*Gallus gallus*) is also quite low, and the total consumption in 2011 was only 18 kg (Table 4.1), corresponding to a decrease of 62% compared with 2010 (Figure 4.11).

In turkeys, the annual consumption is highly variable. In 2011, the consumption was 251 kg which was similar to 2010 level. However, relative to the production, the consumption increased to 1 ADDkg per kg meat produced, representing a 34% increase from 2010. This was still 10% lower than the median for the past decade (web annex A4.4 and Figure 4.11). 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.

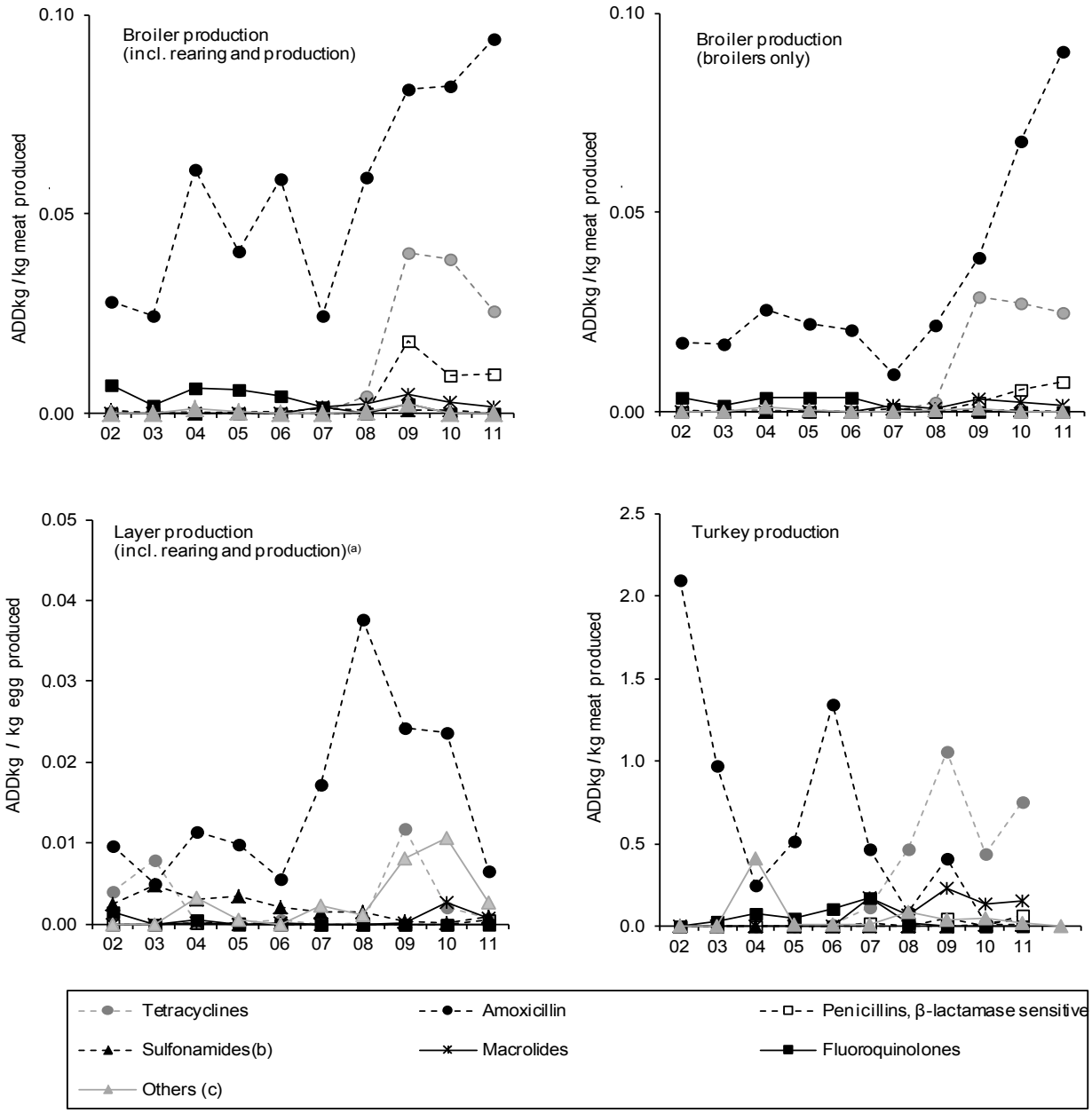
In 2011, the use of tetracyclines increased slightly to 73% of the antimicrobial consumption in turkeys. Tetracyclines have been the drug of choice in the turkey production since 2008. Prior to 2007, amoxicillin constituted 70–99% of the antimicrobial consumption in turkeys, while the use had decreased to 3–4% in 2010–2011. The changes in prescription practice occurred after the marketing of tetracyclines and other agents for use in poultry during 2007–2008. Before 2007, only amoxicillin and fluoroquinolones were approved for use in poultry.

In 2010–2011, fluoroquinolones were used neither in turkeys nor in *Gallus gallus*, and the consumption has decreased importantly since 2006 where fluoroquinolones comprised 7% of the antimicrobial consumption in these species.

Annual production data are not available for game birds. In 2004, the population was estimated at 1 million pheasants, 0.5 million ducks and 0.1 million other birds (mainly guinea fowl). Assuming a constant population and carcass weights of 650 g for pheasants, 750 g for (wild) ducks and 300 g for guinea fowl, the consumption has fluctuated between 1.3–2.9 ADDkg per kg carcass, during 2002–2011 (Figure 4.11 and web annex, Table A4.4).

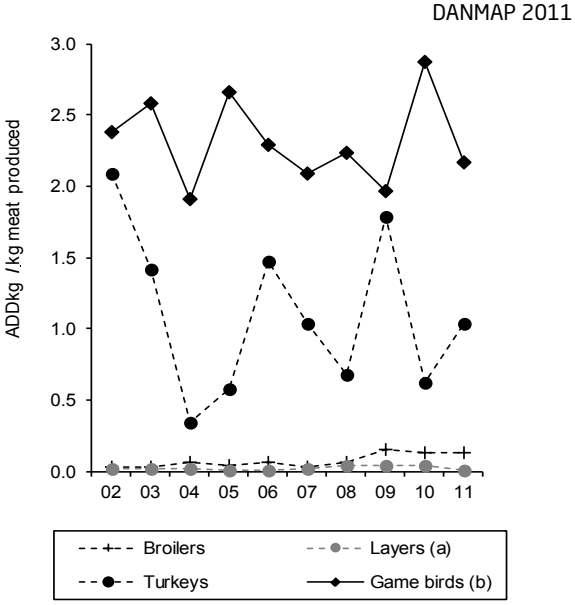
Figure 4.10. Consumption of antimicrobial agents in the poultry production, Denmark

DANMAP 2011



Note: ADDkg is the dose necessary for treating 1 kg live-body-weight
a) For layers and layer rearing, only the production of eggs for consumption is included (not the slaughter/export of hens)
b) Includes sulfaclozin (a coccidiostat/antibacterial) and sulfonamide/trimethoprim combinations
c) Includes primarily fluoroquinolones, but also aminoglycosides, pleuromutilins, QA07AA10 (colistin), QJ01FF (lincosamides, including combinations with spectinomycin), QJ01B (amphenicols) and QJ01R (penicillin/streptomycin combinations)

Figure 4.11. Total consumption of antimicrobial agents in various poultry production species, Denmark



Note: ADDkg is the dose necessary for treating 1 kg body-weight
a) For layers and layer rearing, only the production of eggs for consumption is included (not the slaughter/export of hens)
b) Carcass weight of pheasants estimated at 550 g for hens and 750 g for cocks. Carcass weight for game ducks is estimated at 750 g, and 300 g for guinea fowl. The production was assumed constant at the 2004 level

4.3.4 Antimicrobial consumption in fur animals, aquaculture and pet animals

In 2011, the production of mink increased by 7% to 15 million mink, while the production of chinchillas was unchanged at 34,000 chinchillas. Antimicrobial consumption in fur animals increased to 4.8 tonnes, representing a 30% increase compared with 2010 (Table 4.1). Aminopenicillins remained the most commonly used antimicrobial class in fur animals, comprising 40%, similar to 2010. Macrolides, tetracyclines and sulfonamide-trimethoprim combinations comprised another 39%. The use of fluoroquinolones increased from 0.4 kg in 2010 to 1 kg in 2011.

The antimicrobial consumption in aquaculture decreased by 11% to 2,700 kg in 2011 compared to 2010 (Figure 4.3). Measured in ADDkg, the major class of antimicrobial was sulfonamide/trimethoprim, increasing to 60% the consumption in aquaculture. The consumption of quinolones (oxolinic acid) comprised 30% and florfenicol 10%.

Assuming an unchanged production volume, the consumption in salt water fish was decreased by 50% to 4.5ADDkg in 2011, as compared to 9 ADDkg per kg fish produced in 2009 – 2010 (Figure 4.3). Fish production is very sensitive to water temperatures and the decrease in consumption was partly due to a very cold summer in 2011, but also increasing vaccination intensity has contributed to a gradual 51% decrease in antimicrobial use in salt water aquaculture through 2006–2010 [personal communication: N.H. Henriksen, Danish Aquaculture].

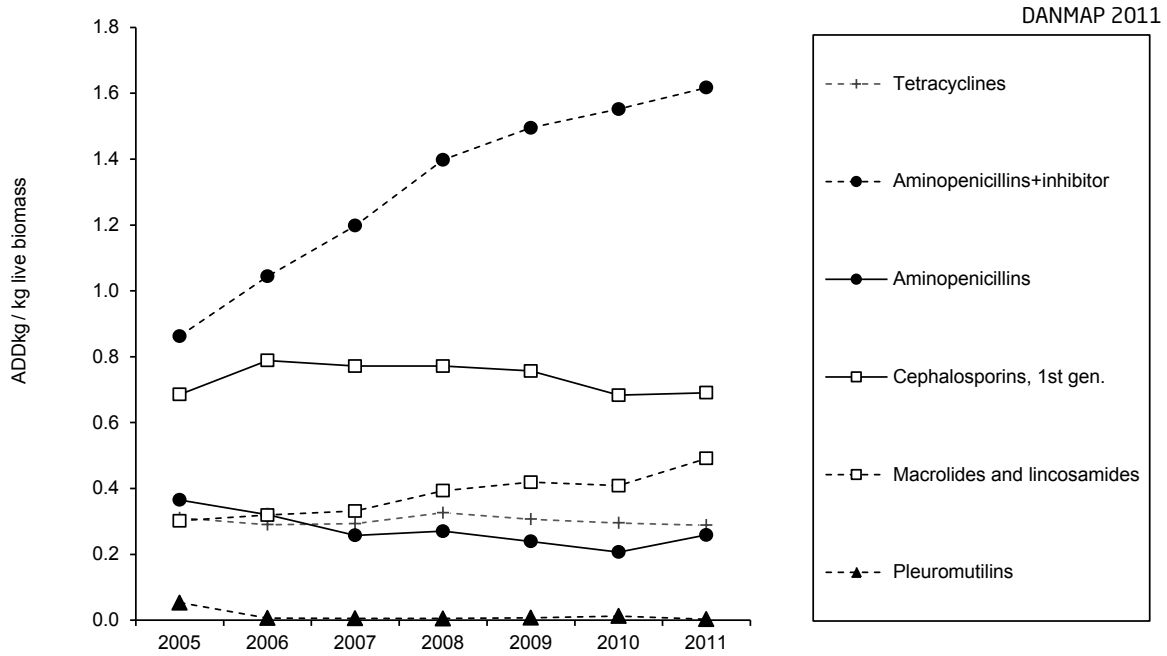
In fresh water fish, the antimicrobial consumption fluctuates less than in salt water, but increased by 12% in 2011 to 2.6 ADDkg per kg fish produced, when assuming the same production volume as in 2010. The increase may partly be explained by a very cold winter followed by a very warm spring in 2011, having a negative impact on the immune competence of the fish. Another explanation may have been a delay in deliverance of vaccines against red mouth disease in late 2010, causing inadequate immune competence against this particular disease in 2011 [personal communication: N.H. Henriksen, Danish Aquaculture].

The consumption of antimicrobial agents in companion animals (pet animals and horses) was an estimated 3 tonnes. The estimation was based on pharmacy data on the prescription for these species, prescription of medicines approved only for oral use in these species, combined with data on sales for companion animal and horse practices, respectively (Table 4.1). This estimate does not include the parenteral and topical use for these species in mixed practice. For pet animals alone, the total use was estimated at 2 tonnes.

In 2011, antimicrobial sales for use in pet animals amounted to more than 5.6 ADDkg per kg live biomass; this estimate does not include parenteral and topical antimicrobial use in mixed practice, causing an underestimation of approximately 10-15%. Consumption of antimicrobial medicines for oral use in dogs and cats amounted to 4.3 ADDkg per kg biomass.
In pet animals (mainly cats and dogs), the prescription for oral treatment measured in ADDkg per kg biomass increased by 8%, as part of a continuous increase of 36% since 2005. This was almost entirely related to an 88% increase in use of amoxicillin with clavulanic acid (Figure 4.12). The use of 1st generation cephalosporins for oral use was almost unchanged in 2011, at a similar level as in 2005.

In pet animals, the consumption of 3rd and 4th generation cephalosporins amounted to 0.32 ADDkg per kg live biomass in 2011. The use of fluoroquinolones amounted to 0.22 ADDkg per kg live biomass. For comparison, the use of cephalsoporins for systemic use was 0.06 ADDkg per kg live biomass in cattle, while the use in pigs is now close to none, and it has not been used in poultry for at least 10 years. The use of fluoroquinolones has been close to none in pigs and cattle since 2003, and close to none in poultry since 2007, due to legal restrictions.

Figure 4.12. Antimicrobial consumption (oral treatments) in cats and dogs, Denmark



The live biomass was estimated based on the assumption of average weight of dogs (20 kg), and cats (4 kg). Census data are available only for 2000 [Statistics Denmark], and the population size was assumed to be constant

In 2011, the consumption of 3rd and 4th generation cephalosporin (parenteral use only) in pet animals was an estimated 3 kg in 2011, corresponding to 5% of the total veterinary consumption of these antimicrobial agents. In addition, 1.3 kg 2nd generation cephalosporins (medicinal products for human use) was used in a few practicies. The use of fluoroquinolones in pet animals was an estimated 12 kg in 2011, corresponding to 51% of the total veterinary use of fluoroquinolones in Denmark.

Regarding amoxicillin-clavulanic acid, 91% of the veterinary consumption was used in pet animals; this combination has a very broad spectrum and should be reserved to 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 frequency using critically important antimicrobial agents is much higher for pet animals compared with food animals. Further more, broad-spectrum antimicrobial agents comprise a much lower proportion of the use in humans compared to the proportion of broad-spectrum antimicrobial agents in pet 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 should be a matter of concern. In particular, the consumption of critically important antimicrobial agents in pet animals could potentially pose an important risk to owners of diseased dogs. Presently, there is no information available about the antimicrobial resistance in pet animals.

In horse practice, sulfonamide-trimethoprim and beta-lactamase sensitive penicillins are used for the vast majority of treatments. However, a recent study has shown a very large variation in prescription habits between practices, with some practices using 3rd generation cephalosporins in a third of the treatments (Textbox 1).

These observations urgently call for treatment guidelines or other means to control the use of critically important antimicrobials in companion animals.

Vibeke Frøkjær Jensen and Birgitte Borck Høg

5. Antimicrobial consumptions in humans

5.1 Introduction

In Denmark, systemic antimicrobial agents for humans are available only on prescription. National and local guidelines, primarily based on resistance patterns, advocate the prudent use of antimicrobial agents, however, there are no restrictions for medical doctors to prescribe antimicrobial agents.

Throughout this section, the antimicrobial consumption in 2011 is compared to that of 2010 and of the last decade (2002). 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. Currently available antimicrobial agents for systemic treatment in humans (and in animals) are listed in Table 3.2.

Narrow- and broad-spectrum agents. Antimicrobial agents have been classified as either narrow-spectrum or broad-spectrum agents according to the spectrum of the activity against Gram-positive and Gram-negative bacteria (Table 5.1).

Defined Daily Dose (DDD). The DDD is defined as the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that DDD is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose [http://www.whoc.no].

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

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

DANMAP 2011

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	Nitrofuran 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 2011 edition of the Anatomical Therapeutic Chemical (ATC) classification system

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.

Packages per 1,000 inhabitants per year. Assuming that one prescription contains one package, this measurement is used as a surrogate for the number of prescriptions or treatments given to the primary health care population.

Treated patients per 1,000 inhabitants per year. Illustrates the number of patients treated in primary health care.

Kilogram. To allow comparison with consumption of antimicrobial agents in animals, total human consumption is also presented in kilograms.

DDD per 100 occupied bed-days (DBD) and DDD per 100 admissions (DAD). Consumption in somatic hospitals is presented in both DBD and DAD to account for hospital activity.

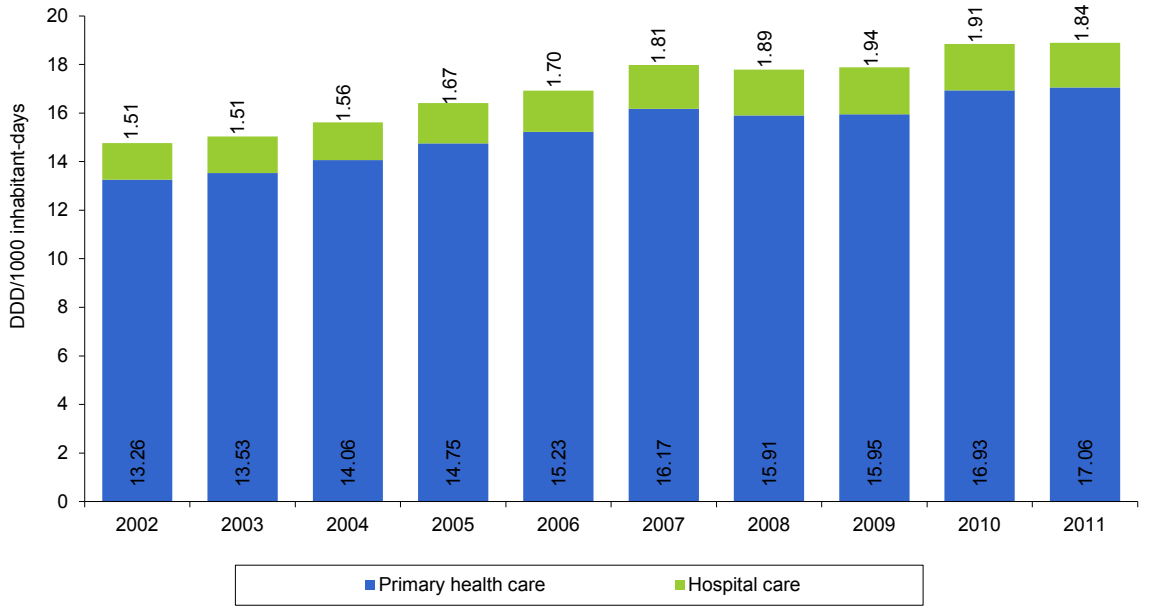
5.2 Total consumption in both primary health care and hospital care

In 2011, the total consumption of antimicrobial agents for systemic use (primary health care and hospital care) remained at the same level as in 2010 (18.84 DID in 2010 compared to 18.90 DID in 2011, Figure 5.1). In primary health care, the reported consumption still remained on an increasing level, in line with that observed during previous years. The consumption in hospital care, as measured by DID, decreased by 3.7% from 2010 to 2011. The total consumption of broad-spectrum agents also remained constant in 2011 (7.76 DID in 2010 compared to 7.80 DID in 2011, Figure 5.2). Primary health care represented 90% of all prescribed DDDs in Denmark in 2011. The consumption observed in primary health care in 2011 was the highest on record since 1995.

The distribution of DIDs between primary health care and hospital care differed between antimicrobial agents (Figure 5.3). For example, penicillins with extended spectrum (J01CA) had a ratio of consumption in primary health care vs. hospital care of around 12/1 while the same ratio for fluoroquinolones (J01MA) and macrolides (J01FA) was 3/1 and 28/1, respectively.

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

DANMAP 2011



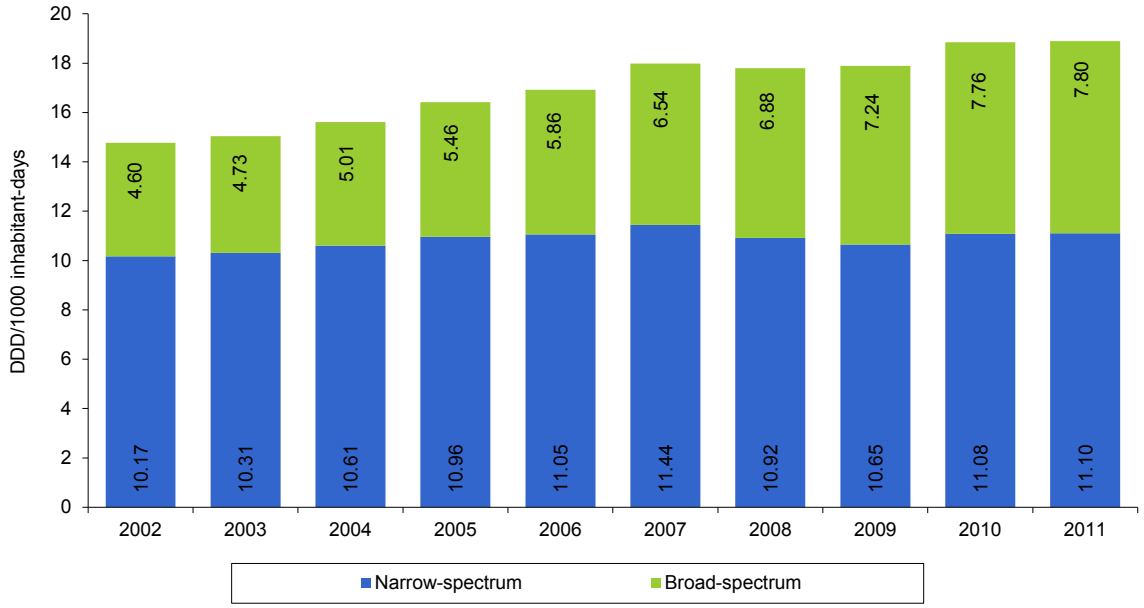
Since 2002, the overall consumption of antimicrobial agents has increased by 28%, or 4.13 DID (Figure 5.1). During the same period, broad-spectrum agents alone have increased by 3.2 DID (70%), comprising 31% of the overall consumption in 2002 and 41% in 2011. The proportion of DDDs prescribed in primary 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 5.3 and Table A5.6 in web annex, respectively.

In 2011, 51 tonnes of antimicrobial agents for systemic use (J01) were used in humans in Denmark. This level is similar to that reported in 2010 but represents an increase of 7.6 tonnes (18%) compared to 2002 (Table A5.6 in web annex).

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

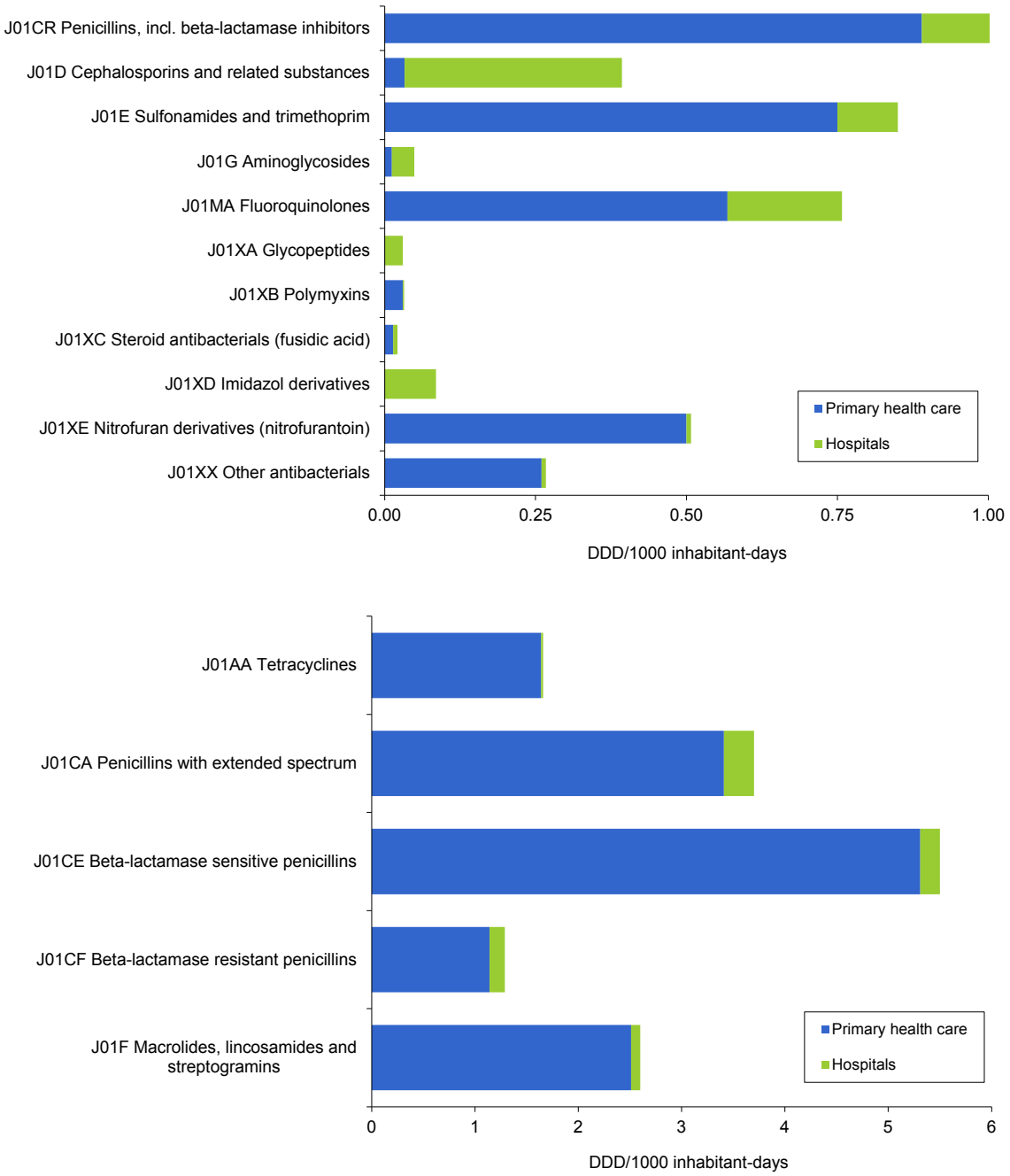
DANMAP 2011



Note: "Narrow-spectrum" includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran 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 2011



5.3 Primary health care

5.3.1 Total consumption in primary health care

In 2011, the total consumption of antimicrobial agents for systemic use (J01) in primary health care remained at the same level as in 2010 (17.06 DID in 2011, compared to 16.93 DID in 2010) but not deviating from the general upwards tendency observed since 1995. Increases were observed for three out of the 19 therapeutic groups (Table 5.2). The most pronounced increases were observed for 'combination penicillins' (0.22 DID) while relatively small upward changes were seen for beta-lactamase sensitive penicillins (0.06 DID) and macrolides (0.03 DID). Consumption decreased within four groups: penicillins with extended spectrum (0.06 DID), tetracyclines (0.05 DID), beta-lactamase resistant penicillins (0.03 DID) and short-acting sulfonamides (0.02 DID).

The consumption of antimicrobial agents in primary health care in 2011 was the highest observed since the initiation of DANMAP in 1995.
Compared to 2010, each treated patient in primary health care used an equal number of DDDs in 2011 (19.4 DDD vs. 19.6 DDD), as discussed in section 5.3.2. However, in 2011 more patients were treated and more packages prescribed than in 2010 (Tables A5.2 and A5.3 in web annex) which explains why the consumption in 2011 is slightly higher even though the number of DDDs used per patient remained constant.

The observed consumption of antimicrobial agents was markedly larger in the second half of 2011 for all antimicrobial agents (J01) in particular macrolides (J01FA) and beta-lactamase sensitive penicillins (J01CE). This pattern is most likely caused by the generally increased burden of lower respiratory tract infections (LRTIs) in late 2011, confirmed by an outbreak of *Mycoplasma pneumoniae* in October – December 2011 (Figure 5.11). 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.
Another driving factor behind the high overall consumption was a 0.89 DID (32%) increase in consumption of 'combination penicillins'. Indeed more patients were treated and more packages prescribed in this therapeutic group in 2011 (web annex, Tables A5.2 and

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

DANMAP 2011

ATC group[a]	Therapeutic group	Year									
		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
J01AA	Tetracyclines	1.04	1.07	1.17	1.28	1.38	1.48	1.54	1.61	1.69	1.64
J01CA	Penicillins with extended spectrum	2.51	2.52	2.63	2.79	2.95	3.25	3.26	3.29	3.47	3.41
J01CE	Beta-lactamase sensitive penicillins	5.00	5.07	5.20	5.28	5.40	5.67	5.30	5.12	5.25	5.31
J01CF	Beta-lactamase resistant penicillins	0.77	0.85	0.92	0.97	1.05	1.09	1.12	1.13	1.17	1.14
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.04	0.05	0.06	0.08	0.12	0.19	0.27	0.45	0.68	0.89
J01D	Cephalosporins and related substances	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.36	0.38	0.41	0.44	0.47	0.49	0.49	0.48	0.51	0.50
J01EB	Short-acting sulfonamides	0.36	0.36	0.36	0.35	0.35	0.31	0.28	0.27	0.26	0.24
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.15	2.13	2.23	2.41	2.31	2.42	2.28	2.21	2.44	2.47
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.18	0.25	0.28	0.33	0.37	0.44	0.51	0.52	0.57	0.57
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	0.02	0.03
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.02	0.02	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	Nitrofuran derivatives (nitrofurantoin)	0.41	0.42	0.43	0.45	0.46	0.47	0.47	0.49	0.51	0.50
J01XX	Other antibacterials (methenamine >99%)	0.34	0.32	0.30	0.28	0.27	0.26	0.27	0.26	0.27	0.26
J01	Antibacterial agents for systemic use (total)	13.26	13.53	14.06	14.75	15.23	16.17	15.91	15.95	16.93	17.06

a) From the 2011 edition of the Anatomical Therapeutic Chemical (ATC) classification system

A5.3) and an increased number of DDDs were supplied to each treated patient (Table 5.3). The possible reasons for this increase are discussed in section 5.3.2.

As observed in 2010, beta-lactamase sensitive penicillins still represented the largest therapeutic group of antimicrobial agents consumed in 2011 (31%), followed by penicillins with extended spectrum (20%) and macrolides (15%) (Figure 5.4). Penicillins accounted for 63% of the total consumption in 2011. Consumption of broad-spectrum agents showed a small upwards change of 0.10 DID (1.5%) compared to 2010 (Figure 5.5).

From 2002 to 2011, antimicrobial consumption (J01) increased by 28.7% from 13.26 DID in 2002 to 17.06 DID in 2011 (Table 5.2). Broad-spectrum agents represented 6.58 DID (38.6%) of the total consumption in 2011 compared to 3.85 DID (29%) in 2002; representing an increase of 78% (Figure 5.5). The consumption of all leading groups of antimicrobial agents was higher in 2011 than a decade earlier, and a steady increase from year to year has been observed for most of these groups (Figure 5.6). Only short-acting sulfonamides (J01EB), combinations of sulfonamides and trimethoprim (J01EE) and 'other antibacterials' (J01XX) were at a lower level in 2011 compared to 2002.

5.3.2 Measures at treated patient level

The total number (J01) of DDDs per treated patient was 19.4 compared to 19.6 in 2010. For substances with the highest consumption (DID), each treated patient received from 11.5 – 21.9 DDDs in 1.5 – 1.6 packages with the exception of tetracyclines (44 DDDs in 1.9 packages) (Table 5.3 and Table A5.4 in web annex).

Three different indicators of antimicrobial consumption at treated patient level in primary health care are available (Figure 5.7). From 2002 to 2011, the largest increases were observed in DDDs per treated patient (24%) and DDDs per prescribed package (20%) (Table 5.3). Steroid antimicrobials (fusidic acid), tetracyclines, 'combination penicillins' and fluoroquinolones showed the largest increases.

The reasons for these changing patterns are unclear, primarily because codes of indication for prescriptions are incomplete. A list of changes in packages and prescription guidelines is presented in DANMAP 2010. In early 2012, an agreement was reached between the Danish Ministry of Health and The Organisation for General Practitioners to remove broad codes of indication ('against infection' and 'against inflammation') from all electronic prescriptions, thereby increasing the likelihood of a practitioner providing more disease- or pathogen-specific information on a prescription.

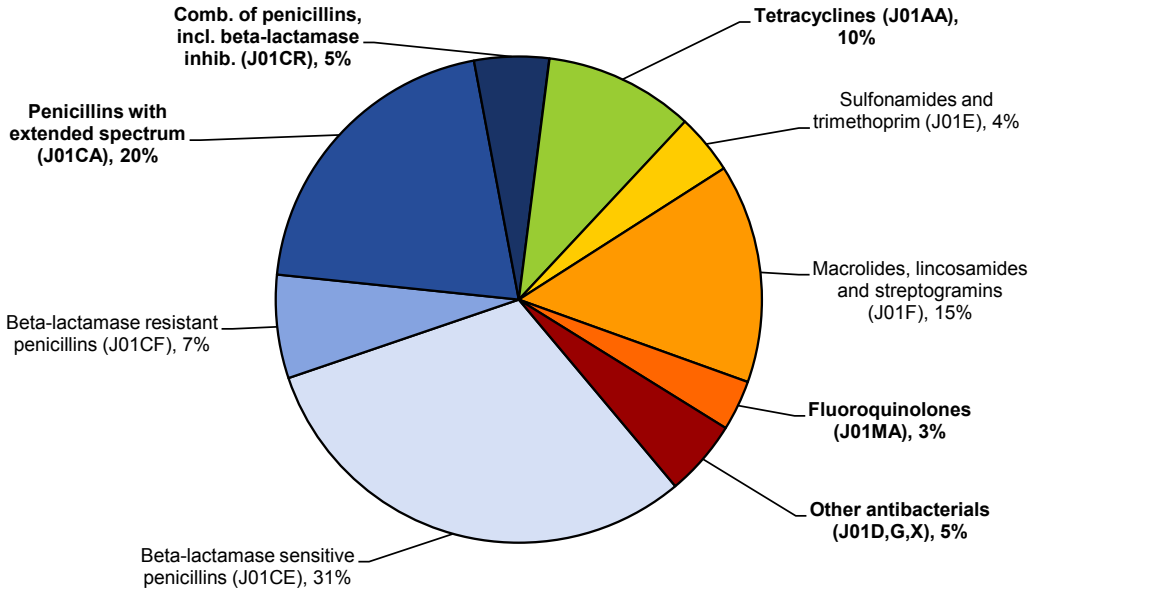
5.3.2 Tetracyclines (J01AA)

In 2011, the overall consumption of tetracyclines decreased by 0.05 DID (2.9%) compared to 2010 (Table 5.2). The most commonly used substance was tetracycline (0.78 DID, 48%) followed by doxycycline (0.47 DID, 29%), lymecycline (0.38 DID, 23%) and oxytetracycline (0.01 DID, 0.03%) (Figure 5.8). Compared to 2010, consumption of tetracycline and lymecycline increased while consumption of doxycycline and oxytetracycline decreased.

Since 2002, a considerable increase in the consumption of tetracyclines has been observed (Table 5.2). As shown in the DANMAP 2007 and 2008 reports, tetracyclines are often prescribed for teenagers and young adults, primarily against acne (tetracycline, J01AA07) and as malaria prophylaxis (doxycycline, J01AA02). Acne usually requires long treatment regimens and hence more DDDs per treated patient, and the continuous increase in DDDs per treated patient may therefore partly be related to increases in the number of acne prescriptions. A total understanding of the observed increase in the past decade, however, is not possible due to incomplete codes of indication.

Figure 5.4. Distribution of the total consumption of antimicrobial agents in primary health care, Denmark

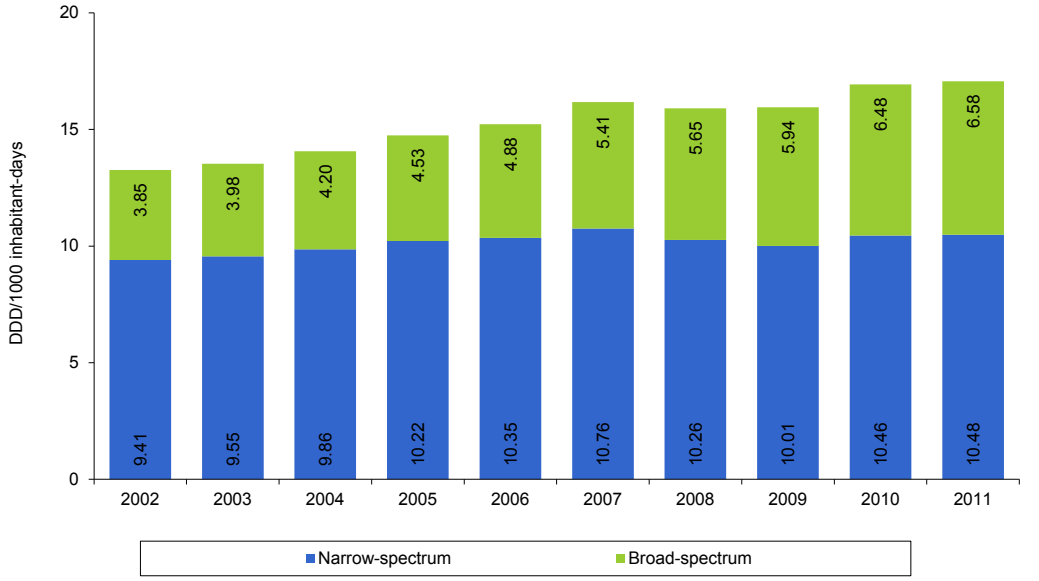
DANMAP 2011



Note: Bold highlights indicate broad-spectrum antibacterial agents

Figure 5.5. Consumption of antimicrobial agents (J01) in primary health care by narrow-spectrum and broad-spectrum agents, Denmark

DANMAP 2011



Note: "Narrow-spectrum" includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran 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 5.6. Consumption of leading antimicrobial groups for systemic use in primary health care, Denmark

DANMAP 2011

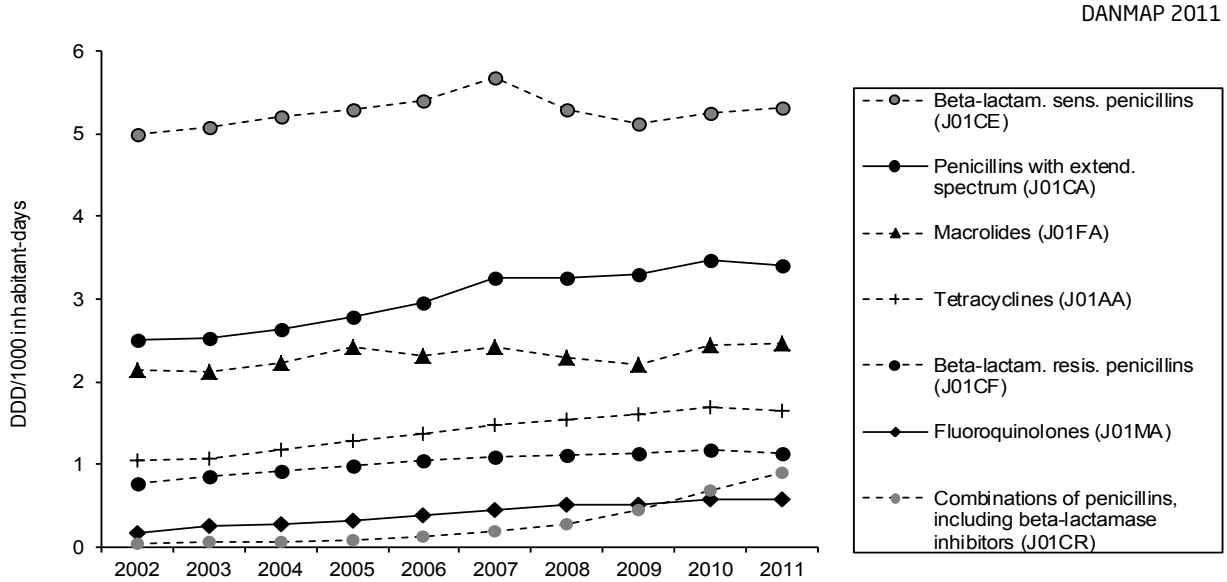


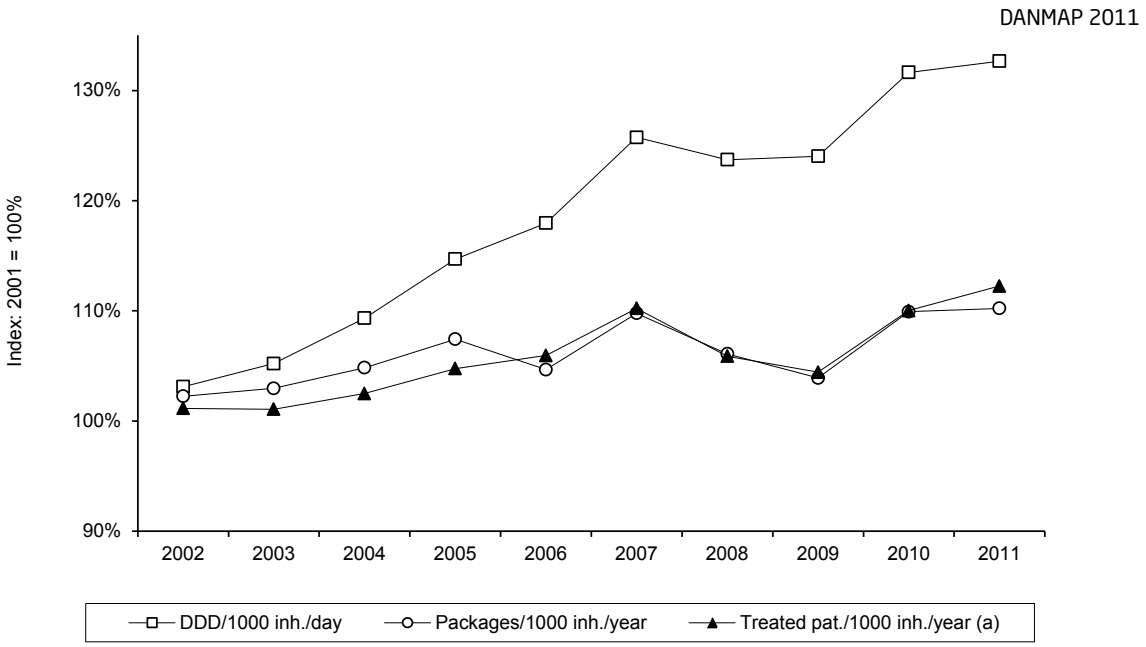
Table 5.3. Number of DDDs and packages per treated patient among leading groups of antimicrobial agents in primary health care, Denmark

DANMAP 2011

ATC group[a]	Therapeutic group	Indicator	Year									
			2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
J01AA	Tetracyclines	DDDs / patient	33.0	34.4	36.9	39.0	40.9	43.0	44.4	45.2	45.9	44.0
		Packages / patient	1.9	1.9	1.9	2.0	1.9	2.0	2.0	2.0	2.0	1.9
		DDDs / package	17.5	18.1	19.0	19.6	21.0	22.0	22.7	22.7	22.7	22.6
J01CA	Penicillins with extended spectrum	DDDs / patient	13.2	13.4	13.6	13.9	14.2	14.4	14.7	14.8	14.9	14.8
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
		DDDs / package	8.2	8.2	8.4	8.5	8.9	9.0	9.2	9.2	9.0	9.2
J01CE	Beta-lactamase sensitive penicillins	DDDs / patient	10.5	10.7	11.1	11.3	11.5	11.7	11.8	11.8	11.8	11.8
		Packages / patient	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.4	1.4
		DDDs / package	7.2	7.3	7.5	7.7	8.0	8.2	8.2	8.4	8.4	8.4
J01CF	Beta-lactamase resistant penicillins	DDDs / patient	11.8	11.8	12.4	12.7	13.0	13.4	13.7	13.9	14.2	13.8
		Packages / patient	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.4
		DDDs / package	7.5	7.4	7.8	8.0	8.6	8.7	9.0	9.1	9.3	9.6
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDDs / patient	14.7	16.6	17.2	16.8	19.3	19.1	19.9	20.4	21.1	21.9
		Packages / patient	1.7	1.8	2.0	2.0	1.8	1.6	1.6	1.5	1.5	1.6
		DDDs / package	8.6	9.1	9.1	9.3	10.7	11.7	12.4	13.3	13.7	14.1
J01FA	Macrolides	DDDs / patient	11.7	12.1	12.4	12.4	12.6	12.4	12.5	12.5	12.2	11.5
		Packages / patient	1.5	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.5
		DDDs / package	7.6	7.8	7.9	8.0	8.3	8.1	8.1	8.1	8.1	7.9
J01MA	Fluoroquinolones	DDDs / patient	8.6	10.3	9.5	9.6	10.3	10.6	11.0	11.2	11.2	11.5
		Packages / patient	1.4	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDDs / package	6.0	6.6	6.4	6.5	6.9	7.0	7.5	7.6	7.6	7.7
J01	Antibacterial agents for systemic use (total)	DDDs / patient	16.0	16.4	17.0	17.5	17.9	17.3	18.9	19.2	19.6	19.4
		Packages / patient	2.0	2.1	2.1	2.1	2.0	1.9	2.1	2.1	2.1	2.1
		DDDs / package	7.8	7.9	8.1	8.3	8.7	8.9	9.1	9.3	9.3	9.3

a) From the 2011 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.7. Indicators of antimicrobial consumption (J01) in primary health care, Denmark



a) Cumulated number of patients treated with anbacterials (ATC-4 level)

5.3.4 Penicillins (J01C)

The overall consumption of penicillins in 2011 showed a small upward change of 0.19 DID (1.8%) compared to 2010 (Table 5.2). In the four main groups, increases in consumption were observed for 'combination penicillins' (30.9%). For the individual substances, the consumption of amoxicillin and enzyme inhibitor (J01CR02) increased by 32% while smaller upward changes were observed for all other substances apart from pivampicillin (J01CA02), amoxicillin (J01CA04) and dicloxacillin (J01CF01) (Figure 5.9). The current guidelines advocate the use of 'combination penicillins' (primarily amoxicillin/clavulanic acid) for broad treatment of respiratory infections, and particularly for patients with exacerbation of chronic obstructive pulmonary disease.

During the past decade (2002 – 2011), the consumption of penicillins (J01C) increased by 2.45 DID (29.5%). This increase was apparent for all four groups of penicillins; penicillins with extended spectrum (0.91 DID, 36.2%), 'combination penicillins' (0.85 DID, 2200%), beta-lactamase resistant penicillins (0.37 DID, 48.1%) and beta-lactamase sensitive penicillins (0.31 DID, 6.2%). Phenoxymethylpenicillin continues to be the most commonly consumed penicillins; however the order has changed among the other substances during the last decade (Figure 5.9).

5.3.5 Macrolides (J01FA)

From 2010 – 2011, the consumption of macrolides showed a small upward change of 0.03 DID (1.2%) (Table 5.2). Within the macrolide group, erythromycin continued a decreasing trend (Figure 5.10) while increases were observed primarily for roxithromycin (0.10 DID) but also for azithromycin (0.04 DID) and clarithromycin (0.04 DID). As in previous years, part of the increased roxithromycin consumption was most likely caused by an outbreak of *M. pneumoniae* (see also section 5.3.1).

Figure 5.8. Consumption of tetracyclines in primary health care, Denmark

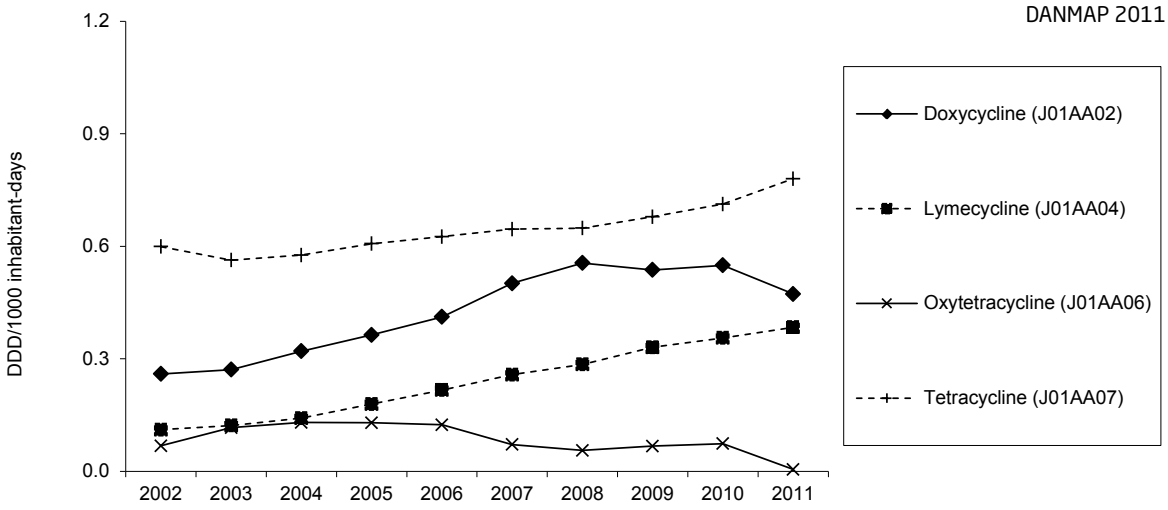
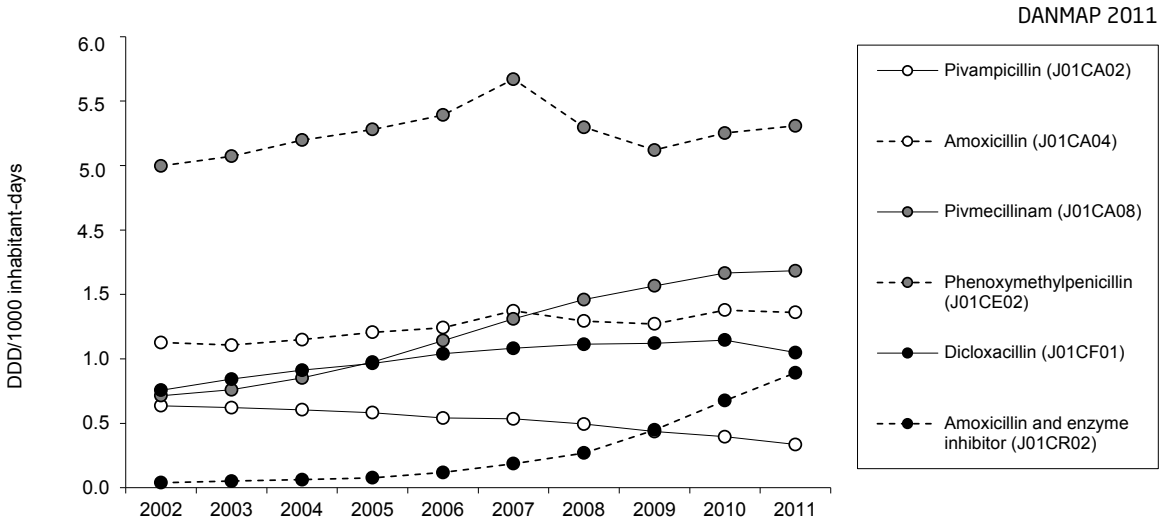


Figure 5.9. Consumption of leading penicillins in primary health care, Denmark



From 2002 to 2011, the consumption of roxithromycin increased noticeably (0.79 DID), with smaller increases also observed for clarithromycin (0.07 DID) and azithromycin (0.02 DID). The consumption of erythromycin decreased substantially (0.54 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 (J01MA)

The consumption of fluoroquinolones in 2011 remained at the same level as observed in 2010 (0.57 DID) (Table 5.2). Ciprofloxacin accounted for the majority of fluoroquinolone consumption (94%), followed by moxifloxacin (3.6%) and ofloxacin (1.9%) (Figure 5.12).

During the past decade, the consumption of fluoroquinolones has increased by 0.40 DID (238%), although with a marked drop in the rate of increase from 2009 and onwards (Table 5.2). As described in 2011 [Jensen and Bjerrum 2011. Ugeskr Læger. 173: 2853–2856] the continuously increasing consumption of ciprofloxacin is most likely due to the introduction of generic versions in Denmark in December 2001, followed by subsequent drops in prices. From a resistance perspective, this increase is particular grounds for concern as ciprofloxacin is strongly associated with resistance.

Figure 5.10. Consumption of macrolides in primary health care, Denmark

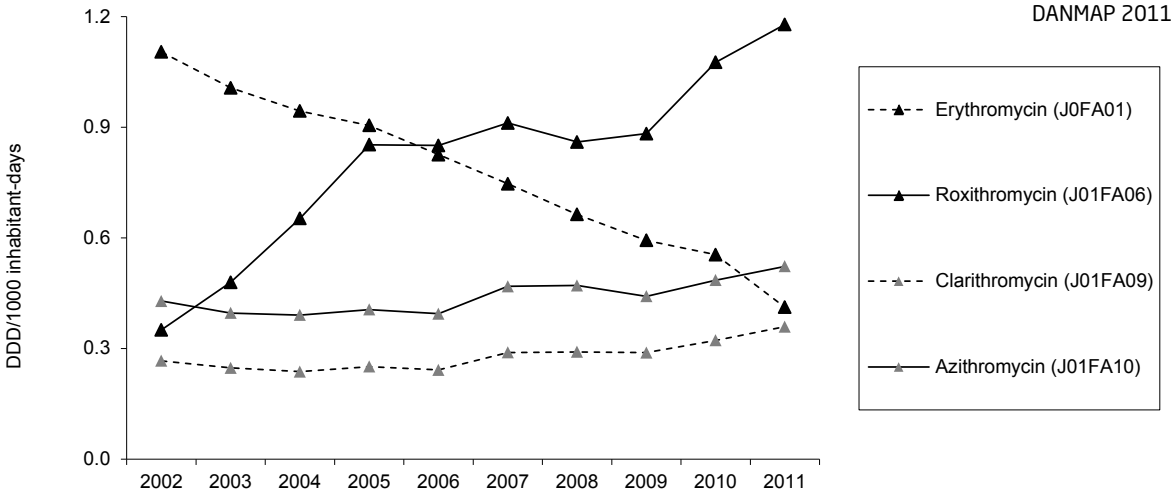


Figure 5.11. Monthly consumption of macrolides and beta-lactamase sensitive penicillins and PCR positive *Mycoplasma pneumoniae* tests in primary health care, Denmark

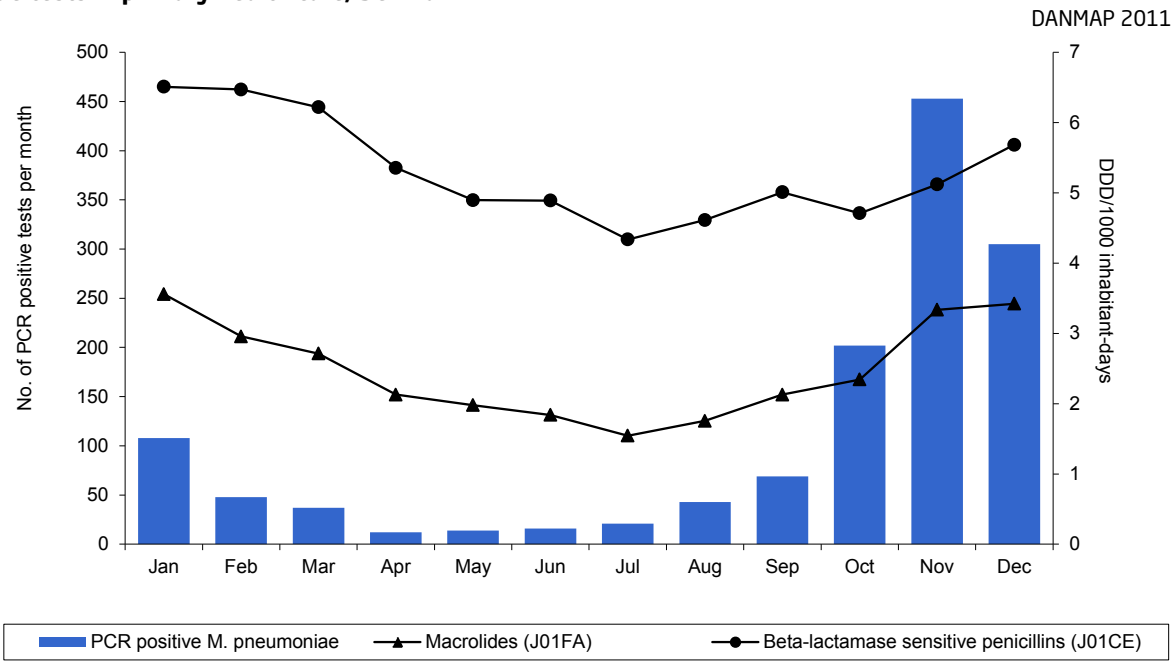
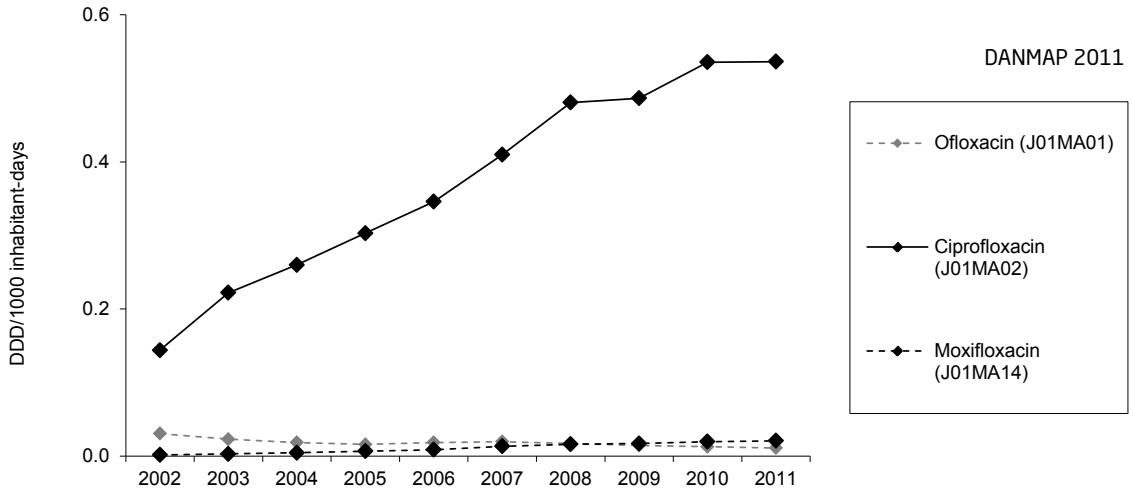


Figure 5.12. Consumption of leading fluoroquinolones in primary health care, Denmark



5.4 Hospital care

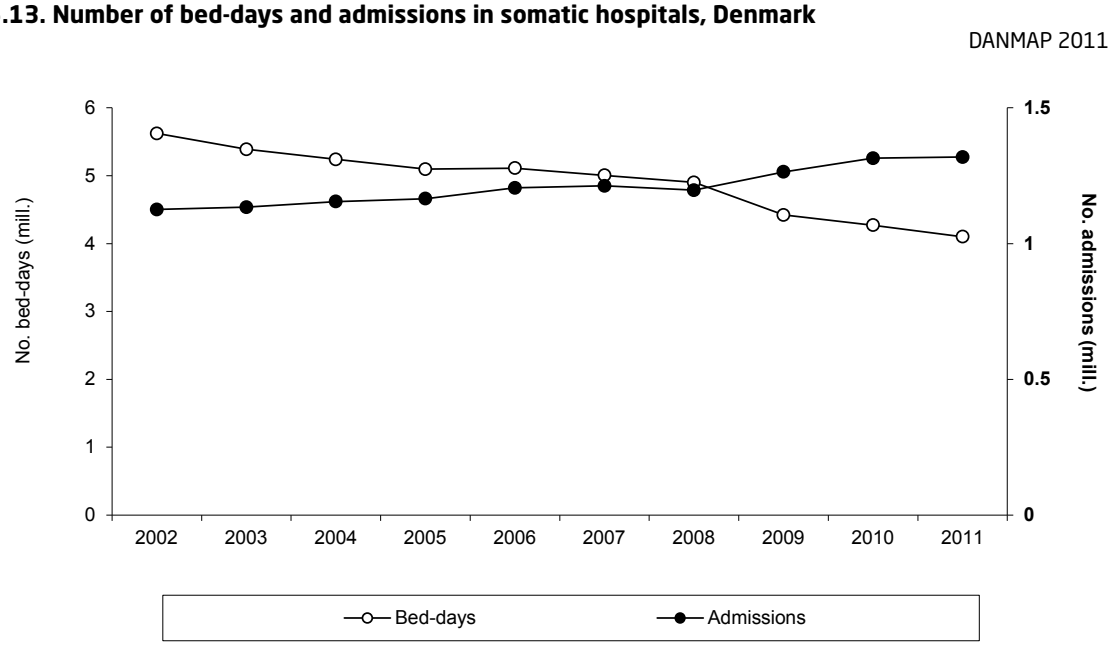
5.4.1 Introduction

The consumption of antimicrobial agents in the hospital sector is presented as both DDD per 100 occupied bed-days (DBD) and DDD per admissions (DAD) to account for hospital activity. Furthermore, data are also presented 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 2011 than in 2002.

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



E-bug: Raising awareness of prudent use of antimicrobial agents in future generations

Antimicrobial resistance is related to use of antimicrobial agents and has previously been connected to hospitals. It is now an increasing community problem. If use of antimicrobial agents could be reduced through preventive hygienic methods, the tide of increasing resistance could be stemmed. e-Bug is a European project for the generation for whom the resistance problem will be very important - the children. e-Bug involves 18 European countries and is partly funded by The Directorate-General for Health and Consumers (DG SANCO) of the European Commission.

The aim of e-Bug is to develop and disseminate across Europe a junior and senior school teaching pack and web site that teaches young people about prudent use of antimicrobial agents, microbes, transmission of infection, preventive hygiene and vaccines. The website (www.e-bug.eu) hosts the lesson plans and complementary games and is easily accessed for children, parents and teachers with access to computers and the internet. The overall goal of the project is to increase young people's understanding of why prudent use of antimicrobial agents is so important when trying to control antimicrobial resistance, and to emphasize the importance of proper hand and respiratory hygiene to help reduce the spread of infection. Within the senior school pack the sexual transmission of infections has also been included, as the peak age of chlamydial infection is in 16–24 year olds.

Teachers, young people and the consortium of 18 countries were closely involved in formulating the learning outcomes and developing the resource activities. Young people helped create the characters and microbe artwork. The material builds on the theory of many learning styles and the explorative approach that creates the understanding of the many factors involved. The resources have been translated, adapted for and disseminated to schools across 10 countries in Europe, and endorsed by the relevant government departments of health and education. The teaching resources have already been used to promote European Antibiotic Awareness Day and better hand and respiratory hygiene in several participating countries. The website has been visited by people from more than 100 countries. In the near future, all teaching resources will be translated into all European Union languages.

Compared to most other European countries, bacterial resistance to antimicrobial agents in primary health care in Denmark is low. The goal of participating in e-Bug was to keep it that way. The Danish "Folkeskole", which is the Danish municipal primary and lower secondary school, uses the principle of differentiated teaching, integrating information technology, and there are no recommended textbooks in the curriculum. The teaching is organized so that it both strengthens and develops an individual student's ability to act and care for themselves and for society in general. e-Bug, in its design, was seen as a tool that could be implemented well in the Danish "Folkeskole", as it fulfills the goals on teaching methods and output. During the initial phase, 2000 e-Bug packs were printed and 100 local education authorities and 1507 school principals were contacted. Since then, e-Bug has been presented to important stakeholders in relevant scientific magazines and at various meetings for teachers and infection control members. Today, the project has been well accepted and the Danish website (www.e-bug.eu/dk) has approximately 350 visits per month.

The development and implementation of e-Bug across Europe is described in detail in a series of articles in Journal of Antimicrobial Chemotherapy, Volume 66 Supplement, 5 June 2011.

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The association between increased antimicrobial consumption and demographic factors in Denmark, 2001 to 2010

Background: The Danish system for monitoring human consumption of antimicrobial agents is one of the few in the world allowing surveillance of all antimicrobial agents prescribed in the primary and hospital sectors. Using these data, it has previously been demonstrated that the consumption of antimicrobial agents in primary health care in Denmark increased by 32% from 2001 to 2010 [DANMAP 2010]. This increase in consumption was mirrored by a concurrent increase in the occurrence of resistant bacteria. The aim of this study was to investigate the demographic factors associated with the increased antimicrobial consumption in Denmark during the past decade.

Materials and methods: Measures of defined daily doses per 1,000 inhabitant-days (DID) for leading antimicrobial groups (ATC-4 level) and substances (ATC-5 level) in primary health care in 2001 and 2010 were obtained from the Danish Medicines Agency, adjusted for population size and compared between years and between age and gender. Data were analysed by t-tests and a multivariate stepwise regression model. Consumption tendencies were assessed with reference to data on visits to a general practitioner (GP).

Results: From 2001 to 2010, the consumption of antimicrobial agents (DID) increased for all age groups and both genders (Figure 1). In both years, females consumed significantly more antimicrobial agents than males. The most marked increases were shown for 15 – 19 year olds and persons older than 65 years. Children up to 9 years had a significantly lower consumption compared to the rest of the population, in both 2001 and 2010. The increase in DIDs for this age group was also significantly lower than the increase in consumption for the rest of the population. The observed difference in DIDs in 2001compared to 2010 varied according to the group (ATC-4 level) and substance (ATC-5 level) (Figure 2). For instance, the consumption of lymecycline and tetracycline increased particularly in 15 – 19 year olds while increases in DIDs for pivmecillinam and amoxicillin/clavulanic acid were observed for people older than 65 years. A multivariate model explained 82% of the observed variation in consumption in 2001 compared to 2010, with 15 – 19 year olds having the most significant effect on the difference in DID between the two years. General increases in consumption for all ages and both genders were shown for roxithromycin and ciprofloxacin. The overall frequency of GP visits increased during the period, but decreases of up to 10% were observed in some age groups (Figure 3).

Discussion and conclusions: During 2001–2010 consumption increases were observed for several leading antimicrobial groups and substances, driven primarily by people older than 65 years and adolescents. The age-related increases observed in tetracyclines and pivmecillinam are most likely related to changes in the treatment of acne and urinary tract infections, respectively. Population-wide increases in the consumption of roxithromycin may partly be explained by the occurrence of several large Mycoplasma pneumoniae outbreaks, while the increase in ciprofloxacin consumption is most likely related to the introduction of generic versions in late 2001 [Jensen et al. 2010. J Antimicrob Chemother. 65:1286-1291]. The increases observed for both roxithromycin and ciprofloxacin are grounds for concern as these substances are broad-spectrum antimicrobial agents and thus strongly associated with selection of resistant bacteria. Although increases in antimicrobial consumption may generally be due to many factors, evidence suggests that increases observed from 2001 to 2010 are a combination of disease-specific factors and not least an inclination of GPs to prescribe more antimicrobial agents per visit and in larger doses. This in itself is not entirely bad news but could rather be a reflection of changed guidelines which advocate increased dosages for shorter periods, in order to decrease the likelihood of selection for resistance. Overall, however, it is important to emphasize the need for not prescribing antimicrobial agents without a confirmed diagnosis.

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Figure 1. Consumption (DID) of antimicrobial agents (ATC-2) over ten years by age and gender

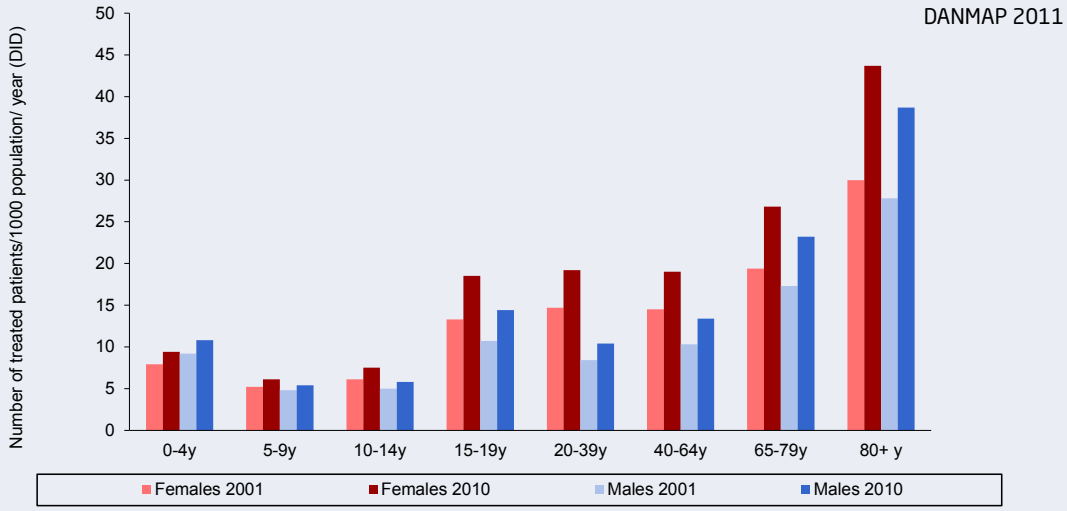


Figure 2. Consumption (DID) of leading antimicrobial groups (ATC-4) in Denmark, 2001 and 2010

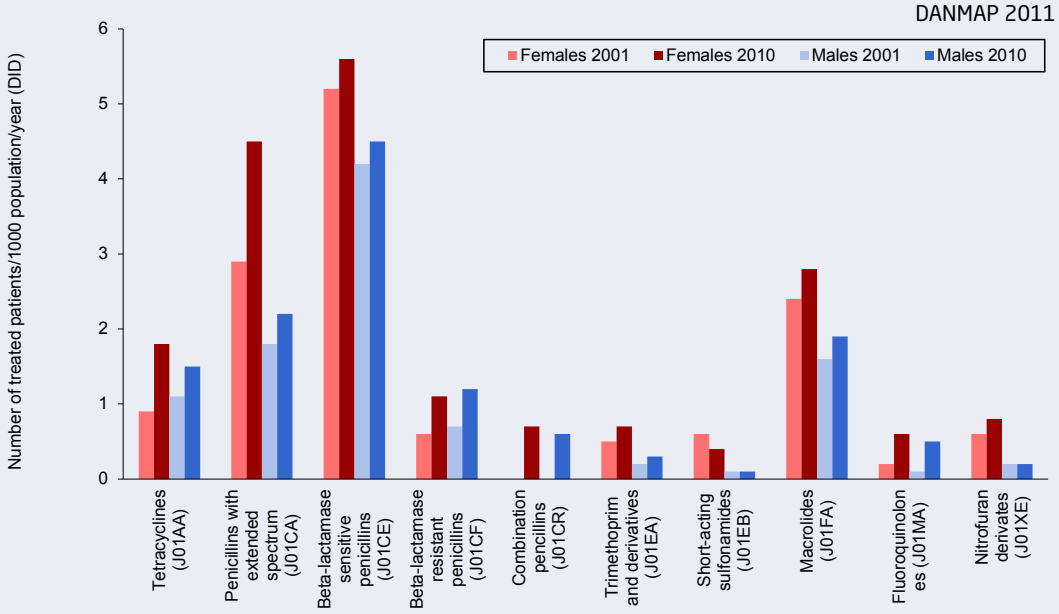


Figure 3. Prevalence of GP visits over ten years by age and gender

The reported sales (consumption) of certain infusion substances by the hospital pharmacies to the Danish Medicines Agency has for several years been inaccurate due to merging of antimicrobial agents and infusion liquids. This is particularly relevant for cephalosporins (J01DB, J01DC, J01DD), carbapenems (J01DH), combinations of sulfonamides and trimethoprim (J01EE) and imidazole derivatives (J01XD). The consumption of these substances has been corrected by direct data collection from all Danish hospital pharmacies.

5.4.2 Total consumption in hospital care - DDD per 1,000 inhabitants per day (DID)

The total consumption of antimicrobial agents in the Danish hospital sector (as defined above) in 2011 was 1.84 DID - a decrease of 3.7% compared to 2010 (Figure 5.14). Broad-spectrum agents represented 66% compared to 67% in 2010.

Since 2002, the total consumption of antimicrobial agents has increased by 0.38 DID (26%). Broad-spectrum agents have increased by 0.47 DID (67%), comprising 66% of the total consumption in 2011 compared to 50% in 2002 (Figure 5.14 and Table A5.6 in web annex).

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

Consumption (DBD) in somatic hospitals compared to 2010

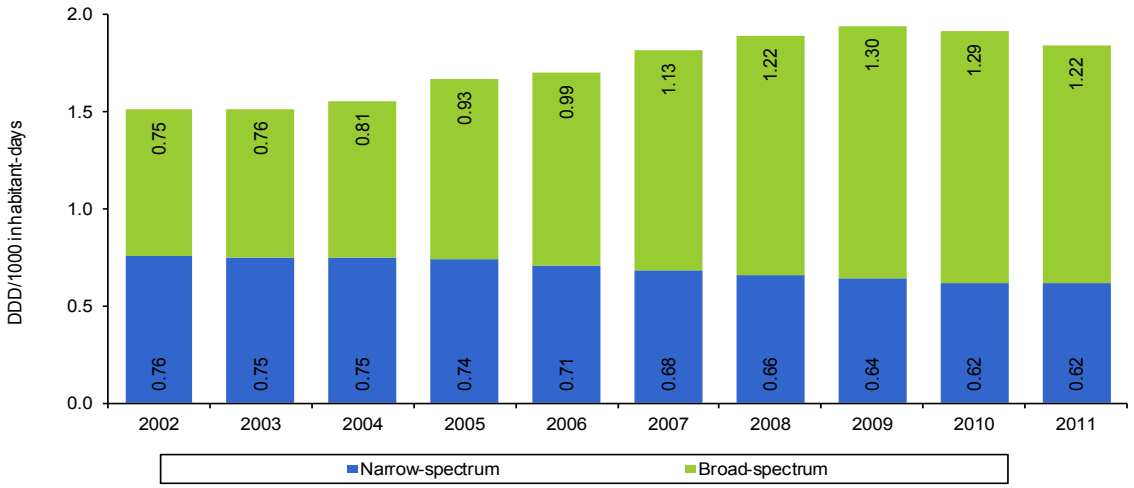
The consumption of antimicrobial agents (J01) in somatic hospitals increased by 3.12 DBD (3.6%) from 2010 to 2011 (Table 5.4). Five therapeutic groups dominated the increasing consumption: linezolid (0.10 DBD, 45.5%), combinations of sulfonamides and trimethoprim (1.07 DBD, 35%), 'combination penicillins' (1.38 DBD, 19%), fluoroquinolones (0.26 DBD, 6.7%), and macrolides (0.17 DBD, 4.7%), but upward changes of ≥ 0.10 DBD were also observed for aminoglycosides, glycopeptides, carbapenems and monobactams. These changes were due both to decreasing numbers of bed-days as well as increasing DDDs.

In two therapeutic groups, consumption decreased ≥ 0.10 DBD: beta-lactamase resistant penicillins (0.41 DBD, 5.3%) and penicillins with extended spectrum (0.20 DBD, 1.4%). These changes were due to a decreasing number of DDDs in relation to a decreasing number of bed-days.

Cephalosporins accounted for 19% of the total consumption of antimicrobial agents in somatic hospitals (Figure 5.15), followed by penicillins with extended spectrum (15%), fluoroquinolones (12%) and beta-lactamase sensitive penicillins (10%).

Figure 5.14. Consumption of antimicrobial agents (J01) in hospital care by narrow-spectrum and broad-spectrum agents, Denmark

DANMAP 2011



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, nitrofuran 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.

Consumption (DBD) in somatic hospitals - the last decade
From 2002 – 2011, the total consumption of antimicrobial agents (J01) increased by 39.11 DBD (76%) (Table 5.4). This increase was due to a 26% increase in the total number of DDDs combined with a 31% decrease in the number of hospital bed-days.

During the past decade, the consumption of broad-spectrum antimicrobial agents in Danish somatic hospitals has increased by 137%, from 25.8 DBD in 2002 to 61.2 DBD in 2011 (Figure 5.16). In 2002, consumption of penicillins with extended spectrum and beta-lactamase sensitive penicillins each represented 22% of the total somatic hospital antimicrobial consumption, compared to 16% and 10%, respectively, in 2011. In the broad-spectrum group, the consumption of fluoroquinolones in somatic hospitals increased by 0.05 DBD (74%) and the consumption of carbapenems increased by 0.03 DBD (283%) during the past decade. Consumption of cephalosporins increased by 133% from 2002–2011.

Although broad-spectrum antimicrobial agents offer treatment for a wider range of pathogens, their potential advantage is now known to be outweighed by the fact that these substances are known to select for resistance (see chapter 8). In this context, it is noteworthy that the rate of increase for the consumption of both cephalosporins and fluoroquinolones has been considerably lower from 2009 onwards.

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

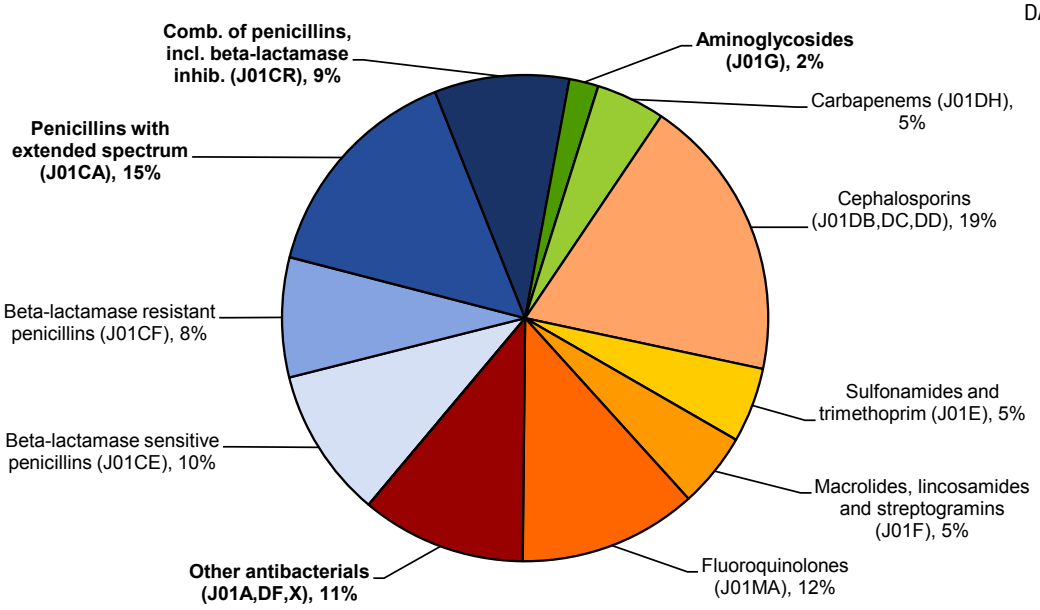
DANMAP 2011

ATC group[a]	Therapeutic group	Year									
		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
J01AA	Tetracyclines	0.32	0.30	0.32	0.33	0.39	0.63	0.78	1.04	1.09	1.18
J01CA	Penicillins with extended spectrum	11.34	11.55	11.51	12.90	13.00	13.42	13.96	15.37	14.61	14.41
J01CE	Beta-lactamase sensitive penicillins	11.37	11.85	12.02	12.17	10.67	10.79	9.98	9.90	9.49	9.32
J01CF	Beta-lactamase resistant penicillins	6.24	6.54	6.78	6.71	6.51	6.70	6.81	7.40	7.71	7.30
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.31	0.49	0.84	1.16	1.83	2.95	4.00	5.65	7.13	7.13
J01DB	First-generation cephalosporins	0.14	0.14	0.17	0.15	0.14	0.13	0.18	0.13	0.13	0.13
J01DC	Second-generation cephalosporins	5.79	6.24	6.91	8.39	9.38	12.31	13.32	15.76	16.21	16.14
J01DD	Third-generation cephalosporins	0.65	0.67	0.67	0.83	0.83	1.03	1.25	1.42	1.26	1.39
J01DF	Monobactams	0.00	0.01	0.00	0.00	0.00	0.04	0.07	0.06	0.09	0.19
J01DH	Carbapenems	0.60	0.68	0.85	1.16	1.38	2.13	2.70	3.15	4.02	4.16
J01EA	Trimethoprim and derivatives	0.41	0.43	0.41	0.41	0.42	0.44	0.44	0.44	0.36	0.36
J01EB	Short-acting sulfonamides	1.23	1.14	1.06	0.99	0.75	0.34	0.35	0.35	0.33	0.25
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	1.46	1.54	1.86	2.11	2.12	1.52	1.95	2.28	3.04	4.11
J01FA	Macrolides	3.21	2.95	2.85	2.89	2.83	3.08	3.06	3.42	3.52	3.69
J01FF	Lincosamides	0.19	0.22	0.23	0.24	0.31	0.35	0.41	0.50	0.47	0.53
J01GB	Aminoglycosides	1.76	1.71	2.00	1.95	1.81	1.79	1.64	1.56	1.71	1.91
J01MA	Fluoroquinolones	3.52	3.90	4.93	6.14	6.74	8.16	9.53	10.71	10.44	10.70
J01XA	Glycopeptides	0.38	0.42	0.47	0.52	0.56	0.63	0.68	0.99	1.07	1.24
J01XB	Polymyxins	0.04	0.03	0.06	0.12	0.12	0.05	0.05	0.07	0.10	0.09
J01XC	Steroid antibacterials (fusidic acid)	0.19	0.22	0.22	0.25	0.28	0.28	0.26	0.31	0.34	0.27
J01XD	Imidazole derivatives	2.12	2.32	2.43	2.62	2.78	2.62	3.27	3.84	3.93	4.19
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.28	0.30	0.28	0.29	0.29	0.28	0.29	0.36	0.31	0.33
J01XX05	Methenamine	0.12	0.08	0.10	0.08	0.11	0.09	0.10	0.09	0.08	0.10
J01XX08	Linezolid	0.04	0.04	0.07	0.15	0.20	0.16	0.21	0.22	0.22	0.32
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.02	0.02
J01	Antibacterial agents for systemic use (total)	51.73	53.77	57.04	62.58	63.47	69.94	75.28	85.03	87.72	90.84

a) From the 2011 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.15. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark

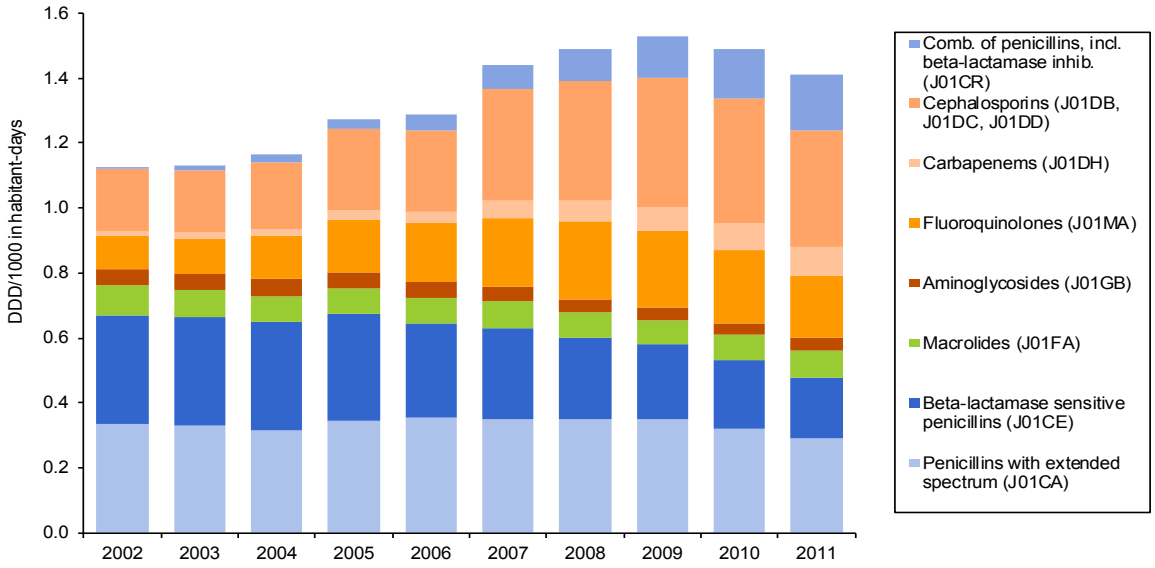
DANMAP 2011



Note: Bold highlights indicate broad-spectrum antibacterial agents

Figure 5.16. Total somatic hospital consumption (DID) by leading groups of antimicrobial agents (J01), Denmark

DANMAP 2011



5.4.4 Somatic hospital consumption - DDD per 100 admissions (DAD)

The total consumption of antimicrobial agents (J01) in Danish somatic hospitals in 2011 remained at the same level as observed in 2010, when expressed as DAD (DDD per 100 admissions) (Table 5.5). Increases in consumption were partly observed for the same therapeutic groups as when expressed in DBD: linezolid (0.27 DAD, 37.5%), combinations of sulfonamides and trimethoprim (2.91 DAD, 29.5%), 'combination penicillins (3.32 DAD, 14.3%) and aminoglycosides (0.39 DAD, 6.9%). A decreased consumption was observed for beta-lactamase resistant penicillins (2.33 DAD, 9.3%), beta-lactamase sensitive penicillins (1.85 DAD, 6%), penicillins with extended spectrum (2.69 DAD, 5.7%) and second-generation cephalosporins (2.46 DAD, 4.7%).

From 2002 – 2011, the consumption of antimicrobial agents (J01) increased by 9.3%, from 258.46 DAD in 2002 to 284.89 DAD in 2011. As observed in previous years, this increase was primarily driven by a 28% increase in the number of DDDs but counterbalanced by a 17% increase in the number of hospital admissions.

The increase in consumption observed when expressed as DBD contrasts the no change observed when consumption is expressed as DAD. This illustrates the necessity for using different indicators to express the consumption of antimicrobial agents in hospitals.

Katrin Gaardbo Kuhn and Maja Laursen

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

DANMAP 2011

ATC group[a]	Therapeutic group	Year									
		2002	2003	2004	2005	2006	2007	2008[b]	2009	2010	2011
J01AA	Tetracyclines	1.58	1.43	1.45	1.45	1.67	2.59	3.19	3.63	3.55	3.66
J01CA	Penicillins with extended spectrum	56.68	54.88	52.22	56.43	55.13	55.39	57.18	53.76	47.46	44.77
J01CE	Beta-lactamase sensitive penicillins	56.79	56.33	54.53	53.20	45.26	44.55	40.90	34.61	30.83	28.98
J01CF	Beta-lactamase resistant penicillins	31.18	31.11	30.77	29.33	27.60	27.64	27.89	25.86	25.04	22.71
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	1.56	2.35	3.82	5.09	7.77	12.17	16.37	19.74	23.15	26.47
J01DB	First-generation cephalosporins	0.72	0.67	0.76	0.67	0.60	0.55	0.72	0.46	0.43	0.41
J01DC	Second-generation cephalosporins	28.95	29.66	31.36	36.70	39.76	50.81	54.55	55.12	52.65	50.19
J01DD	Third-generation cephalosporins	3.25	3.17	3.06	3.62	3.53	4.24	5.10	4.98	4.10	4.33
J01DF	Monobactams	0.02	0.02	0.02	0.02	0.00	0.18	0.27	0.21	0.29	0.60
J01DH	Carbapenems	2.98	3.24	3.85	5.05	5.86	8.78	11.08	11.01	13.07	12.55
J01EA	Trimethoprim and derivatives	2.07	2.05	1.86	1.78	1.78	1.81	1.80	1.56	1.17	1.11
J01EB	Short-acting sulfonamides	6.16	5.44	4.82	4.32	3.18	1.41	1.43	1.21	1.09	0.78
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	7.31	7.32	8.44	9.21	8.98	6.28	7.98	7.96	9.88	12.79
J01FA	Macrolides	16.03	14.03	12.92	12.64	12.01	12.70	12.53	11.97	11.45	11.47
J01FF	Lincosamides	0.94	1.05	1.04	1.05	1.31	1.46	1.69	1.74	1.52	1.63
J01GB	Aminoglycosides	8.79	8.14	9.07	8.55	7.68	7.39	6.71	5.45	5.56	5.95
J01MA	Fluoroquinolones	17.60	18.53	22.38	26.87	28.58	33.66	39.04	37.45	33.92	33.30
J01XA	Glycopeptides	1.88	1.97	2.12	2.28	2.38	2.61	2.77	3.48	3.47	3.87
J01XB	Polymyxins	0.20	0.14	0.27	0.54	0.53	0.22	0.21	0.24	0.32	0.28
J01XC	Steroid antibacterials (fusidic acid)	0.97	1.04	1.01	1.11	1.19	1.17	1.05	1.09	1.12	0.85
J01XD	Imidazole derivatives	10.57	11.03	11.02	11.47	11.81	10.83	13.39	13.43	12.76	13.03
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.42	1.41	1.26	1.28	1.24	1.17	1.19	1.27	1.01	1.02
J01XX05	Methenamine	0.61	0.37	0.45	0.36	0.46	0.38	0.43	0.31	0.27	0.32
J01XX08	Linezolid	0.22	0.21	0.34	0.64	0.86	0.68	0.84	0.76	0.72	0.99
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.06	0.07	0.05
J01	Antibacterial agents for systemic use (total)	258.46	255.59	258.81	273.67	269.18	288.70	308.39	297.36	284.89	282.53

a) From the 2011 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

6. Resistance in zoonotic bacteria

Zoonoses are infections and diseases that are transmissible between animals and humans, either via direct contact or indirectly via contaminated food. Zoonotic bacteria such as *Salmonella* and *Campylobacter* can develop resistance to antimicrobial agents as a result of treatment of the animals, which subsequently may lead to limitation of treatment possibilities or treatment failure of human infections. A more detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in Textbox 4 and in the Annual Report on Zoonoses in Denmark 2011 [www.food.dtu.dk].

6.1 *Salmonella*

Salmonella is an important zoonotic pathogen of great economic impact in both animals and humans. Human salmonellosis is typically characterised by watery, sometimes bloody diarrhoea, abdominal pain, fever, headache and nausea after an incubation period of 12 to 36 hours. Extraintestinal infections or post-infection complications such as reactive arthritis can occur

The common reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals. In animals, infections are often sub-clinical. Transmission of *Salmonella* to humans often happens through food that has been contaminated with *Salmonella,* which has subsequently been allowed to multiply due to e.g. inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food. In Denmark, as well as in the 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 and poultry meat, while *S.* Typhimurium cases are mostly associated with the consumption of contaminated pork, beef as well as poultry meat.

Clonal dissemination plays an important role for the spread of antimicrobial resistant *Salmonella* spp., particularly within *S.* Typhimurium. Examples of this are the rapid, global dissemination of the monophasic variants of *S.* Typhimurium, which are resistant to ampicillin (A), streptomycin (S), sulfonamide (Su) and tetracycline (T), often referred to as the ASSuT-profile. Presumably also as a consequence of clonal dissemination, certain phage types appear to be strongly associated with particular resistance patterns, as is observed with the monophasic *S.* Typhimurium DT193 and DT120 strains.

However, DT193 and DT120 are also commonly found in other lineages, for instance lineages derived from DT104 which are typically resistant to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su) and tetracycline (T), known as the 'classic DT104 pattern' or the ACSSuT-profile. For a detailed presentation of the relationship between phage types and the ASSuT and ACSSuT resistance profiles, please see DANMAP 2010.

Table 6.1. Phage type distribution (%) among *Salmonella* Typhimurium[a] from pigs[b], Danish pork and human cases[c], Denmark

DANMAP 2011

Phage type	Pigs	Pork	Human			
	%	Danish %	Domestic sporadic %	Domestic outbreak %	Travel abroad %	Unknown origin %
DT 193	13	20	21	0	47	28
DT 120	27	24	16	100	10	16
DT 104	3	8	10	0	5	7
DT 12	14	4	3	0	1	6
DT U292	1	0	8	0	1	5
DT U302	7	2	2	0	1	0
DT 170	6	2	3	0	0	1
DT 1	0	0	2	0	5	6
DT 135	1	0	2	0	3	4
DT 17	5	4	0	0	0	1
DT 8	0	0	3	0	3	2
Other/ unspc.	23	35	27	0	23	24
Number of isolates	162	49	202	21	73	83

a) Include isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-
b) Some of the isolates from pigs (n = 40) and humans (n = 4) included in the analysis of resistance was not phage typed in 2011
c) The isolate was categorised as 'domestic sporadic' if the patient did not travel one week prior to the infection and was not reported as being part of an outbreak

Annual Report on Zoonoses in Denmark 2011

Background: The Annual Report on Zoonoses presents a summary of the trends and sources of zoonotic infections in humans and animals, as well as the occurrence of zoonotic agents in food and feeding stuffs in Denmark in 2011. The report is based on data compiled according to the zoonoses directive 03/99/EEC, supplemented by data obtained from national surveillance and control programmes and data from relevant research projects. The report is available at www.food.dtu.dk.

Surveillance of *Salmonella* and *Campylobacter*: 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. Eggs from *Salmonella* positive laying hen flocks are heat treated or destroyed, and meat from broiler flocks found *Salmonella* positive at the ante-mortem control is heat treated. An extensive *Salmonella* surveillance and control programme is also running in the Danish pig production. At herd level, slaughter pig herds are monitored using serological testing of meat juice samples collected at slaughter, and high-risk herds are slaughtered under special hygienic precautions. Breeder and multiplier herds are monitored using serological testing of blood samples collected at farm, and fecal samples are collected in high-risk herds in order to assess the serotype distribution. In sow herds receiving animals from high-risk breeder and multiplier herds, fecal samples are collected as well. At the slaughterhouses, swabs samples from pig and cattle carcasses and neck skins from broiler carcasses are collected after chilling.

Since January 2010, a mandatory surveillance of *Campylobacter* in broiler flocks at the farm has been in place. To the extent possible, positive flocks are allocated to the production of frozen products. At the slaughter houses and at retail level, *Campylobacter* in fresh broiler meat are surveyed.

Finally, a case-by-case based control programme for *Salmonella* and *Campylobacter* in Danish and imported broiler meat, turkey meat, beef and pork ready for retail has been in place since 2007. Samples are collected at cold storages, slaughter houses, processing and catering facilities as well as at the border control.

The occurrence of *Salmonella* and *Campylobacter* in the primary production, at the slaughter houses and in Danish and imported fresh meat ready for retail are presented in Table 1.

Table 1. Occurrence of *Salmonella* and *Campylobacter* in pig, cattle and broiler production in Denmark, 2011

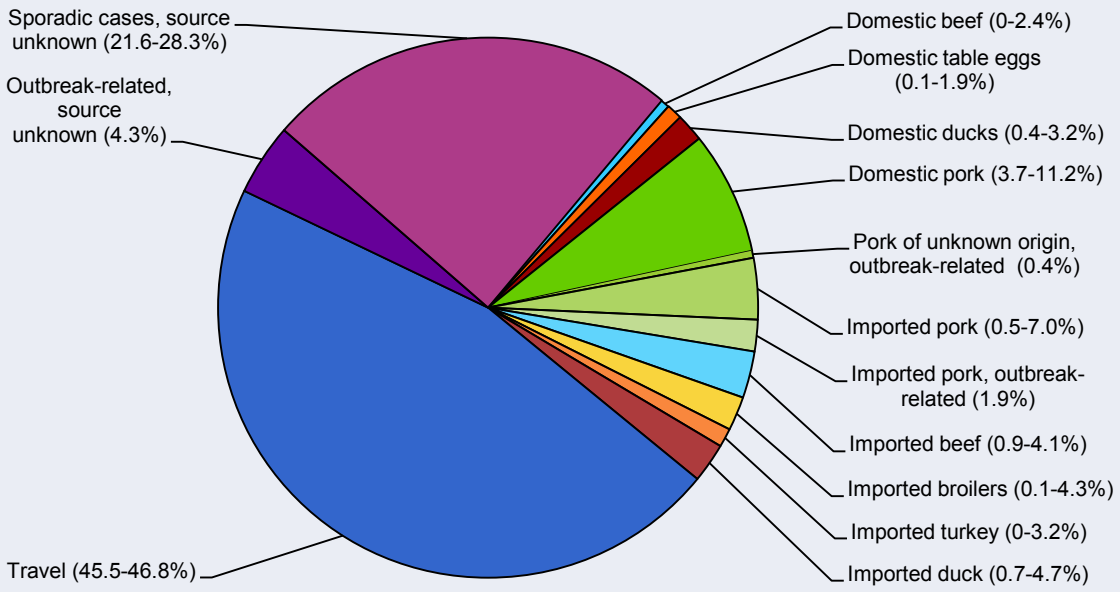
DANMAP 2011

		Primary production		At slaughter		Danish meat, ready for retail		Imported meat, ready for retail	
		herds/flocks		Carcasses/samples		Batches		Batches	
Bacteria	Animal species	Tested	% pos	Tested	% pos	Tested	% pos	Tested	% pos
Salmonella	Cattle[a]	-	-	7635	0.4	110	2.7	110	1.8
	Pigs[ab]	7427	4.8	22025	1.3	259	9.7	256	10.5
	Broilers	3795	1.2	306	0.0	96	1.0	212	5.7
Campylobacter	Broilers[c]	3379	14.4	1095	12.7	265	12.8	211	25.1

Source: Annual Report on Zoonoses in Denmark 2011
a) Data from slaughterhouses where 50 animals or more were slaughtered pr month (>99% of all tested carcasses). Pooled swab samples from five carcasses, where the percent positive carcasses was estimated using a conversion factor adjusting for the pooling
b) Slaughter pig herds are monitored using serological testing af meat juice samples collected at slaughter. If the seroprevalence during a three months period exceed a certain limit, herds will be classified as level 2 (medium risk) or level 3 (high risk) and defined as *Salmonella* positive
c) Detection limit for samples tested at slaughter is <10 cfu/g and detection limit for samples for batches ready for retail is <100 cfu/g

Figure 1. Estimated sources[a] of 1,166 cases of human salmonellosis, Denmark

DANMAP 2011



Source: Annual Report on Zoonoses in Denmark 2011
a) Sporadic and outbreak-related cases with unknown source include all sources not in the model. E.g. one outbreak with 43 cases where the source was tomatoes imported from Italy was included as "outbreak-related, source unknown"

Human infections: Human salmonellosis and campylobacteriosis are notifiable in Denmark, and all cases are reported to the national database at SSI.

Campylobacter is the most frequently reported foodborne pathogen in Denmark. In 2011, the number of human Campylobacteriosis cases (73.1 cases per 100,000 inhabitants) remained at the same level as in 2010. Since 2007, approximately one third of the cases have been related to travel. Consumption and handling of broiler meat is assumed to be the major source of human campylobacteriosis (estimated more than 50% of domestic sporadic cases), however other sources such as contaminated water, vegetables and direct contact to farm animals cattle exist.

In 2011, the number of human salmonellosis decreased by 27% compared to 2010 (from 28.7 to 21.0 cases per 100,000 inhabitants). Very few *Salmonella* outbreaks were reported and there were no outbreaks caused by *S.* Enteritidis. For the first time in more than a decade none of the *Salmonella* outbreaks could be related to meat of Danish origin. As in previous years, almost half of the human cases of salmonellosis were due to travel (Figure 1) and more than 70% of the *S.* Enteritidis cases were acquired abroad. The majority of the *S.* Typhimurium cases were still acquired in Denmark. For the sporadic cases not related to travel, Danish pork was estimated to be the most important source although decreasing markedly compared to the previous year, mainly because there were no outbreaks related to Danish pork in 2011. The relative importance of eggs as a source of infection continued to decrease and was the source with lowest estimated number of cases next to Danish produced beef. No cases were attributed to Danish broiler meat.

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Some phage types (e.g. DT12 and DT66) appear to acquire resistance very slowly - if at all. Rather, such phage types appear to be displaced by resistant phage types if a selection pressure is applied due to changes in the use of antimicrobial agents.

The distribution of *S.* Typhimurium phage types among the isolates from pigs, Danish pork and human cases included in the DANMAP report 2011 is presented in Table 6.1.

In this report, *S.* Typhimurium include the monophasic variants with antigenic formulas *S.* 4,5,12:i:- and *S.* 4,12:i:-, as recently recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826]. Isolates verified as monophasic variants of *S.* Typhimurium from animals, meat and humans have been included for all years presented in this report. If an isolate was resistant to three or more of the ten different antimicrobial classes included in the test panel, it was considered multi-resistant (see definition of multi-resistance and the included antimicrobial classes in section 10.5).

6.1.1 *Salmonella* in animals

For pigs and poultry, the isolates originated mainly from the national surveillance programmes. Comparison of resistance levels to previous years should be done cautiously, because the origin of the pig isolates collected in 2011 differ from previous years. The number of *Salmonella* isolates from the national surveillance has decreased and in 2011, implementation of a new *Salmonella* programme in the pig production meant that relatively few pen-faecal samplings were to be conducted. New initiatives were commenced, and *Salmonella* isolates from samples taken at medicine- and welfare control visits as well as the samples collected for the DANMAP programme of healthy pigs at slaughter were susceptibility tested.

Among the *Salmonella* isolates tested for antimicrobial resistance in 2011, one isolate per farm was randomly selected and included. Insufficient numbers of *S.* Typhimurium and *S.* Enteritidis isolates (<15) were obtained for Danish broilers and layers and for Danish cattle; therefore the results of the susceptibility testing are not presented. This also applies for the susceptibility results for *S.* Enteritidis in pigs. MIC distributions among *S.* Typhimurium from pigs in 2011 are shown in the web annex (Table A6.1).

In 2011, *Salmonella* was isolated from 420 pig herds, where 45% were *S.* Typhimurium and 37% *S.* Derby. Considering the large proportion of *S.* Derby it is important to mention, that the majority of the isolates were fully sensitive and that relatively few human *S.* Derby cases are reported in Denmark [Annual Report on Zoonoses in Denmark 2011]. Only two *S.* Enteritidis isolates were found in pigs.

Salmonella Typhimurium in pigs

In 2011, the *S.* Typhimurium isolates from pigs originated from samples taken in a number of different contexts: 1) from farms where the national surveillance had detected medium and high sero-prevalence (n = 107), 2) from the DANMAP samples collected from healthy pigs at slaughter (n = 50), 3) from control visits in herds with animal health problems and high antimicrobial consumption (n = 12), and 4) from pig herds investigated due to clinical disease (n = 33). As in previous years, all available isolates (e.g. one isolate per farm) were included to estimate the occurrence of resistance in *Salmonella* from pigs. However, this year the proportion of isolates from diagnostic submissions or control visits was relatively high (22%). Since these isolates are likely to originate from animals with a history of antimicrobial therapy, a relatively higher frequency of resistance is to be expected, when compared to isolates from healthy animals (e.g. the DANMAP samples).

Among all the susceptibility tested *S.* Typhimurium isolates from pigs (n = 202), 32% of the isolates were of the monophasic variants of *S.* Typhimurium. Not all isolates had been phage typed (n = 162), but the most common phage types were DT120 (27%) and DT193 (13%) (Table 6.1). Overall, 33% of the susceptibility tested *S.* Typhimurium isolates from pigs were fully sensitive, whereas 53% were found to be multi-resistant. The monophasic variants of *S.* Typhimurium isolates represented 60% (64/107) of all multi-resistant isolates . The highest occurrence of resistance was to streptomycin (57%), followed by sulfonamide (55%), tetracycline (54%) and ampicillin (51%) (Table 6.2).

The isolates included in the DANMAP report were previously obtained through the national surveillance in the pig production. However, 2011 was the first year that *Salmonella* was also isolated from samples collected specifically for DANMAP from healthy pigs at slaughter.

The level of resistance among the isolates from randomly selected healthy animals (DANMAP samples, Table 6.2), was generally lower than the resistance level among all available isolates (Table 6.2). Among the *S.* Typhimurium isolates from healthy animals, 44% of the tested isolates were fully sensitive, whereas 42% were multi-resistant. Less than half (43%, 9/21) of the multi-resistant *S.* Typhimurium isolates were of the monophasic variants.

Resistance to cephalosporins, ciprofloxacin or nalidixic acid in Danish pigs have been reported occasionally in the previous years, however the last two years resistance towards these antimicrobial classes have not been detected.

The levels of resistance to ampicillin, neomycin, sulfonamide and tetracycline were significantly higher in 2011 than in 2010. Since 2000, there has been a parallel increase in resistance to ampicillin, sulfonamide and tetracycline (Figure 6.1).

In 2008 and 2009, a temporary reduction in tetracycline resistance was observed but has not been fully explained; although it was partly related to a reduction in DT104 (consequent to a national eradication programme) and other phage types often resistant to tetracycline. However, in 2010 and 2011 the level of resistance to tetracycline increased again (Figure 6.1 and 6.2), which is mainly explained by an increase in the monophasic DT193 isolates with the ASSuT-profile.

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

DANMAP 2011

	Pigs		Pork	Human			
Antimicrobial agent	%	DANMAP only %	Danish %	Domestic sporadic %	Domestic outbreak %	Travel abroad %	Unknown origin %
Tetracycline	54	(42)	65	53	90	74	54
Chloramphenicol	8	(4)	10	14	0	28	9
Florfenicol	6	(4)	8	9	0	23	6
Ampicillin	51	(36)	71	55	0	69	52
Ceftiofur	0	(0)	0	1	0	12	0
Cefotaxime	0	(0)	0	-	-	-	-
Sulfonamide	55	(42)	67	59	0	70	67
Trimethoprim	8	(8)	10	2	0	9	8
Apramycin	2	(0)	0	0	0	1	0
Gentamicin	2	(0)	0	2	0	14	0
Neomycin	8	(12)	6	1	0	0	1
Spectinomycin	17	(10)	22	14	0	14	11
Streptomycin	57	(44)	71	60	5	68	60
Ciprofloxacin	0	(0)	0	2	0	16	6
Nalidixic acid	0	(0)	0	1	0	7	4
Colistin	0	(0)	0	0	0	3	0
Number of isolates	202	(50)	49	203	21	74	85

a) Include verified monophasic *S.* Typhimurium-like isolates
b) In 2011, the *S.* Typhimurium isolates from pigs originated the national surveillance (n = 107), control visits in pig herds with animal health problems and high antimicrobial consumption (n = 12), clinical investigations (n = 33) and also from the DANMAP samples collected from randomly selected healthy pigs at slaughter (n = 50). The distribution of resistant isolates among the 50 DANMAP samples are also presented seperately
c) The isolate was categorised as 'domestic sporadic' if the patient did not travel one week prior to the infection and was not reported as being part of an outbreak

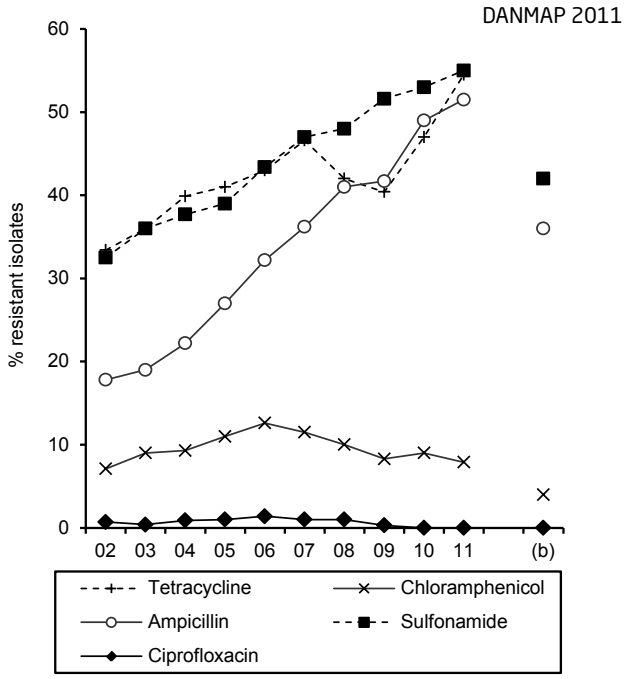
The usage of tetracycline for pigs decreased in second half of 2010 and in 2011. However, the occurrence of tetracycline resistance in *Salmonella* from pigs continued to increase significantly, thereby supporting the theory that clonal spread is responsible for the current increasing level of resistance, particularly by the spread of the multi-resistant clones of the monophasic *S.* Typhimurium variants (Figure 6.3).

From 2007 to 2011, the proportion of *S.* Typhimurium isolates with the ASSuT-profile parallels the significantly increasing proportion of phagetypes DT120/193 and monophasic variants of *S.* Typhimurium (Figure 6.3).

Overall during the period 2008 - 2011[1], the proportion of multi-resistant isolates increased significantly, while the proportion of fully sensitive isolates decreased significantly (Figure 6.3).

1) Data on resistance to colistin and trimethoprim were not available for 2007, thus the proportion of multi-resistant or fully sensitive were not calculated

Figure 6.1. Resistance (%) in *Salmonella* Typhimurium[a] from pigs, Denmark



a) The numbers of isolates varies between years (n = 216–736). Include monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-.
b) Presents the distribution of resistance in isolates from healthy pigs at slaughter in 2011 (n = 50). Please note that the occurrence of tetracycline and sulfonamide is identical

6.1.2 *Salmonella* in meat

Salmonella isolates from Danish and imported broiler meat, turkey meat, beef and pork are collected and susceptibility tested as part of national surveillance and control programmes. One isolate was selected from each *Salmonella* positive batch of imported fresh meat or one carcass per herd.

In 2011, a total of 49 *S.* Typhimurium isolates from Danish pork (carcasses at slaughter) was included, and the MIC distributions are shown in the web annex (Table A6.2).

Insufficient numbers of *S.* Typhimurium isolates (<15) were available for Danish broiler meat, Danish beef, imported beef, imported turkey and imported pork and the results of the susceptibility testing are not presented in this report. Also due to a low number of isolates, resistance data for *S.* Enteritidis in meat were also excluded.

The vast majority of Danish turkeys are exported for slaughter; therefore, no *Salmonella* isolates were available for susceptibility testing from Danish turkey meat in 2011.

***Salmonella* Typhimurium in pork**

Among the 49 susceptibility tested *S.* Typhimurium isolates available from Danish pork, the most dominant phage types were DT120 (24%) and DT193 (20%) and 43% of the isolates were of the monophasic variant of *S.* Typhimurium (Table 6.1 and Figure 6.3).

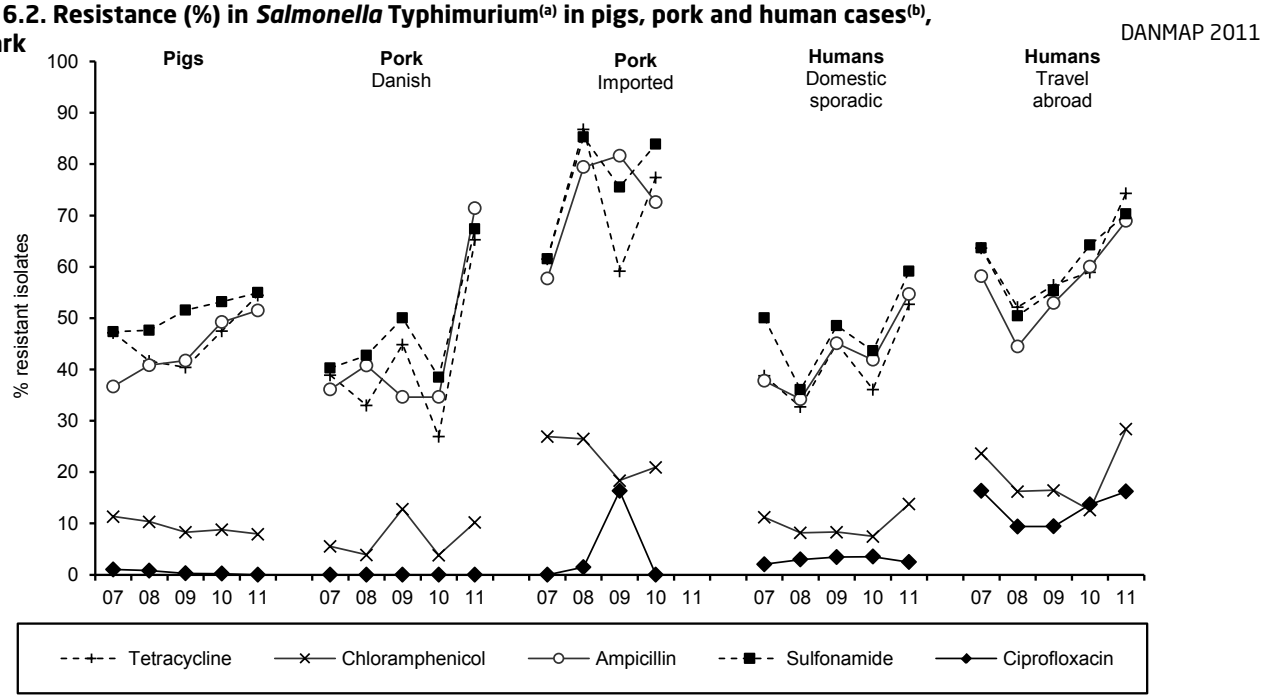
Overall, 14% of the susceptibility tested *S.* Typhimurium isolates from Danish pork were fully sensitive, whereas 67% were found multi-resistant. Resistance to ampicillin (71%), streptomycin (71%), sulfonamide (67%) and tetracycline (65%) was most common (Table 6.1). In 2011, the monophasic variants of *S.* Typhimurium represented 64% (21/33) of all multi-resistant isolates and 67% (14/21)of the total number of isolates with the ASSuT-profile.

As in 2010, none of the *S.* Typhimurium isolates from Danish pork were resistant to cephalosporins or fluoroquinolones. Cephalosporin resistance in pork has not been reported since 2008, where it was found in one isolate.

When comparing the occurrence of resistance in *S.* Typhimurium from Danish pork in 2011 to the level in 2010, significant increases were found for tetracycline, ampicillin, sulfonamide, streptomycin, spectinomycin and trimethoprim. In 2011, the level of resistance towards tetracycline, ampicillin and sulfonamide in the isolates from Danish meat was comparable to the levels found in imported pork during the period 2007–2010. This comparison could not be made in 2011 data, due to insufficient numbers of *S.* Typhimurium isolates from imported pork. However, in previous years the *S.* Typhimurium isolates from Danish pork have had significantly lower resistance to these three antimicrobial agents compared to isolates from imported pork (Figure 6.2).

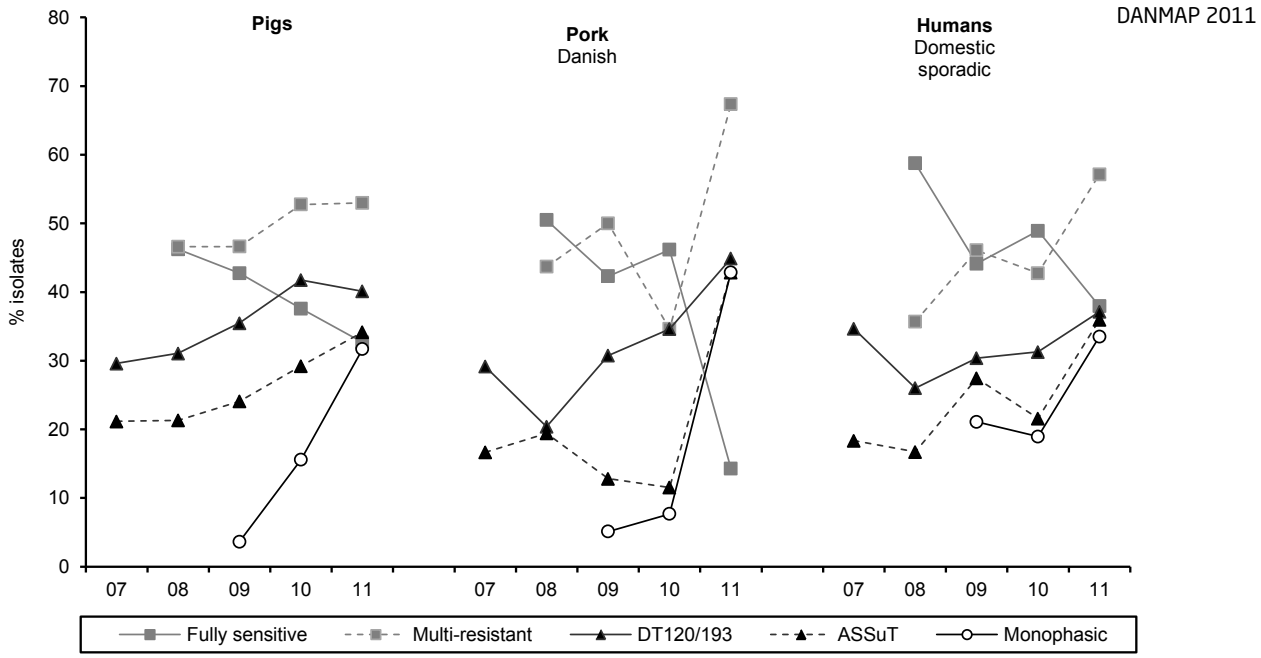
The proportion of *S.* Typhimurium isolates from Danish pork with the ASSuT-profile was significantly higher in 2011 (43%) than in 2007 (17%) (Figure 6.3). This coincided with a significant increase in the occurrence of phagetypes DT120 and DT193 as a result of a significantly increasing proportion of monophasic variants of *S.* Typhimurium (Figure 6.3). As for the isolates from pigs, the proportion of fully sensitive isolates decreased over the period 2008–2011 (Figure 6.3). From 2010 to 2011, there was a significant increase in the proportion of multi-resistance (from 35% to 67%) as a concequence of the increased occurrence of monophasic variants of *S.* Typhimurium (8% to 43%).

Figure 6.2. Resistance (%) in *Salmonella* Typhimurium[a] in pigs, pork and human cases[b], Denmark



a) The number of isolates varies between years (pigs n = 202–581, Danish pork: n = 26–103, imported pork: n = 26–68, domestic sporadic human cases: n = 98–269 and travel related human cases: n = 55–117). Include isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-. Data for imported pork in 2011 was not presented due to insufficient number of isolates
b) The isolate was categorised as 'domestically acquired' if the patient did not travel one week prior to the infection and as 'travel abroad reported' if the patient travelled one week prior to the infection

Figure 6.3. Occurrence (%) of multi-resistance[a b], phagetypes DT120/193[c] and monophasic variants[d] in *Salmonella* Typhimurium in pigs, pork and human cases[e], Denmark



Note: The number of isolates varies between years (pigs: n = 202–581, Danish pork: n = 26–103, domestic sporadic human cases: n = 98–269). Include isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-.
a) An isolate was assumed multi-resistant if resistant to three or more of the ten different antimicrobial classes included in the test panel, see definition in section 10.5. Data on resistance to colistin and trimethoprim were not available for 2007, thus the proportion of multi-resistant or fully sensitive were not calculated
b) 'ASSuT' isolates are resistant to ampicillin, streptomycin, sulfonamide and tetracycline, but can also include resistant to other antimicrobial agents except chloramphenicol
c) Presenting the proportion of DT 120/193 among phage typed isolates. Only in 2011, some of the isolates from pigs (n = 40) and humans (n = 4) was not phage typed
d) The recording of monofasic strains in the databases were not fully implemented in 2007 and 2008, thus data are not presented
e) The isolate was categorised as 'domestically acquired' if the patient did not travel one week prior to the infection

6.1.3 *Salmonella* in humans

In 2011, 1,166 cases of human salmonellosis (corresponding to 21.0 cases per 100,000 inhabitants) were reported, representing a decrease of 27% compared to 2010. The estimated sources of human salmonellosis in 2011 are presented in Textbox 4.

SSI collected travel information from the patients diagnosed with salmonellosis by phone interviews. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period before getting ill. Patients who had travelled were also asked about their destinations. Depending on the available information, patients were divided into three categories: 1) domestically acquired, 2) travel abroad reported and 3) unknown origin. The latter category were cases, where no travel information had been reported to the general practitioners, and where no phone interview was conducted. In 2011, travel information was obtained for 80% of the *Salmonella* cases.

Outbreaks of human salmonellosis are reported in the 'Annual Report on Zoonoses in Denmark in 2011' [www.dtu.food.dk]. All human cases associated with a detected outbreak were considered 'outbreak-related' in this report.

MIC distributions among *S.* Typhimurium and *S.* Enteritidis from human cases in 2011 are shown in the web annex (Tables A6.3 and A6.4).

Salmonella Typhimurium in humans

Among the reported human *S.* Typhimurium isolates with valid results from susceptibility testing of all antimicrobial agents included in the test panel (n = 383), 19% were categorised as travel-related and 58% as being domestically acquired. In 2011, there was one outbreak of *S.* Typhimurium DT120 including 21 cases; so of the domestically acquired cases, 10% was outbreak related and 90% were of sporadic origin. The origin of the remaining 23% of the *S.* Typhimurium isolates was unknown (Table 6.2).

Irrespectively of the origin of the infection, the most commonly reported phage types in human *S.* Typhimurium isolates were DT193 and DT120 (Table 6.1).

Among the human sporadic domestic cases (n = 203), 38% of the *S.* Typhimurium isolates were fully sensitive whereas 57% were multi-resistant. The monophasic variants of *S.* Typhimurium represented 59% (68/116) of all multi-resistant isolates. For the travel-related cases (n = 74) and cases of unknown origin (n = 85), 15% and 27% of the *S.* Typhimurium isolates were fully sensitive, whereas 68% and 56% were multi-resistant, respectively.

Resistance to cephalosporins was only found among isolates from nine travel-associated cases (12%) and one sporadic domestic case (1%). As observed in 2010, a marked difference in ciprofloxacin resistance was found between domestically acquired infections (2%) and travel related infections (16%). The higher level of ciprofloxacin resistance in the travel-associated *S.* Typhimurium infections may reflect a higher consumption of fluoroquinolones in production animals in the countries of destination.

The pattern of resistance in the human *S.* Typhimurium isolates in 2011 continued the trend observed the previous years. As observed in human isolates from the rest of Europe, resistance to ampicillin, streptomycin, sulfonamide and tetracycline were dominating (the ASSuT-profile). The occurrence of resistance to these four antimicrobial agents was similar among travel-related cases and cases with unknown origin. In the domestic sporadic cases resistance was markedly lower for all four antimicrobial agents than what was observed in the travel-related cases and the cases of unknown origin (Figure 6.2).

As a consequence of the significantly increasing occurrence of monophasic variants of *S.* Typhimurium, the proportion of fully sensitive human *S.* Typhimurium isolates of domestic sporadic origin has decreased significantly from 50% in 2008 to 38% in 2011, and the proportion of multi-resistant isolates increased significantly from 36% to 57% (Figure 6.3).

Salmonella Enteritidis in humans

Among the reported human *S.* Enteritidis isolates with valid results from susceptibility testing of all antimicrobial agents included in the test panel (n = 288), 58% of the infections were associated with travelling abroad, 23% was domestically acquired infections and the remaining 19% had an unknown origin. No *S.* Enteritidis outbreaks were recorded in 2011, wherefore all cases with domestically acquired infections were considered to be of sporadic origin (Table 6.3).

The level of resistance among the human *S.* Enteritidis isolates was generally lower than observed in human *S.* Typhimurium isolates. The majority of *S.* Enteritidis isolates originating from domestic sporadic cases (74%), travel-related cases (56%) and cases of unknown origin (58%) were fully sensitive, whereas one (2%) domestic sporadic case, ten (6%) travel-related cases and two (4%) case of unknown origin were multi-resistant. Resistance to cephalosporins was only found in one of isolates from the travel-associated cases.

Overall in 2011, 18% of the domestic sporadic isolates were ciprofloxacin and nalidixic acid resistant. This is a 10% increase compared to 2010, meaning that the occurrence of fluoroquinolone resistance in the domestic sporadic isolates is approaching the level observed in isolates from the travel-related cases (24%) and of unknown origin (27%) (Table 6.3). The major source of S. Enteritidis is poultry, however fluoroquinolones have not been used in Danish table egg production (parents and layers) since 2004, and the prevalence of *Salmonella* is low in Danish layers [Textbox 4].

Table 6.3. Resistance (%) in *Salmonella* Enteritidis from human cases[a], Denmark

DANMAP 2011

Antimicrobial agent	Humans		
	Domestic sporadic %	Travel abroad %	Unknown origin %
Tetracycline	3	6	5
Chloramphenicol	0	0	0
Florfenicol	0	0	0
Ampicillin	5	7	13
Ceftiofur	0	1	0
Cefotaxime	-	-	-
Sulfonamide	2	4	4
Trimethoprim	2	2	2
Apramycin	0	1	0
Gentamicin	0	0	0
Neomycin	0	0	0
Spectinomycin	2	1	2
Streptomycin	0	3	2
Ciprofloxacin	18	24	27
Nalidixic acid	18	22	27
Colistin	0	1	0
Number of isolates	66	167	55

a) The isolate was categorised as 'domestically acquired' if the patient did not travel one week prior to the infection, and as 'travel abroad reported' if the patient travelled one week prior to the infection

6.1.4 *Salmonella* in a farm to fork perspective

In 2011, the occurrence of resistance in the *Salmonella* isolates from pigs, Danish pork and humans were markedly influenced by increased occurrence the monophasic variants of *S.* Typhimurium, carrying the ASSuT resistance profile. Figure 6.3 shows the distinct association between the occurrence of the ASSuT-profile, the phagetypes DT120/DT193 and the monophasic variants of *S.* Typhimurium. This tendency is observed throughout the farm to fork pathway, where the proportion of multi-resistance has increased dramatically, while the number of fully sensitive isolates has decreased.

Despite the reduced consumption of tetracycline in pigs in 2011, the occurrence of tetracycline resistance in *Salmonella* from pigs and Danish pork continued to increase significantly due to the increased occurrence of the multi-resistant clones of the monophasic *S.* Typhimurium variants.

S. Typhimurium isolates sampled from healthy pigs at slaughter is assumed more representative of the general Danish population of finishers than the overall collection of pig isolates. However, significantly higher occurrence of resistance was found for ampicillin, streptomycin, sulfonamide and tetracycline in *S.* Typhimurium isolates from Danish pork compared to isolates from Danish pigs (healthy pigs at slaughter only).

The *Salmonella* source attribution model (Textbox 4) estimated that 7% (86 sporadic cases) of the human salmonellosis cases in 2011 could be attributed to Danish pork. Of the 75 *S.* Typhimurium cases attributed to pork, 53% were of the monophasic variant, almost exclusively multi-resistant phagetypes DT120 and DT193. All of the 37 sporadic *S.* Typhimurium cases attributed to imported pork (86% of all sporadic cases attributed to imported pork) were estimated to be of multi-resistant monophasic variants (all DT120 or DT193). Additionally, seven *S.* Typhimurium cases were attributed to poultry, all of the monophasic variant.

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Whole Genome Sequencing of *Salmonella* Typhimurium from Danish pigs for identification of acquired antimicrobial resistance

Background: The cost of Whole Genome Sequencing (WGS) continues to decline, making it increasingly available in routine diagnostic laboratories and as a supplement for traditional methods for resistance gene identification. The use of a genotypic method for diagnostic purposes may have the disadvantages that unknown mechanisms or point mutation are not detected, on the other hand a genotypic method may detect resistance genes not expressed in the bacteria. For the traditional susceptibility testing methods the result depends on testing condition and interpretation criteria and a high level of standardisation is required. One limitation for diagnostic laboratories to use WGS is interpretation of the sequence results. The aim of this study was to compare analyses of WGS data with traditional phenotypic determination of antimicrobial resistance among *S.* Typhimurium from Danish pigs.

Materials and methods: Antimicrobial susceptibility tests were made on *S.* Typhimurium isolates from Danish pigs (n = 50) as part of the DANMAP program. The isolates originate from different farms and were collected randomly in the first half of 2011. Total DNA was purified from the isolates and sequenced on the Illumina platform, paired-end reads. The reads was assembled de novo prior to prediction of the resistance profile and N50 values of the assembly were used for quality control of the DNA sequences. The web-server ResFinder [www.genomicepidemiology.org] was used to identify acquired antimicrobial resistance genes in the WGS data. A threshold equal to 98% identity was selected and results were compared with the phenotypic antimicrobial susceptibility testing results.

Results: One isolate was excluded from the study because of poor WGS data (N50 = 196), making it impossible to test in ResFinder. The rest of the isolates were assembled to draft genomes with high N50 values (average 238,833 ± 12,079, 95% CI). ResFinder identified resistance genes in 32 (65%) of the isolates, all genes were found with a >99% identity match to resistance genes in the database. The susceptibility tests found that the same 32 isolates were phenotypically resistant and their resistance profiles matched the ResFinder results 100% (Table 1).

Table 1. Number of isolates with phenotypic resistance according to the antimicrobial agent DANMAP 2011

Antimicrobial agent	Total no. of isolates with phenotypic resistance	% Isolates with matching resistance gene found with ResFinder	Genes found by ResFinder (n)
Apramycin	0	-	-
Gentamicin	0	-	-
Neomycin	4	100	*aph(3')-Ia* (2), *aph(3')-Ic* (2)
Spectinomycin	8	100	*aadA1* (5), *aadA2* (2), *aadA13* (1)
Streptomycin	25	100	*strA/strB* (19), *aadA1* (5), *aadA2* (2), *aadA13* (1)
Amoxicillin/clavulanic acid	0	-	-
Ampicillin	23	100	bla_{TEM-1}(21), bla_{CARB-2}(2)
Ceftiofur	0	-	-
Cefotaxime	0	100	-
Chloramphenicol	2	100	*floR* (2)
Florfenicol	2	100	*floR* (2)
Ciprofloxacin	0	-	-
Nalidixan	0	-	-
Sulfamethoxazole	26	100	*sul1* (9), *sul2* (20)
Tetracycline	22	100	*tet(B)* (19), *tet(G)* (2), *tet(A)* (1)
Trimethoprim	2	100	*dfrA1* (1), *dfrA14* (1)
Colistin	0	-	-

In most cases only one resistance gene was found for each of the detected resistances except for three isolates that contained various genes conferring the same resistance (streptomycin, spectinomycin and sulfamethoxazole). All three isolates contained both *sul1* and *sul2*, in all three cases both genes were found with a 100% identity match and were located at different positions in the genome. In addition, two of the three isolates contained both *strA/strB* (streptomycin resistance) and *aadA1* (streptomycin and spectinomycin resistance). The rest of the isolates with streptomycin and spectinomycin resistance (n = 8) did not contain the *strA/strB* genes, but only an *aadA* gene.

The results indicate that ResFinder based on WGS can predict the resistance profile for at least nine of the tested antimicrobial agents and did not produce false positives for the last eight tested. WGS may be a useful tool for diagnostic and surveillance purposes in the future.

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Textbox 6

Tentative colistin epidemiological cut-off value for *Salmonella* spp.

Background: The interpretive criteria are crucial when determining bacteria as either resistant or sensitive to an antimicrobial drug. The European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2011) presents a clinical breakpoint and an epidemiological cut-off value of >2 mg/L for colistin, based on a few isolates whereas *Salmonella* isolates with a Minimal Inhibitory Concentration (MIC) ≤2 mg/L should be reported as sensitive or wildtype. The epidemiological cut-off value is defined based on MIC population distributions of *Salmonella* spp. [EUCAST 2011, www.EUCAST.org].

In a study we determined MIC population distributions for colistin for *Salmonella* on subtype level. Furthermore, also if differences in MIC for colistin could be explained by mutations in *pmrA* or *pmrB* encoding proteins involved in processes that influence the binding of colistin to the cell membrane [Agersø et al 2012. Foodborne Pathog Dis. 2012 9:367-9].

Materials and methods: During 2008–2011, *Salmonella* isolates of human (n = 6,583) and of animal/meat origin (n = 1,931) were collected. The isolates were serotyped and susceptibility tested towards colistin (range 1 -16 mg/L). Moreover, 37 isolates were tested for mutations in *pmrA* and *pmrB* by PCR and DNA sequenced.

Results: MIC distribution for colistin at serotype level showed that *S*. Dublin followed by *S*. Enteritidis were less susceptible than 'other' *Salmonella* serotypes originating from humans and *S*. Typhimurium of animal and meat origin. Among the human isolates, MIC was ≤1 mg/L for 98.9% of 'other' *Salmonella* serotypes, 61.3% of *S*. Enteritidis and 12.1% of *S*. Dublin isolates. Among the animal and meat isolates, MIC was ≤1 mg/L for 99.4% of S. Typhimurium, (Figure 1).

Interestingly, *S*. Dublin and *S*. Enteritidis belong to the same O-group (O:1, 9,12) suggesting that surface lipopolysaccharides (LPS) of the cell (O-antigen) play a role in colistin susceptibility.

The epidemiological cut-off value >2 mg/L for colistin suggested by EUCAST is placed inside the distribution for both *S*. Dublin and *S*. Enteritidis. All tested *S*. Dublin isolates, regardless of MIC colistin value, had identical *pmrA* and *pmrB* sequences. Missense mutations were found only in *pmrA* in one *S*. Reading isolates and in *pmrB* in one *S*. Concord isolate both with MIC≤1 for colistin.

In conclusion, our study indicates that missense mutations are not necessarily involved in increased MICs for colistin. Increased MICs for colistin seemed to be linked to specific serotypes (*S*. Dublin and *S*. Enteritidis). Therefore, we recommend that *Salmonella* with MIC >2 mg/L for colistin are evaluated on serovar level.

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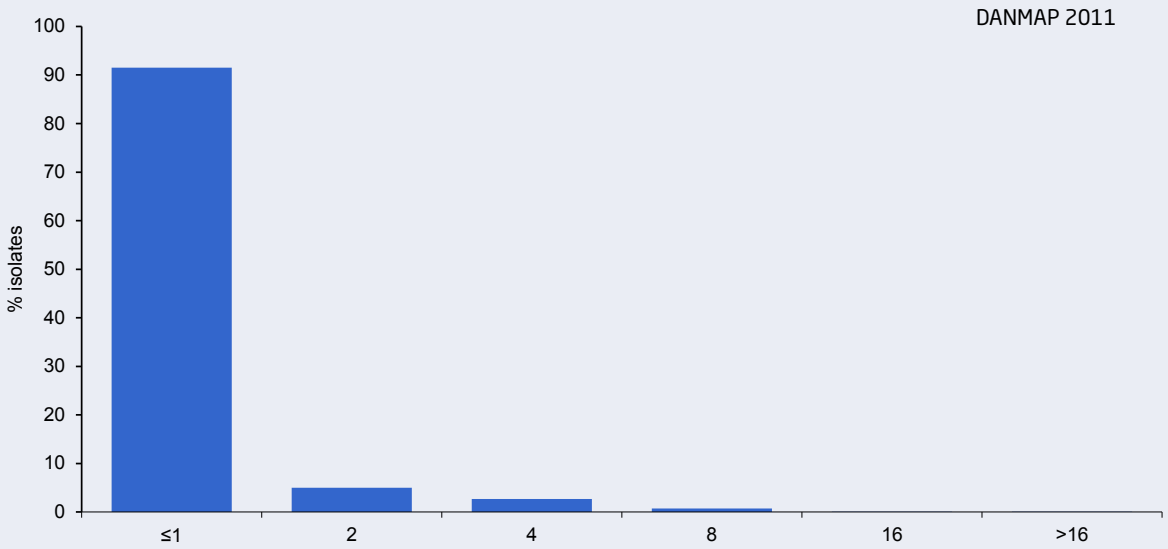
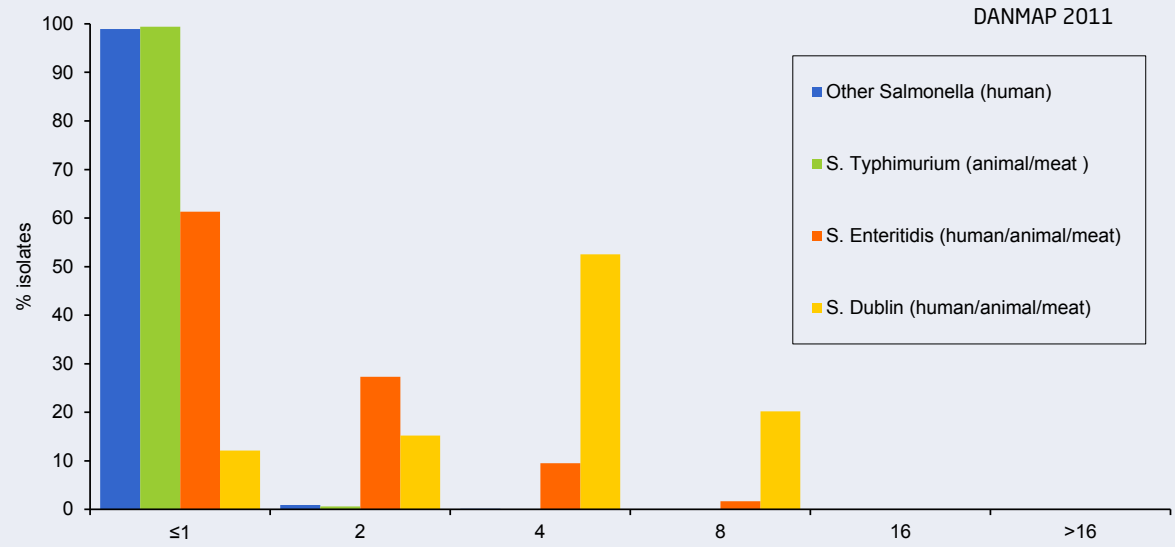
Figure 1. MIC distribution (%) of colistin determined for all *Salmonella* serovars[a], Denmark



a) Total number of *Salmonella* isolates include isolates from humans, animals and meat (n = 198)

Figure 2. MIC distribution (%) of *Salmonella* serovars[a] from humans and animals/meat, Denmark



Note: Number of isolates included: *S.* Dublin = 198, *S.* Enteritidis = 1,247, *S.* Typhimurium from animals and meat = 1,795 and 'other' *Salmonella* serovars from humans = 5,274. Other *Salmonella* serovars of human origin constitutes all included serovars except *S.* Dublin and *S.* Enteritidis

6.2 *Campylobacter*

Campylobacter are the most commonly reported cause of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union (see Textbox 4).

Human campylobacteriosis is caused by thermotolerant *Campylobacter* spp. The species most commonly associated with human infections is *C. jejuni* followed by *C. coli*, but other species are also known to cause infections in humans. In Denmark, 85-95% of the human campylobacteriosis cases are caused by *C. jejuni*.

The incubation period is typically two to five days and common clinical symptoms include watery, sometimes bloody diarrhoea, abdominal pain, fever, headache and nausea. Usually, infections are self-limiting and last only a few days. Infrequently, extraintestinal infections or post-infection complications such as reactive arthritis and neurological disorders may occur. *C. jejuni* has become the most recognized antecedent cause of Guillain-Barré syndrome, which is a polio-like form of paralysis that can result in respiratory failure and severe neurological dysfunction and even death.

Thermotolerant *Campylobacter* are widespread in nature and the most important reservoirs are the alimentary tract of wild and domesticated birds and mammals. They are prevalent in production animals such as poultry, cattle, pigs and sheep; in pets, including dogs and cats; in wild birds and environmental water sources. The bacteria can readily contaminate various foodstuffs. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infection. Furthermore, source attribution models have attributed the majority of human cases to the broiler reservoir. The main focus of this chapter is therefore *C. jejuni* isolates obtained from the broilers and broiler meat.

An isolate was considered multi-resistant if it was found resistant to three or more of the six different antimicrobial classes included in the test panel (see definition of multi-resistance and the included antimicrobial classes in Section 10.5).

6.2.1 *Campylobacter jejuni* in animals

Samples from healthy animals were collected at slaughter for the DANMAP programme and all analyses were performed at the National Food Institute. Only one isolate per farm was included in the report. *C. jejuni* from pigs were not included due to the very low number of isolates found in pigs.

MIC distributions among *C. jejuni* from cattle and broilers in 2011 are shown in the web annex (Table A6.5).

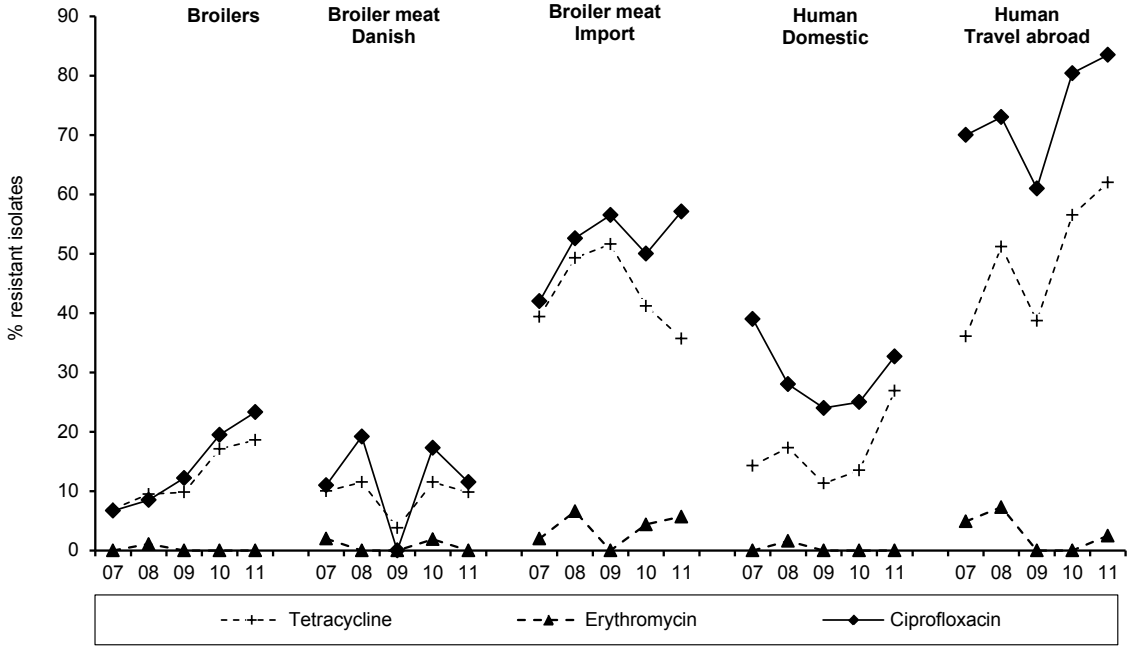
Table 6.4. Resistance (%) in *Campylobacter jejuni* from animals, Danish broiler meat, imported broiler meat and in domestic and travel related human cases, Denmark 2011

DANMAP 2011

Antimicrobial agent	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestically acquired	Travel abroad
	%	%	%	%	%	%
Tetracycline	4	19	10	36	27	62
Chloramphenicol	0	0	0	1	0	1
Erythromycin	0	0	0	6	0	3
Gentamicin	0	0	0	0	1	1
Streptomycin	0	5	2	1	4	5
Ciprofloxacin	20	23	11	57	33	84
Nalidixic acid	20	23	11	57	33	84
Number of isolates	95	43	61	70	104	79

Figure 6.4. Resistance (%) in *Campylobacter jejuni*[a] from broilers, broiler meat and human cases[b], Denmark

DANMAP 2011



a) The number of isolates varies between years (broilers: n = 41–94, Danish broiler meat: n = 26–114, imported broiler meat: n = 62–152, domestic sporadic human cases: n = 52–185 and travel related human cases: n = 31–79)
b) The isolate was categorised as 'domestically acquired' if the patient did not travel one week prior to the infection and as 'travel abroad reported' if the patient travelled one week prior to the infection

Broilers

A total of 43 *C. jejuni* from broilers were isolated and susceptibility tested (Table 6.4). Of these, 74% were fully sensitive to the antimicrobial agents tested and only one isolate was found multi-resistant.

In isolates from Danish broilers, the highest levels of resistance were found for ciprofloxacin (23%) and tetracycline (19%). A significant increase in resistance to ciprofloxacin and tetracycline has been observed over the last decade from less than 10% resistant isolates in 2001 to 23% and 19% in 2011, respectively (Figure 6.4).

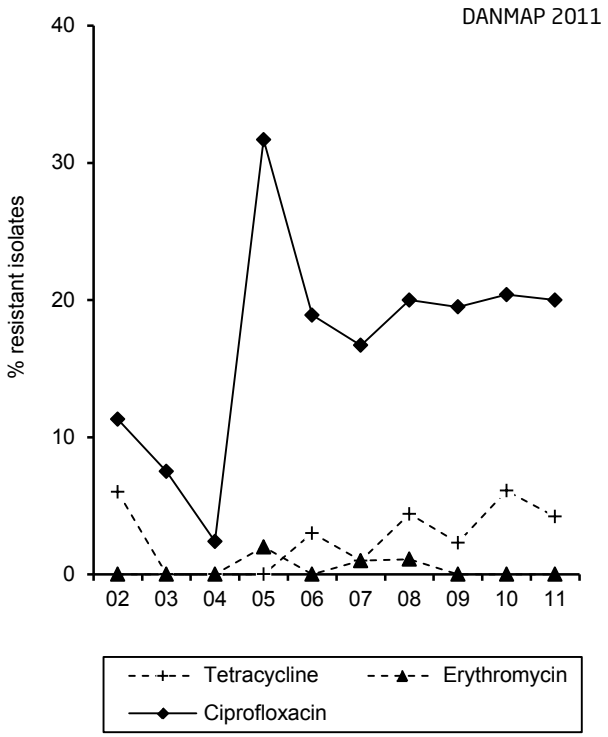
Since 2009, fluoroquinolones have not been used in rearing and parent flocks for the broiler production and considerable decreases have also been observed in the consumption of amoxicillin, penicillins, sulfonamides and macrolides in these types of flocks. In broiler flocks, the consumption of tetracycline increased considerably from 2008 to 2010, but decreased from 2010 to 2011. However, the use of tetracycline remains higher than before 2008. Second to amoxicillin, tetracycline was the most commonly used antimicrobial agent in Danish broilers in 2010 and 2011; this may be an explanation for the increasing tetracycline resistance in *C. jejuni* from Danish broilers. In contrast to what was observed for the broiler rearing flocks, the consumption of amoxicillin and penicillins in broiler flocks increased importantly from 2010 to 2011 (Figure 4.10).

Cattle

For cattle, 95 *C. jejuni* isolates were susceptibility tested; 78% of the *C. jejuni* isolates from cattle were fully sensitive to the antimicrobial agents tested and no isolates were multi-resistant.

Resistance to ciprofloxacin among *C. jejuni* from cattle has remained virtually unchanged since 2008, at a level around 20% (Figure 6.5). As described in previous DANMAP reports, a significant increase in the level of fluoroquinolone resistance occurred in 2005 despite low consumption of fluoroquinolones in cattle since 2003. As in previous years, only few of the fluoroquinolone resistant isolates were also resistant to tetracycline in 2011, indicating that co-selection by tetracycline (one of the major drugs for treatment of calves) was not the explanation for the high occurrence 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 the occurrence of resistance has been moving north since 2007, and in 2011, 95% of the ciprofloxacin resistant *C. jejuni* isolates originated from farms distributed throughout Jutland.

Figure 6.5. Resistance (%) in *Campylobacter jejuni* from cattle, Denmark



Since 2005, the level of resistance to tetracycline has fluctuated, but overall a general increase in the resistance to tetracycline has been observed in isolates obtained from cattle (Figure 6.5).

6.2.2 *Campylobacter jejuni* in meat

In 2011, the Danish Veterinary and Food Administration (DVFA) collected samples for *Campylobacter* testing from meat sold at wholesale and retail outlets. The isolates were species identified in the regional laboratories of DVFA and tested for antimicrobial susceptibility at the National Food Institute.

MIC distributions among *C. jejuni* from broiler meat in 2011 are shown in the web annex (Table A6.6).

Broiler meat

From broiler meat, 131 *C. jejuni* isolates (61 Danish, 70 imported) were susceptibility tested. One isolate per sample was reported (Table 6.4). The percentage of isolates that were fully sensitive to the antimicrobial agents tested increased among the isolates from Danish broiler meat from 2010 to 2011(from 75% to 87%). In contrast, the percentage of fully sensitive isolates from imported meat decreased from 37% in 2010 to 27% in 2011, making the difference in antimicrobial resistance between Danish and imported meat even more pronounced than in 2010. Multi-resistance was found in only one isolate obtained from imported broiler meat.

The observed resistance to ciprofloxacin and tetracycline has fluctuated over the last five years, and in 2011, the resistance increased to approximately 11% and 10%, respectively, returning to the same levels as reported in 2007. As in previous years, resistance to ciprofloxacin and tetracycline was significantly higher in *C. jejuni* from imported broiler meat compared to Danish broiler meat (Figure 6.4). Erythromycin resistance has remained at a very low level in both domestic and imported broiler meat for almost a decade.

6.2.3 *Campylobacter jejuni* in humans

Campylobacter continued to be the most frequent cause of bacterial intestinal infections in 2011. A total of 4,068 human laboratory confirmed cases of campylobacteriosis were reported (73.1 per 100,000 inhabitants), similar to the year before [Annual report on Zoonoses in Denmark 2011]. For surveillance of antimicrobial resistance among human cases, isolates from three out of five geographical regions in Denmark (North Denmark Region and the regions of Southern Denmark and Zealand) were selected and tested in 2011.

Since 2007, SSI has 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 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 infection.

MIC distributions among *C. jejuni* from humans in 2011 are shown in the web annex (Table A6.7).

In 2011, 183 *Campylobacter jejuni* isolates were submitted to SSI for susceptibility testing continuously over the year. The isolates were randomly selected from all *Campylobacter* isolated from stool samples in the geographical regions mentioned above. Among the tested isolates, 104 (57%) were from travel-associated cases and 79 (43%) were considered to be domestically acquired. Among the isolates from domestically acquired infections, 82% were fully sensitive to the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower (9%) among isolates from travel associated cases (Table 6.4). Multi-resistance was found in three domestically acquired infections (4%) and five (5%) infections associated with travel.

In 2011, the level of resistance to ciprofloxacin in *C. jejuni* isolates from domestically acquired human infections remained in between the level of resistance for isolates obtained from Danish broiler meat and imported broiler meat. The consumption of imported broiler meat has continued to increase in Denmark, from 17% in 2003 to 45% in 2010. However, while, the overall consumption of broiler meat increased in 2011, the percentage of imported meat decreased slightly to 35% (Table 3.2). Imported broiler meat contributes to the relatively high occurrence of ciprofloxacin resistance in *C. jejuni* isolates (33%) from domestically acquired human infections, however, the extent of this contribution is unknown.

The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel associated *C. jejuni* isolates (84% and 62%, respectively) compared to isolates from domestically acquired infections (27% and 33%, respectively) (Figure 6.4). For the other antimicrobial agents tested, no significant differences in resistance levels were observed. Ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with severe bacterial gastroenteritis. Fluoroquinolones are also used in animal husbandry. In Denmark, however, 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*.

6.2.4 *Campylobacter coli* in animals

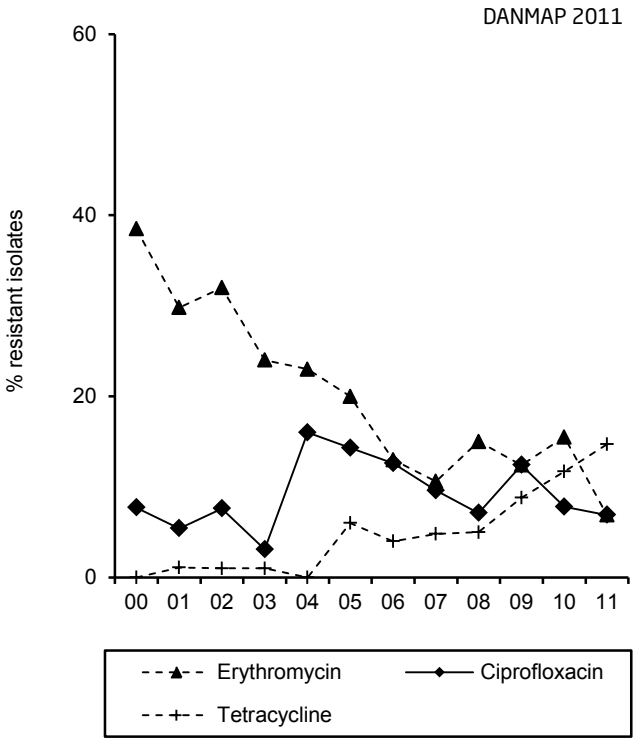
For animals, antimicrobial resistance among *C. coli* is reported only for pig isolates in this report. MIC distributions and the occurrence of antimicrobial resistance among *C. coli* from pigs are shown in the web annex (Table A6.8).

In 2011, 102 randomly selected *C. coli* isolates were susceptibility tested. Only one isolate per herd was tested; 36% were found fully sensitive and four isolates were found multi-resistant. As in the previous three years, no significant changes in fluoroquinolone resistance were observed among *C. coli* from pigs from 2010 to 2011. Fluoroquinolone resistance was detected in approximately 7% of the tested isolates (Figure 6.6) despite low consumption of fluoroquinolones in pigs since 2003 (web annex, Table A4.2).

A continuous decrease in erythromycin resistance in *C. coli* was observed after withdrawal of the growth promoter tylosin from the Danish pig production in 1998–1999. Between 2006 and 2010, the level of erythromycin resistance remained stable between 10-15%, but from 2010 to 2011, erythromycin resistance in *C. coli* from pigs decreased significantly from 15% to 7% (Figure 6.6). In contrast, an increasing trend has been observed in the occurrence of resistance to tetracycline in *C. coli* from Danish pigs over the past decade, especially during 2004–2011. Overall, the consumption of tetracycline has also increased from 2001 to 2009 (web annex, Table A4.1). The dramatic decrease in use of tetracyclines in the pig production in 2011 (by 27%) has not affected the level of tetracycline resistance in *C. coli* isolates from pigs.

In 2011, streptomycin resistant *C. coli* isolates from pigs remained at the same high level as in 2010 (61% in 2011 compared with 62% in 2010). The increase observed since 2009 may reflect that isolates coincidentally originated

Figure 6.6. Resistance (%) in *Campylobacter coli* from pigs, Denmark



from producers with high prevalences of diseases typically treated with streptomycin (limb, joint, CNS and skin). It is, however, noteworthy that the consumption of penicillin-streptomycin combinations for finisher pigs also increased during 2009-2010. It should also be noted that the increase in streptomycin consumption represents only a very small fraction of the total consumption (web annex, Table A4.2).

6.2.5 *Campylobacter coli* in meat

From samples of meat, only one isolate per sample was susceptibility tested and reported. In 2011, 20 *C. coli* isolates were obtained from imported broiler meat. Due to an insufficient number of samples (< 15), no *C. coli* isolates from Danish broiler meat were included in this report. Only two isolates from imported broiler meat were fully sensitive to the antimicrobial agents tested, while five of the isolates were multi-resistant. The MIC values are shown in the web annex (Table A6.9).

6.2.6 *Campylobacter coli* humans

No *C. coli* isolates from human cases were included in the DANMAP report 2011, since only a small number of *C. coli* were received at SSI.

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7. Resistance in indicator bacteria

Indicator bacteria (*Enterococcus faecium, Enterococcus faecalis* and *Escherichia coli*) have been included in the DANMAP programme since 1995. These bacteria were selected as indicators for prevalence of antimicrobial resistance for several reasons. They are ubiquitous, and present as major commensals in both the animal and human reservoirs. They may acquire antimicrobial resistance as response to selective pressures. Furthermore, they have the potential for transferring resistance to pathogenic bacteria.

Most of the antimicrobial agents that were used for growth promotion in Denmark (banned successively from 1994–1999) had an effect on Gram-positive bacteria such as enterococci. Today, many of the antimicrobial agents used in veterinary clinical therapy are broad spectrum agents that are also active against Gram-negative bacteria, such as *Salmonella* and *E. coli.* Exceptions are penicillins, lincosamides, macrolides and pleuromutilins that are most active against Gram-positive bacteria.

Enterococci are included in the DANMAP programme to monitor the persistence of resistance after the ban of growth promoters and to monitor changes in antimicrobial resistance in Gram-positive bacteria. *E. coli* are included to monitor resistance in Gram-negative bacteria.

7.1 Enterococci

Enterococcus faecium and *Enterococcus faecalis* were isolated from healthy broiler and pigs at slaughter for the DANMAP programme. More over, enterococci were also isolated from meat sold at wholesale and retail outlets. If an isolate was resistant to three or more of the ten different antimicrobial classes included in the test panel, it was considered multi-resistant (see definition of multi-resistance and the included antimicrobial classes in section 10.5).

The MIC distributions and occurrence of resistance among *E. faecium* and *E. faecalis* are presented in the web annex (Tables A7.1, A7.2, A7.3 and A7.4).

7.1.1 *Enterococcus faecium* in animals

For samples from pigs and broilers, only one isolate per farm was included. A randomly selected subsample of isolates from broilers (n = 107) and pigs (n = 116) were susceptibility tested and reported (Table 7.1).

Table 7.1. Resistance (%) among *Enterococcus faecium* from animals and meat of Danish and imported origin, Denmark 2011

DANMAP 2011

Antimicrobial agent	Broilers	Pigs	Broiler meat		Beef meat	Pork meat
	Danish %	Danish %	Danish %	Imported %	Imported %	Danish %
Tetracycline	5	62	10	34	0	7
Tigecycline	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0
Penicillin	3	23	2	28	0	0
Ampicillin	3	10	2	27	0	0
Erythromycin	15	33	19	61	0	15
Gentamicin	0	1	0	0	0	0
Kanamycin	1	25	0	16	0	4
Streptomycin	4	41	0	28	0	4
Ciprofloxacin	0	0	0	0	0	0
Vancomycin	0	1	0	0	0	0
Quinupristin/dalfopristin	1	2	1	12	0	0
Salinomycin	55	0	54	25	0	0
Linezolid	0	0	0	0	0	0
Teicoplanin	0	1	0	0	0	0
Number of isolates	107	116	83	64	16	27

Broilers
Among the *E. faecium* isolates from broilers, 38% were fully sensitive to the antimicrobial agents tested and four isolates (4%) were multi-resistant. The highest occurrence of resistance was found for salinomycin (55%) followed by erythromycin (15%) and tetracycline (5%) (Table 7.1).

Salinomycin is widely used as a coccidiostat in the broiler production. Presently, no cut-off value is recommended by EUCAST for salinomycin, and the value used here is equivalent to the one used in DANMAP 2010. Significant reductions in resistance to salinomycin (from 75% to 55%) and to streptomycin (from 13% to 4%) have been seen over the past five years (Figure 7.1).

From 2010 to 2011, the antimicrobial resistance to erythromycin among *E. faecium* isolated from broilers decreased significantly from 26% to 15% (Figure 7.1), while the overall consumption of macrolides decreased by 30% (web annex, Table A4.4). Resistance towards 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.

Pigs
Among the *E. faecium* isolates from pigs, 31% were fully sensitive to the antimicrobial agents tested and 32% were multi-resistant. The highest occurrence of resistance was found for tetracycline (62%), followed by streptomycin (41%), erythromycin (33%) and kanamycin (25%) (Table 7.1). Both streptomycin and kanamycin belong to the aminoglycosides.

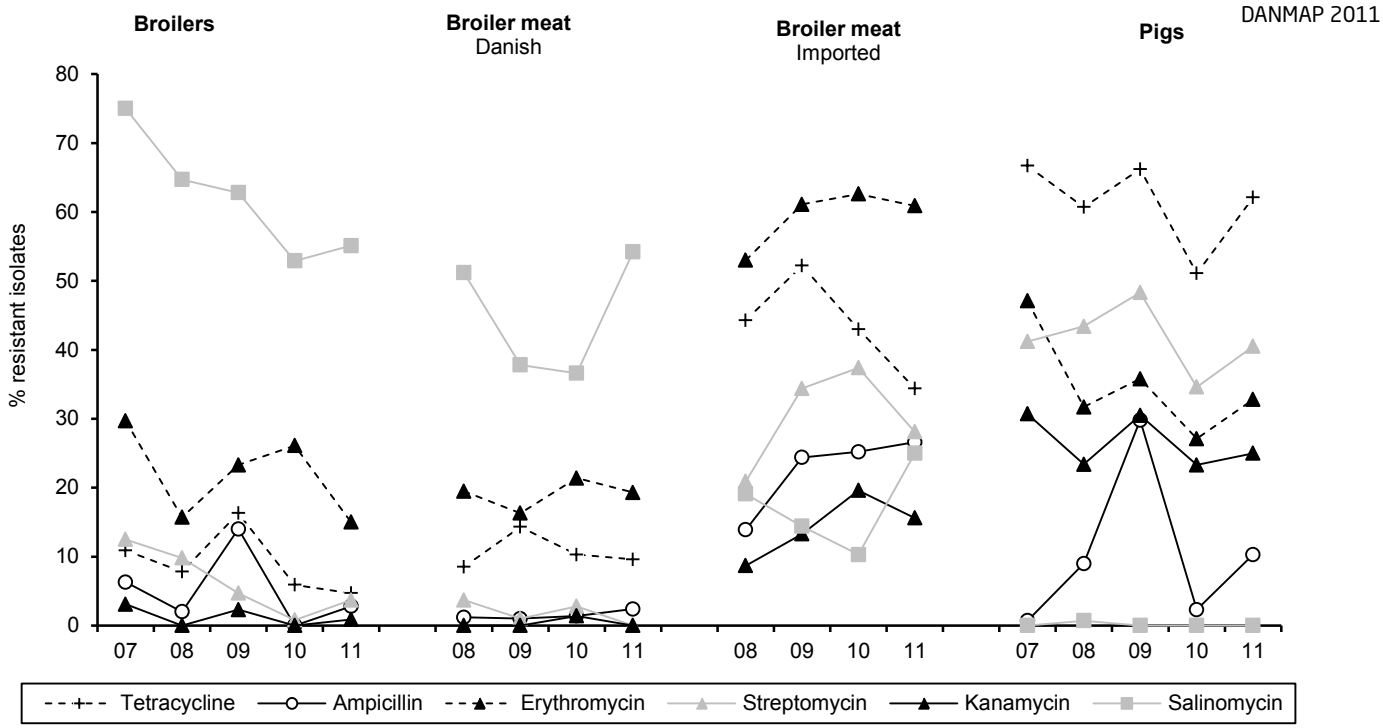
From 2010 to 2011, significant increases in antimicrobial resistance among isolated *E. faecium* from pigs were seen for ampicillin and penicillin; both belonging to the beta-lactams. The increase in resistance to the beta-lactams (ampicillin and penicillin) did not correlate with the usage of beta-lactams in pigs.

Despite usage of streptogramins and avoparcin as growth promoters has been banned since 1994, resistance to vancomycin and quinupristin/dalfopristin remained at a low level among *E. faecium* isolated from pigs (Table 7.1).

Of the streptomycin resistant isolates, 98% (46/47) were also resistant to tetracycline, indicating co-resistance between these antimicrobial agents..

In 1995, the consumption of tetracyclines in pigs was reduced markedly, but has since then increased gradually until 2010 (web annex, Figure A7.1). During this period, the level of tetracycline resistance was not directly linked to the tetracycline consumption. In contrast, the level of erythromycin resistance among *E. faecium* isolates from pigs was strongly correlated with the marked decrease in consumption of macrolides in 1999 (web annex, Figure A7.2).

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



Note: The number of isolates varies between years (broilers: n = 43–119, Danish broiler meat: n = 83–164, imported broiler meat: n = 64–230, pigs: n = 116–153). Data from broiler meat was not available from 2007 and data from pork was excluded due to few isolates

7.1.2 *Enterococcus faecium* in meat

E. faecium were isolated from broiler meat (83 Danish, 64 imported), pork (27 Danish and 16 imported) and beef (16 imported) sampled at wholesale and retail outlets in 2011. Only one isolate per positive sample was susceptibility tested and reported (Table 7.1).

Broiler meat

Among the *E. faecium* isolates from Danish and imported broiler meat, 33% and 20% were fully sensitive to the antimicrobial agents tested, whereas 1% and 38% were found multi-resistant, respectively. For Danish broiler meat, the highest occurrence of resistance was found for salinomycin (54%), erythromycin (19%) and tetracycline (10%), whereas resistance to erythromycin (61%), tetracycline (34%) and beta-lactams (penicillin 28% and ampicillin 27%) was most common among the isolates from imported broiler meat.

From 2010 to 2011, a significant increase in antimicrobial resistance towards salinomycin was seen among isolates from imported broiler meat (Figure 7.1).

When comparing *E. faecium* isolates from Danish and imported broiler meat, significantly higher resistance to tetracycline, penicillin, ampicillin, erythromycin, kanamycin and streptomycin was found in isolates from imported broiler meat. In contrast, resistance to salinomycin was higher among isolates from Danish broiler meat compared to isolates from imported broiler meat. These significant differences have also been observed in previous years (Figure 7.1).

Pork and beef

The number of samples from Danish pork (n = 27) and imported beef (n = 16) was quite low, however among isolates from Danish pork, resistance to erythromycin was most common (15%) followed by resistance towards tetracycline (7%). The isolates from imported beef were fully susceptible to all antimicrobial agents tested.

7.1.3 *Enterococcus faecalis* in animals

For samples from pigs and broilers, only one isolate per farm was included. A randomly selected subsample of isolates from broilers (n = 110) and pigs (n = 117) were susceptibility tested and reported (Table 7.1).

Broilers

Among *E. faecalis* isolates from broilers, 76% were fully sensitive to the antimicrobial agents tested and four isolates (4%) were multi-resistant. The highest occurrence of resistance was observed for tetracycline (17%) followed by erythromycin (15%), streptomycin (4%) and salinomycin (3%) (Table 7.2). As for *E. faecium* isolates from broilers, resistance to erythromycin among the *E. faecalis* isolates decreased significantly from 2010 to 2011. During this period, the overall consumption of macrolides decreased by 30%, even though the consumption already was at a low level (web annex, Table A4.4).

As in previous years, the level of salinomycin resistance was significantly lower in *E. faecalis* than in *E. faecium* isolates from broilers.

As for streptomycin resistance among *E. faecium* isolated from pigs, high (100%) co-resistance between streptomycin, tetracycline and erythromycin was detected among *E. faecalis* from broilers (only four isolates).

Pigs

Among *E. faecalis* isolates from pigs, 13% were fully sensitive to the antimicrobial agents tested and 42% were multi-resistant. The highest occurrence of resistance was observed for tetracycline (85%) followed by erythromycin (54%), streptomycin (37%), kanamycin (32%) and gentamicin (21%) (Table 7.2). From 2010 to 2011, antimicrobial resistance to the aminoglycosides gentamicin and kanamycin increased significantly. This increase could not be related to increased use of aminoglycosides.

All chloramphenicol resistant isolates (27/27) were co-resistant to both tetracycline and erythromycin, while 94% (35/37) of the kanamycin resistant isolates and 96% (24/25) of the gentamicin resistant isolates were resistant to tetracycline and erythromycin. For isolates resistant to both aminoglycosides, 100% (22/22) were resistant to both tetracycline and erythromycin.

The level of tetracycline resistance among *E. faecalis* isolates from pigs did not reflect the general changes in in tetracycline consumption during 1995 to 2011 (web annex, Figure A7.1). In contrast, the level of erythromycin resistance among *E. faecalis* isolates from pigs was strongly correlated with the marked decrease in consumption of macrolides in 1999. During the following decade, the consumption of macrolides to pigs increased slowly, and a corresponding significant increase in erythromycin resistance was observed (web annex, Figure A7.2).

7.1.4 *Enterococcus faecalis* in meat

E. faecalis was isolated from pork (133 Danish, 45 imported), broiler meat (34 Danish, 69 imported) and beef (20 Danish, 30 imported). Only one isolate per sample was susceptibility tested and reported (Table 7.2).

Broiler meat

Among the susceptibility tested *E. faecalis* isolates from Danish and imported broiler meat, 68% and 29% were fully sensitive to the antimicrobial agents tested, whereas 6% (n = 2) and 35% were found multi-resistant, respectively. The highest occurrence of resistance was found for tetracycline (26% and 67%) and erythromycin (18% and 49%) among the isolates from Danish and imported broiler meat, respectively.

When comparing *E. faecalis* isolates from Danish and imported broiler meat from 2011, a significantly lower occurrence of resistance to tetracycline, erythromycin, kanamycin and streptomycin was observed. This tendency

Table 7.2. Resistance (%) among *Enterococcus faecalis* from animals and meat of Danish and imported origin, Denmark 2011

DANMAP 2011

Antimicrobial agent	Broilers	Pigs	Broiler meat		Beef		Pork meat	
	Danish %	Danish %	Danish %	Imported %	Danish %	Imported %	Danish %	Imported %
Tetracycline	17	85	26	67	20	17	17	36
Tigecycline	0	0	0	0	0	0	0	0
Chloramphenicol	0	23	0	6	5	3	4	7
Penicillin	0	0	0	0	0	0	0	0
Ampicillin	0	0	0	0	0	0	0	0
Erythromycin	15	54	18	49	5	7	8	11
Gentamicin	0	21	0	1	0	3	2	4
Kanamycin	0	32	0	29	10	7	5	7
Streptomycin	4	37	6	33	10	10	5	7
Ciprofloxacin	0	0	0	4	0	0	0	0
Vancomycin	0	0	0	0	0	0	0	0
Salinomycin	3	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0	0
Number of isolates	110	117	34	69	20	30	133	45

was also observed in previous years in isolates from Danish broiler meat (Figure 7.2).

Pork

Among *E. faecalis* isolates, 83% of the isolates from Danish pork were fully sensitive to the antimicrobial agents tested, while 44% of the isolates from imported pork were fully sensitive. For both Danish and imported pork, 7% of the isolates were found multi-resistant. Also, for *E. faecalis* from pork, the highest occurrence of resistance was found for tetracycline (17% and 36%) and erythromycin (8% and 11%) among the isolates from Danish and imported origin, respectively.

Compared to 2010, a significantly higher occurrence of antimicrobial resistance to erythromycin and streptomycin was observed in *E. faecalis* isolates from Danish pork. In contrast, no significant changes in levels of resistance were observed in *E. faecalis* isolates from imported pork. Compared to Danish pork, a significantly higher occurrence of resistance to tetracycline was found in *E. faecalis* from imported pork.

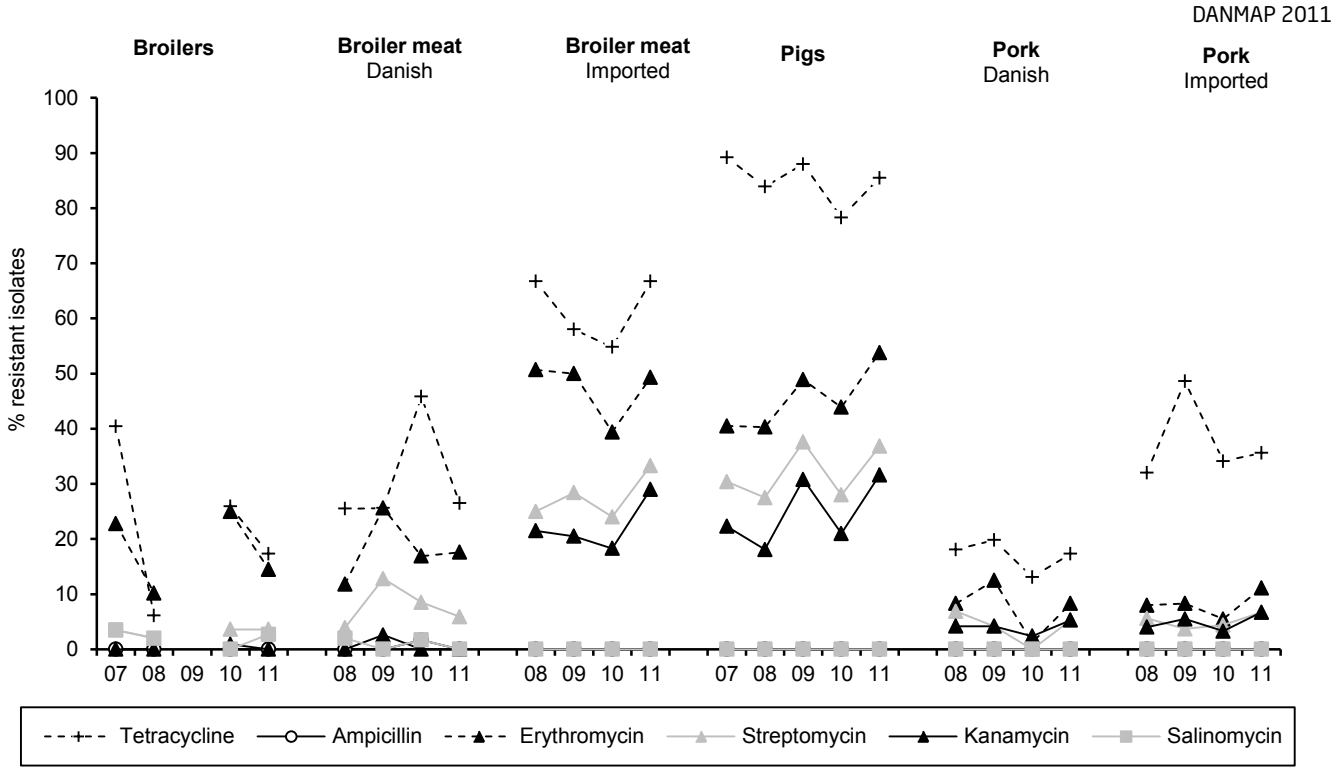
Beef

The number of samples from Danish and imported beef was relatively low, resulting in a low number of isolates available for susceptibility testing. Most of the isolates (80%) from both Danish and imported beef were fully sensitive to the antimicrobial agents tested, whereas very few isolates (1 and 3, respectively) were multi-resistant. Resistance to tetracycline was most common and found in 20% of the Danish isolates and 17% of imported isolates.

7.1.5 Comparison of resistance in animals

Among isolates from pigs, significantly higher levels of resistance were seen in *E. faecium* (tetracycline, penicillin, ampicillin, erythromycin, and kanamycin) and *E. faecalis* (tetracycline, chloramphenicol, erythromycin, gentamicin, kanamycin and streptomycin) than in isolates from broilers. However, a higher level of resistance to salinomycin was found in *E. faecium* from broilers. These differences probably reflect the relatively higher consumption per live biomass, and thus the higher selection pressure, in pigs compared to broilers (Figure 4.3).

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



Note: The number of isolates varies between years (broilers: n = 49–112, Danish broiler meat: n = 34–102, imported broiler meat: n = 69–288, pigs: n = 117–157, Danish pork: n = 84–144, imported pork: n = 45–250). Data from meat was not available from 2007, and broiler data from 2009 was excluded due to few isolatesfrom 2007, and broiler data from 2009 was excluded due to few isolates

7.1.6 *Enterococcus* in a farm to fork perspective

The level of resistance in *E. faecium* and *E. faecalis* from Danish broiler meat reflects the levels observed in the Danish broiler flocks, as there were no significant differences between the observed antimicrobial resistance in isolates from animals and meat. In contrast, significantly lower occurrence of resistance was found for tetracycline, kanamycin and streptomycin in *E. faecium* and *E. faecalis* isolates from Danish pork compared to isolates from Danish pigs. Significantly lower occurrence of antimicrobial resistance was also found for penicillin in *E. faecium* isolates and for chloramphenicol and gentamicin in *E. faecalis* isolates from Danish pork compared to Danish pigs.

The level of antimicrobial resistance in *E. faecium* and *E. faecalis* isolates was significantly higher in imported broiler meat compared to Danish broiler meat. This includes tetracycline, erythromycin, kanamycin and streptomycin for both *E. faecium* and *E. faecalis*, and in addition ampicillin and penicillin for *E. faecium*. Only *E. faecium* from Danish broiler meat had a higher occurrence of resistance to salinomycin than what was observed among the isolates from imported meat. These significant differences have also been observed in previous years.

Lars Bogø Jensen and Lars Stehr Larsen

7.2 Indicator *Escherichia coli*

E. coli isolates from healthy animals originated from faecal samples collected for the DANMAP programme at the time of slaughter. *E. coli* from meat originated from meat sampled at wholesale and retail outlets, collected randomly in all regions of Denmark by the Danish Veterinary and Food Administration Regional Laboratories in a centrally coordinated programme. Samples from healthy humans have not been collected since 2008. If an isolate was resistant to three or more of the ten different antimicrobial classes included in the test panel, it was considered multi-resistant (see definition of multi-resistance and the included antimicrobial classes in section 10.5).

7.2.1 Indicator *Escherichia coli* in animals

Only one isolate per farm was susceptibility tested, and a random selection of isolates from broilers (n = 134), cattle (n = 93) and pigs (n = 157) , were included in the report.

MIC distributions among *E. coli* from broilers, cattle and pigs are shown in the web annex (Table A7.5). Trends in resistance to selected antimicrobial agents in isolates from production animals during 2002–2011 are presented in Figure 7.5.

In 2011, resistance levels were generally higher in isolates from pigs compared to broilers and cattle; however, resistance to quinolones (nalidixic acid and ciprofloxacin) was higher in isolates from broilers (9%) than from cattle or pigs (0-1%) (Table 7.3).

Broilers

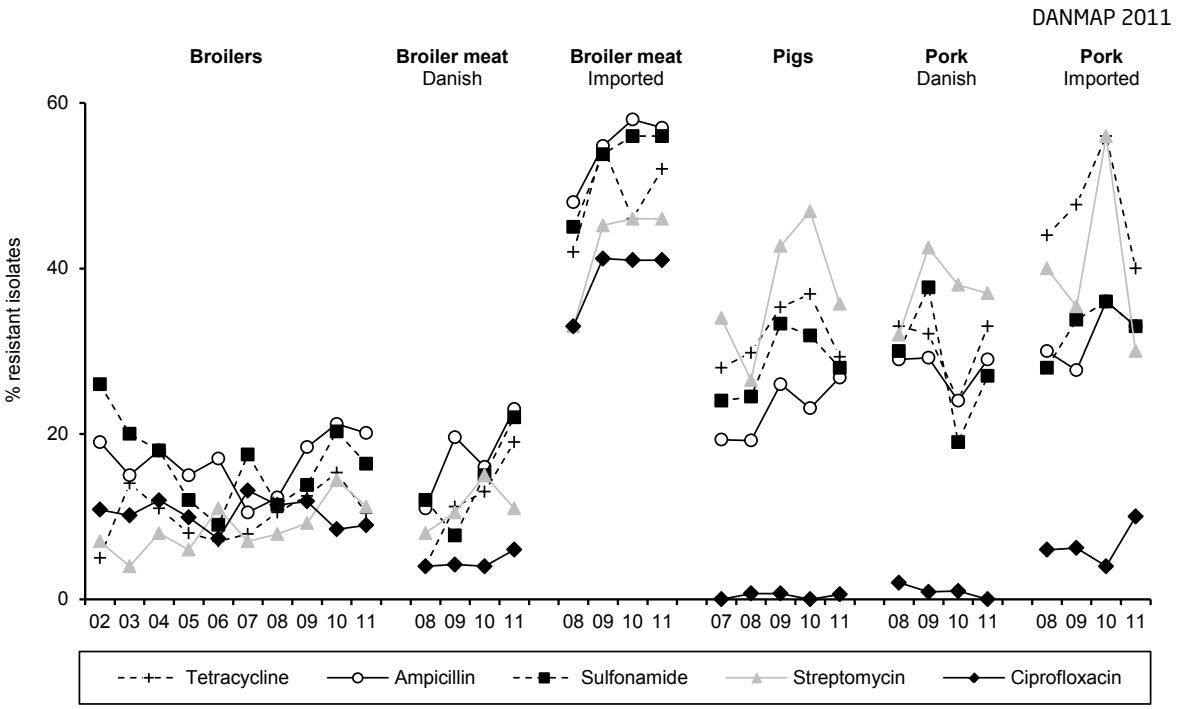
Among *E. coli* isolates from broilers, 57% were fully sensitive and 16% were multi-resistant, similar to levels in 2010. No significant changes in the occurrences of resistance were observed in 2011 compared to 2010 (Figure 7.5). The highest occurrence of resistance in broiler isolates was seen for ampicillin (20%, Table 7.3), a resistance that confers cross-resistance to amoxicillin which has been the most frequently used antibacterial agent in the broiler production for at least a decade (Figure 4.10).

Among the *E. coli* isolates from broilers, one isolate was resistant to ceftiofur.

In 2011, 9% of the *E. coli* isolates from broilers were resistant to fluoroquinolones, and the occurrence has varied between 7% and 13% during the last 10 years. Until 2007, fluoroquinolone consumption in poultry was relatively higher than for the other animal species in Denmark, because the number of antimicrobial agents approved for poultry were limited. In the broiler production (including parent flocks), the highest fluoroquinolone consumption per broiler was observed during 2004–2007, reaching a maximum in 2006. Since 2008, the fluoroquinolone usage in the poultry production has been very low.

The highest occurrence of ciprofloxacin resistance in *E. coli* from broilers was observed in 2007, but the level of resistance has not changed significantly over the past decade (Figure 7.5).

Figure 7.5. Resistance (%) in indicator *Escherichia coli* from broilers and pigs and meat thereof, Denmark



Note: The number of isolates varies between years (broilers: n = 114–152, Danish broiler meat: n = 113–148, imported broiler meat: n = 140–304, pigs: n = 150–160, Danish pork: n = 66–108, imported pork: n = 30–96)

Table 7.3. Resistance (%) among *Escherichia coli* from and meat of Danish and imported origin, Denmark 2011

DANMAP 2011

Antimicrobial agent	Broilers	Cattle	Pigs	Broiler meat		Beef		Pork meat	
	Danish %	Danish %	Danish %	Danish %	Imported %	Danish %	Imported %	Danish %	Imported %
Tetracycline	10	5	29	19	52	5	14	33	40
Chloramphenicol	0	2	4	2	19	0	7	2	20
Florfenicol	0	2	0	0	1	0	2	0	3
Ampicillin	20	2	27	23	57	5	9	29	33
Cephalothin	-	-	-	-	-	-	-	-	-
Ceftiofur	1	0	1	2	7	0	0	0	3
Cefpodoxime	-	-	-	-	-	-	-	-	-
Cefotaxime	1	0	1	2	7	0	0	0	3
Sulfonamide	16	3	28	22	56	0	5	27	33
Trimethoprim	10	0	21	12	38	0	5	24	30
Apramycin	0	0	0	0	1	0	0	0	0
Gentamicin	0	0	0	0	3	0	0	0	0
Neomycin	1	0	3	4	12	0	0	2	3
Spectinomycin	3	0	20	2	31	0	2	15	10
Streptomycin	11	5	36	11	46	3	9	37	30
Ciprofloxacin	9	0	1	6	41	0	5	0	10
Nalidixic acid	9	0	1	6	39	0	5	0	10
Colistin	0	0	0	0	4	0	0	0	0
Number of isolates	134	93	157	122	140	37	44	92	30

Cattle

Among the included animal species, the level of resistance in indicator *E. coli* was lowest in isolates from cattle, where 92% of the isolates were fully sensitive and only 3% (n = 3) were multi-resistant, a level comparable to 2010. In isolates from cattle, the level of resistance was similar to previous years. The most frequent types of resistance (Table 7.3) were resistance to tetracycline (5%) and streptomycin (5%).

In 2011, none of the *E. coli* isolates from cattle were resistant to fluoroquinolones and cephalosporins.

Pigs

From pigs, 49% of the *E. coli* isolates were fully sensitive and 32% were multi-resistant, a level comparable to 2010. In *E. coli* from pigs, resistance to ampicillin (27%), streptomycin (36%), sulfonamide (28%) and tetracycline (29%) was common (Table 7.3), and 11% of the included isolates had the ASSuT resistance profile (resistant to ampicillin, streptomycin, sulfonamide and tetracycline).

For a decade, the total consumption of antimicrobial agents for pigs has increased, but since 2009, large decreases have been observed for the consumption of tetracyclines (32%), penicillins with extended spectrum (23%), streptomycin (12%) and sulfonamides (12%) (Figure 4. 4). However, a time lapse is often seen between changes in consumption and a consequent change in resistance in *E. coli* [Jensen *et al.* 2006. J Antimicrob Chemother. 58: 101–107].

Streptomycin resistance in *E. coli* isolates from pigs decreased significantly in 2011 compared to 2010 (47% vs. 36%, Figure 7.5). Co-resistance between streptomycin and tetracycline and/or sulfonamide resistance is very frequently observed. In 2010, this co-resistance occurred in 99% (74/75) of the streptomycin resistant isolates from pigs, whereas this proportion was reduced to 75% (42/56) in 2011, coinciding with an overall (non-significant) reduction in tetracycline and/or sulfonamide resistance. The decrease in resistance to streptomycin in indicator *E. coli* from pigs may therefore be an effect of the dramatic decrease (by 27%) in use of tetracyclines in the pig production in 2011 (Figure 4.5).

Among the *E. coli* isolates from pigs, two isolates were resistant to both ceftiofur and cefotaxime.

The low level of fluoroquinolone resistance in *E. coli* from Danish pigs and cattle probably reflects the low consumption of fluoroquinolones since 2002–2003, when the use in production animals was restricted by law.

7.2.2 Indicator *Escherichia coli* in meat

Broiler meat

Indicator *E. coli* were isolated from broiler meat (158 Danish, 177 imported), beef (32 Danish, 39 imported) and pork (68 Danish, 70 imported) sampled at wholesale and retail outlets in 2011. Only one isolate per positive sample was susceptibility tested and reported (Table 7.3).

MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates collected from broiler meat, beef and pork are presented in the web annex (Table A7.6).

Among the *E. coli* isolates from Danish and imported broiler meat, 56% and 16% were fully sensitive, whereas 18% and 61% were found to be multi-resistant, respectively. For *E. coli* from Danish broiler meat, the observed resistance to trimethoprim increased significantly from 4% in 2010 to 12% in 2011 (Figure 7.5). The level of resistance in isolates from Danish broiler meat was zero or very low-moderate for all tested agents, with the highest level found for ampicillin (23%) (Table 7.3). The increasing trend in resistance to sulfonamide observed from 2009 (8%) to 2010 (15%) continued through 2011 (19%).

In imported broiler meat, the level of resistance was significantly higher for 11 of the 16 tested antimicrobial agents as compared to *E. coli* from Danish broiler meat (tetracycline, chloramphenicol, ampicillin, colistin, sulfonamide, trimethoprim, neomycin, spectinomycin, streptomycin, ciprofloxacin and nalidixic acid) (Table 7.3).

In 2011, three ceftiofur resistant isolates (2%) were found in Danish broiler meat, as compared to 2010 when the first isolate was found in broiler meat by the non-selective methods. Consequently, the level found in Danish broiler meat was no longer significantly lower than in imported broiler meat (7%). Occurrence determined by nonselective methods is an indicator of the prevalence in the bacterial population, whereas occurrence shown by selective methods is an indicator of the presence at sampling level (e.g. in the animals or meat samples). See Textbox 7 for results using selective methods.

The occurrence of fluoroquinolone resistance in broiler meat was unchanged. The level of fluoroquinolones resistance was significantly higher in imported meat (41%) than in Danish broiler meat (6%) (Table 7.3).

Pork

Among indicator *E. coli* isolates from Danish and imported pork, 49% and 30% were fully sensitive whereas 35% and 33% were found to be multi-resistant, respectively. From 2010 to 2011, no significant changes in resistance in *E. coli* from Danish pork were observed. In imported pork, a decrease in occurrence of resistance to streptomycin from 56% in 2010 to 30 % in 2011 was observed (Figure 7.5).

In 2011, significantly lower resistance to chloramphenicol, ciprofloxacin and nalidixic acid was found in isolates from Danish pork compared to imported pork (Table 7.3).

In imported pork, 10% of the *E. coli* isolates were resistant to fluoroquinolone, and 3 % were resistant to ceftiofur in 2011 (Table 7.3). Fluoroquinolone resistance remained low (none observed in 2011) in Danish pork, probably due to the low fluoroquinolone consumption in Danish pigs since 2002 (see web annex, Table A4.2). The occurrence of cephalosporin resistant isolates in Danish pork has remained at a very low level (see also Textbox 7).

In contrast, the use of both fluoroquinolones and cephalosporins has been increasing for animal production in some European countries during 2005–2009 [European Medicines Agency, 2011] and internationally, the occurrence of cephalosporin resistance (ESBL) in food is increasing [European Food Safety Agency, 2011].

Beef

Among the susceptibility tested indicator *E. coli* isolates from Danish and imported beef, 92% and 80% were fully sensitive whereas 3% (one isolate) and 7% were found to be multi-resistant, respectively. No significant changes in resistance levels were observed from 2010 to 2011 and no significant differences were found between imported and Danish beef. In imported beef, two indicator *E. coli* isolates (5%) were found resistant to fluoroquinolones, whereas no fluoroquinolone resistant isolates were found in Danish beef (Table 7.3).

7.2.3 Indicator *E. coli* in a farm to fork perspective

For most of the tested antimicrobial agents, the level of resistance in Danish meat reflected the level of resistance in the corresponding animal species with one exception: The occurrence of tetracycline resistance among *E. coli* from Danish broiler meat (19%) was significantly higher than what was found among Danish broilers (10%). This difference might be caused by contamination from other sources at the slaughterhouse. In Denmark, the level of resistance is generally very low among broilers, and therefore contamination is likely to cause an increase in resistance level.

Vibeke Frøkjær Jensen and Lars Stehr Larsen

Occurrence of Extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* after selective enrichment with ceftriaxone in meat and food producing animals

Background: Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide. This resistance type seems to be related to food producing animals and may spread to humans via food. 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 at farm level, in pigs and cattle at slaughter and in meat at retail (see section 11.2 for definition of ESBL).

Materials and methods: During February 2011 through October 2011, pools of five stool samples (n = 78) were collected in pig farms and fecal samples were taken from pigs (n=777) and cattle (n=186) at slaughter. From January through November 2011, samples of broiler meat (132 Danish, 152 imported), beef (122 Danish, 115 imported) and pork (225 Danish, 99 imported) were collected randomly in retail stores and outlets in all regions of Denmark. The samples were randomly selected; and for pig farms each farm was sampled only once. For cattle and pigs sampled at slaughter, one animal represented one farm and no herds were sampled more than once in the same month. *E. coli* was isolated from 1 g of pooled stool sample, 1 g of feces 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 PCR, micro array and DNA sequencing.

Results: None (0/78) of the pig stool samples contained ceftriaxone resistant *E. coli* and this was significantly lower than in 2010 (Figure 1). For pigs at slaughter 3.6% (28/777) contained ceftriaxone resistant *E. coli*, which were significantly lower than in 2009 and 2010. The most commonly detected gene was CTX-M-1 but also CTX-M-14 and CTX-M-15 genes often detected in *E. coli* causing human infections were found. In cattle, the prevalence of ceftriaxone resistant *E. coli* were at the same level (10.2% , 19/186), as in 2010 and CTX-M-1 followed by CTX-M-14 was most frequently found (Figure 1).

From meat samples, the highest prevalence of ceftriaxone resistant *E. coli* was found among imported broiler meat (48%, 73/152). But the prevalence in Danish broiler meat (44%, 58/132) had increased significantly when compared to 2010 (8.6%) now being at the same level as imported broiler meat. The most commonly detected gene in broiler meat was, as found in previous years, CMY-2 followed by CTX-M-1. The prevalence of ceftriaxone resistant *E. coli* was 4% in imported pork and remained even lower in beef and Danish pork [0-0.9%] (Figure 2).

Discussion and conclusions: The results showed that the voluntary ban of cephalosporin usage in the Danish pig production had an effect on reducing ceftriaxone resistant *E. coli* in the pigs at farm level and at slaughter. The prevalence of ceftriaxone resistant *E. coli* in cattle was unchanged in 2011 compared to 2010, probably because 3rd and 4th generation cephalosporins are still used for intramammary and systemic treatment in cattle. The high prevalence of ceftriaxone resistant *E. coli* found in Danish broiler meat was surprising since cephalosporins are not used in the Danish broiler production. However, this high prevalence may be due to extended spectrum penicillins being increasing used for treatment in the Danish broiler production as extended spectrum penicillins may select for ceftriaxone resistant *E. coli* being introduced via breeding animals.

CTX-M-1 was the most common ESBL-gene in pigs and cattle, but also CTX-M-14 and CTX-M-15 was found; these genes are often found in ESBL *E. coli* causing human infections. A more detailed analysis of CTX-M-14 and CTX-M-15 isolates from pigs and pork showed isolates from porcine origin to belong to STs that had previously given rise to infections in humans. Furthermore, the genes were transferable to a ST131 *E. coli* recipient of pork origin suggesting that pigs and pork can be a reservoir of human pathogenic ESBL-producing *E. coli* and a reservoir of transferable CTX-M-14 and CTX-M-15 genes [Hammerum et al, 2012, J. Antimicrobial Chemother., Ahead of print].

Broiler meat from both Danish and imported sources seemed to be the most important meat sources and CMY-2 was the most common type in broiler meat. CMY-2 was also found in ESBL *E. coli* causing human infections (See Textbox 9) but it is unknown whether the prevalence of *E. coli* with CMY-2 causing human infections has increased.

Yvonne Agersø

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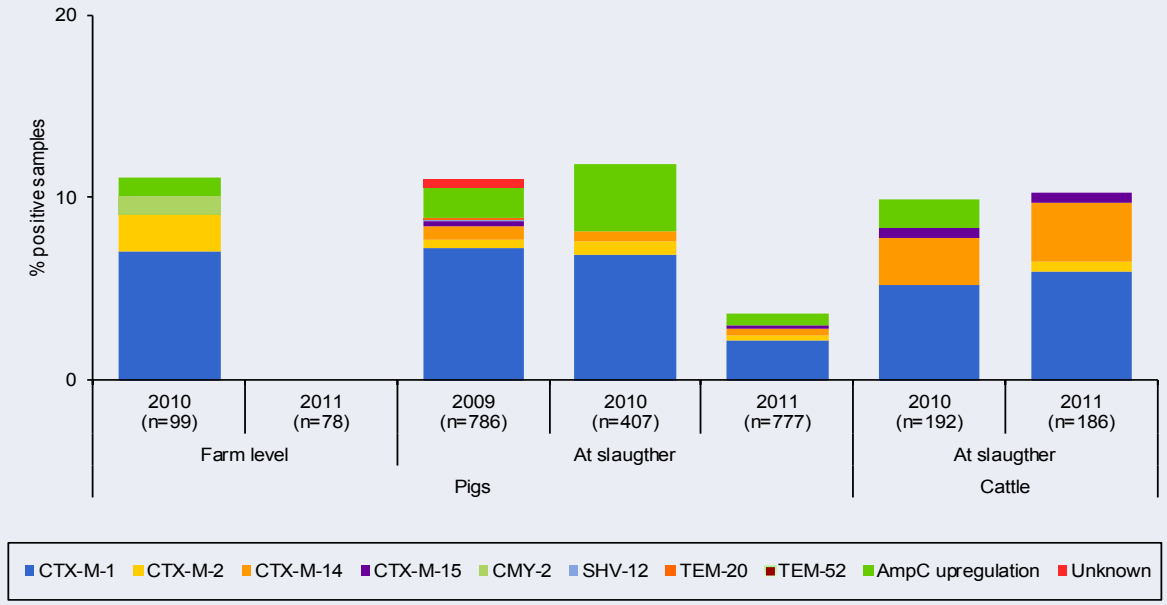
Figure 1. Occurrence (%) of ESBL-producing *Escherichia coli* and genes in pigs and cattle from samples[a] collected at farm and slaughterhouse level, Denmark

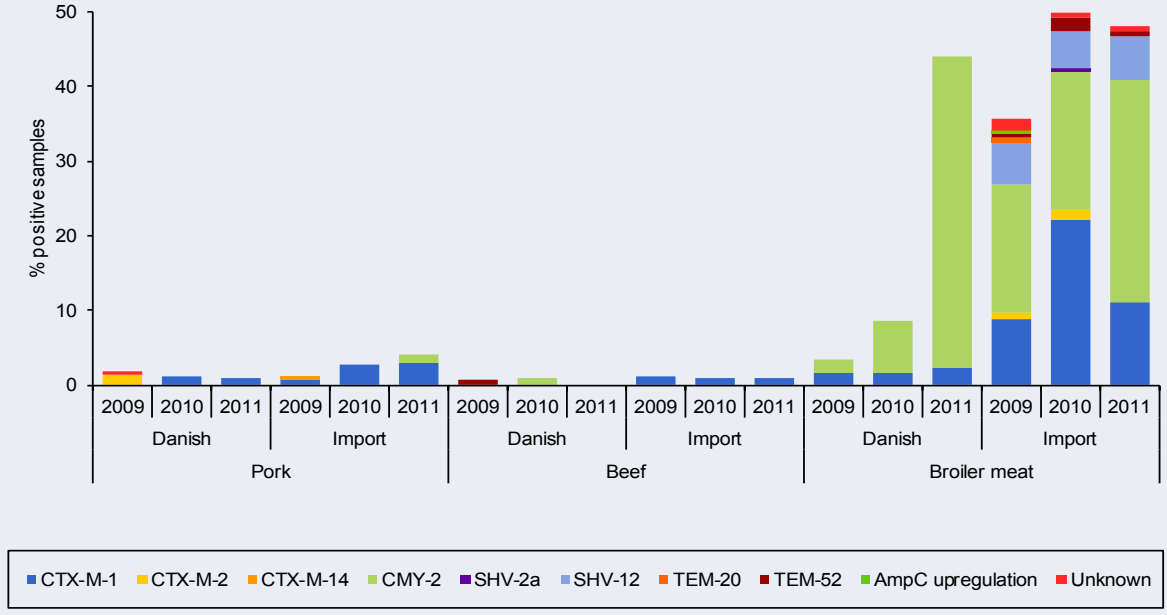
DANMAP 2011



a) *E. coli* was isolated after selective enrichment 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-producing *Escherichia coli* and genes in meat[ab], Denmark

DANMAP 2011



a) *E. coli* was isolated after selective enrichment with ceftriaxone (1 µg/ml). The genetic background for ESBL resistance was revealed by use of PCR, micro array and DNA sequencing
b) Each year, approximately 1,000 samples are collected evenly distributed between the six categories of meat

RESISTANCE IN HUMAN CLINICAL BACTERIA 8

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* cause a variety of intestinal and extra-intestinal infections such as diarrhoea, urinary tract infections, meningitis, and bloodstream infections. For *E. coli*, this report includes data from 12 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. Data on antimicrobial resistance in blood and urine isolates of *E. coli* in hospitals were obtained from 12 of the 13 Danish DCM working with hospital isolates; 11 DCM 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).

***E. coli* blood isolates obtained from hospitalised patients**

The antimicrobial susceptibility of approximately 3,600 *E. coli* isolates from blood was reported in 2011 (Table 8.1 and Figure 8.1).

In 2011, cefuroxime (2nd generation cephalosporin) resistance was 9% (min. 6%, max. 24%) the same as reported in 2010. Likewise, 8% (min. 6%, max. 21%) of the isolates were resistant to 3rd generation cephalosporin (reported as resistance to ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) similar to the level in 2010. The occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 368 *E. coli* blood isolates from hospitalised patients (7%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes; ESBL-producing *E. coli* blood isolates from October 2011 were investigated for the genetic background of their ESBL-production, see Textbox 9.

In 2011, ciprofloxacin resistance was 14% (min. 12%, max. 31%) and nalidixic acid resistance was 21% (min. 17%, max. 24%), which is the same level as in 2010.

The level of fluoroquinolone 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 2010 [EARS-Net 2010].

Aminoglycoside (gentamicin) resistance was 6% (min. 2%, max. 15%) in 2011, which is at the same level as in 2010. This was above the level reported to EARS-Net by the other Nordic countries in 2010 [EARS-Net 2010]. Mecillinam resistance was 10% (min. 5%, max. 17%) in 2011, which is at the same level as in 2010.

Over the last decade, resistance to cefuroxime has increased from 2% in 2002 to 9% in 2011. Resistance to 3rd generation cephalosporins has only been reported since 2008. Resistance to fluoroquinolones has also increased over the last ten years; ciprofloxacin resistance increased from 3% in 2002 to 14% in 2011, and nalidixic acid resistance from 6% in 2002 to 21% in 2011. Aminoglycoside (gentamicin) resistance has increased from 1% in 2002 to 6% in 2011. The increased frequency of resistance in *E. coli* blood isolates parallels the increased consumption of broad spectrum antimicrobial agents [Textbox 8].

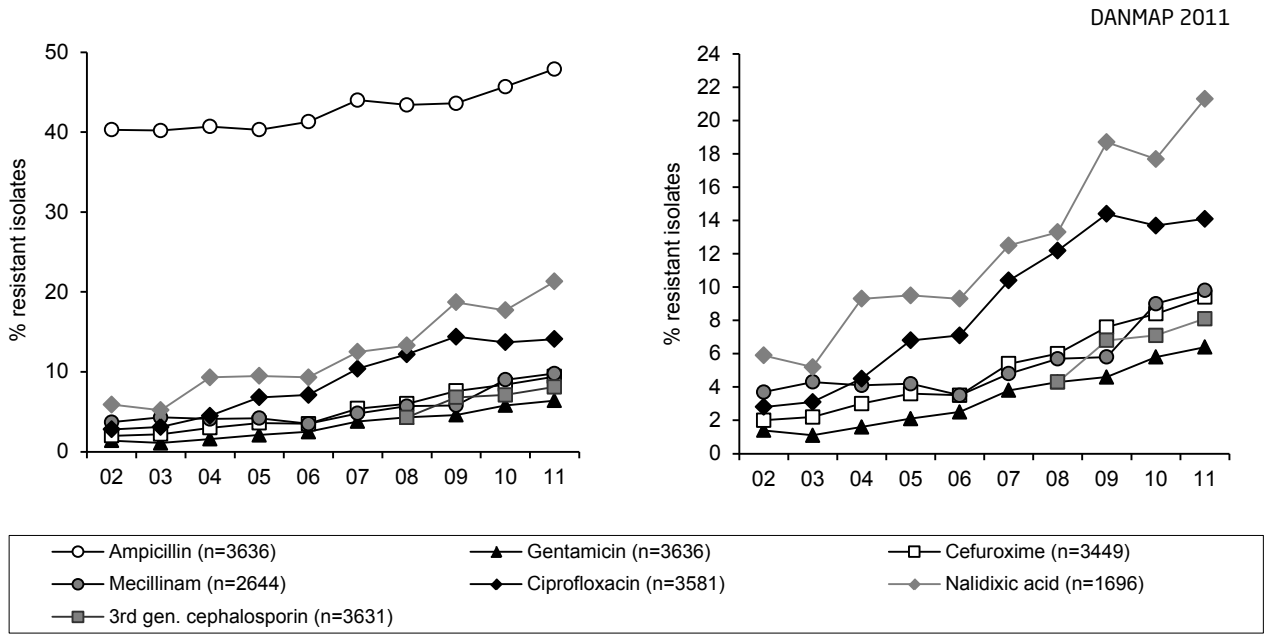
Table 8.1. Resistance (%) in *Escherichia coli* isolates from humans, Denmark 2011

DANMAP 2011

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Ampicillin	48	42	41
Mecillinam	10	7	6
Sulfonamide		35	35 #
Gentamicin	6	4 *	3
Ciprofloxacin	14	13 *	11
Nalidixic acid	21	17 *	15
Cefuroxime	9	5	3
3rd generation cephalosporins [a)]	8	5	3
Meropenem	0		
Max. number of isolates tested	3636	36389	32117

*) An asterisk indicates a significant increase from 2010 to 2011
#) A number sign indicates a significant decrease from 2010 to 2011
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 2011

In 2011, carbapenem (meropenem) resistance was not observed in *E. coli* blood isolates.

E. coli urine isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 36,000 *E. coli* isolates obtained from hospitalised patients with a urinary tract infection was reported in 2011 (Table 8.1 and Figure 8.2).

In 2011, cefuroxime (2nd generation cephalosporin) resistance was 5% (min. 4%, max. 9%) the same as reported in 2010. The occurrence of 3rd generation cephalosporin resistance (reported as resistance to cefpodoxime or cefotaxime) was 5% (min. 4%, max. 8%) similar to the level in 2010. The occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 4068 *E. coli* urine isolates from hospitalised patients (5%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes; ESBL-producing *E. coli* blood isolates from October 2011 were investigated for the genetic background of their ESBL-production, see Textbox 9.

Fluoroquinolone resistance increased significantly from 2010 (ciprofloxacin 12%, nalidixic acid 15%) to 2011 (ciprofloxacin 13%, nalidixic acid 17%). Over the last decade, an increase in the occurrence of fluoroquinolone resistance has also been seen; ciprofloxacin resistance has increased from 2% in 2002, nalidixic acid resistance has increased from 6% in 2002.

Aminoglycoside (gentamicin) resistance increased significantly from 3.7% in 2010 to 4.4% in 2011.

Carbapenem resistance is not mandatory reportable in Denmark and only one DCM reported data on all isolates; therefore no calculation of the occurrence of carbapenem resistance could be made. In 2011, carbapenem (meropenem) resistance was observed in five *E. coli* urine isolates from hospitalised patients. The mechanism behind the carbapenem resistance in these isolates is not known since the isolates were not sent to SSI for further investigation.

E. coli urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 32,000 *E. coli* isolates obtained from patients with a urinary tract infection from primary health care was reported in 2011 (Table 8.1 and Figure 8.3).

In 2011, cefuroxime (2nd generation cephalosporin) resistance was 3% (min. 3%, max. 5%) the same as reported in 2010. The occurrence of 3rd generation cephalosporin resistance (reported as resistance to cefpodoxime or cefotaxime) was 3% (min. 3%, max. 5%) similar to the level in 2010. The occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 4037 *E. coli* urine isolates from hospitalised patients (3%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes; ESBL-producing *E. coli* blood isolates from October 2011 were investigated for the genetic background of their ESBL-production, see Textbox 9.

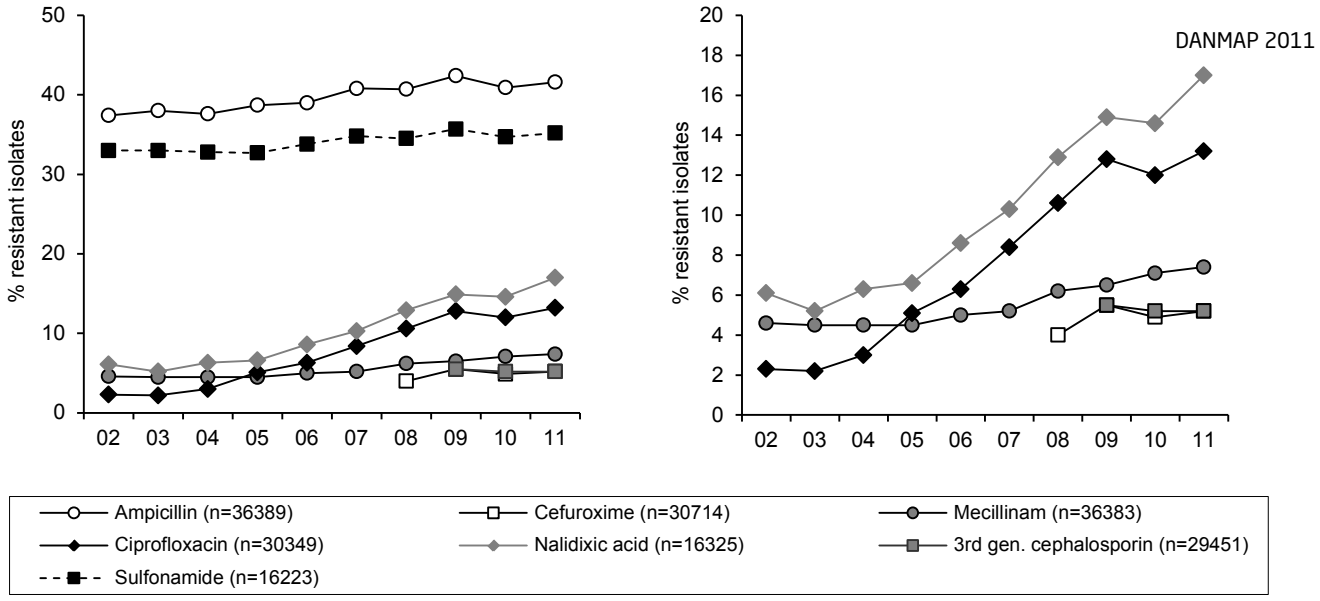
Over the last decade, the occurrence of resistance to fluoroquinolones has increased; ciprofloxacin resistance from 1% in 2001 to 11% in 2011, and nalidixic acid resistance from 5% in 2001 to 15% in 2011, respectively.

The occurrence of sulfonamide resistance decreased significantly from 37% in 2010 to 35% in 2011.

In 2011, carbapenem (meropenem) resistance was observed in three *E. coli* urine isolates from primary health care. The carbapenem resistant isolates were not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since the DCM reported data on selected isolates only.

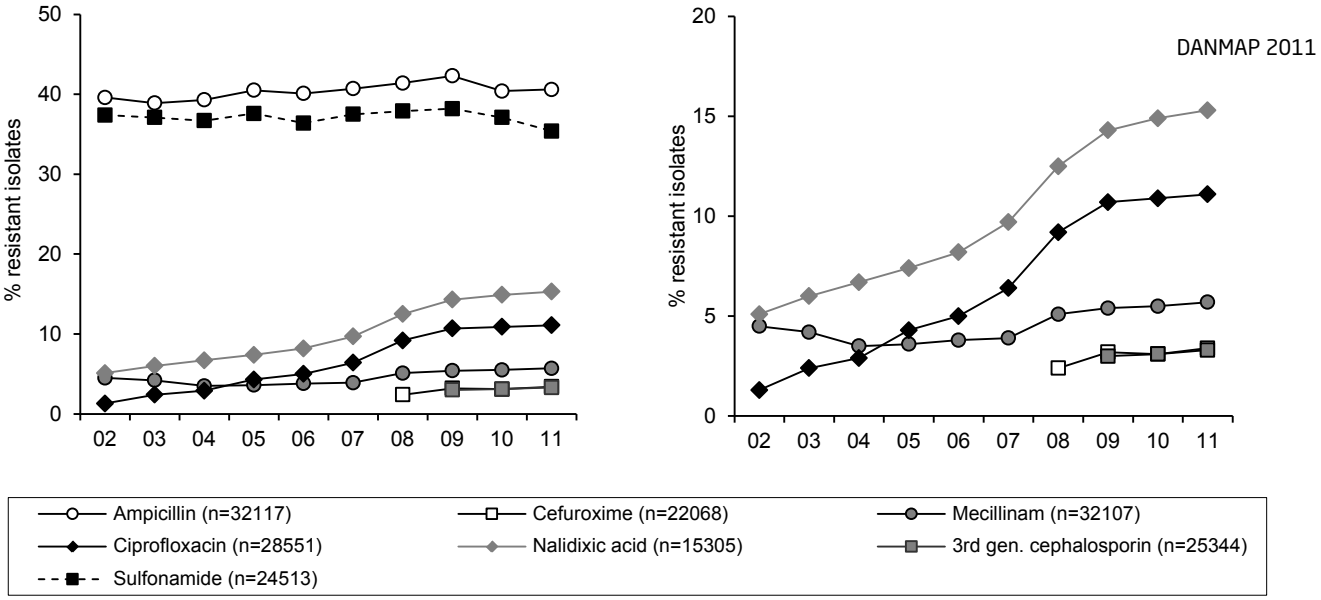
Line Skjøt-Rasmussen, Stefan S. Olsen and Anette M. Hammerum

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 2011

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

Increasing consumption of antimicrobial agents in Denmark parallels increasing resistance in *Escherichia coli* bloodstream isolates

Background: *Escherichia coli* is one of the most important causes of nosocomial infections. In hospitals, *E. coli* bloodstream infections are often treated with beta-lactams (mainly cephalosporins and carbapenems) and fluoroquinolones. However, resistance to these compounds has been reported with increasing frequency in Europe in recent years. Until 2006, the occurrence of 3rd generation cephalosporin resistant *E. coli* from bloodstream infections in Denmark was low [EARRS Annual Report 2006]. To monitor the ESBL-prevalence in Danish patients, three studies have been carried out; these results are presented in Textbox 9. In 2009, we reported on the consequences of the increased antibacterial consumption in Danish hospitals (2001–2007) [Jensen *et al.* 2009. J Antimicrob Chemother. 63: 812-5]. We recently reported on the development of antimicrobial consumption in Danish hospitals (2007–2010) and resistance among *E. coli* from bloodstream infections (2008–2010) [Skjøt-Rasmussen *et al.* 2012. Int J Antimicrob Agents, 40: 86–8].

Materials and methods: From 2008 through 2010, data on gentamicin, ciprofloxacin, 3rd generation cephalosporin (ceftazidime, ceftriaxone, or cefotaxime) and carbapenem resistance in *E. coli* blood isolates were collected from 9 of 14 Departments of Clinical Microbiology (DCM) in Denmark (DCM at Hvidovre, Rigshospitalet, Hillerød, Odense, Esbjerg, Vejle, Aarhus, Viborg and Aalborg Hospital). An average of 2300 *E. coli* blood isolates per year was collected from the catchment area representing approximately 2/3 of the Danish population (3.7 million people).
Consumption data for the included hospitals were obtained from Statens Serum Institut (formerly the Danish Medicines Agency). Since an increase in the level of resistance is often observed after the consumption of antimicrobial agents takes place, consumption data from 2007 were included in our study.

Results: The level of resistance to gentamicin, ciprofloxacin and 3rd generation cephalosporins increased significantly from 2008 through 2010 (Figure 1a). Resistance to gentamicin increased significantly from 4.3% to 6.5%, ciprofloxacin from 9.7% to 13.6%, and 3rd generation cephalosporins from 3.9% to 7.5%. The level of multi-resistance (gentamicin, ciprofloxacin, 3rd generation cephalosporins) increased significantly from 1.3% in 2008 to 2.4% in 2010 (Figure 1a). None of the isolates were carbapenem resistant.
From 2007–2010, the consumption of broad spectrum antimicrobial agents also increased (Figure 1b). This was seen for 2nd generation cephalosporins (12.01 to 15.35 DDD/100 bed-days in 2007 compared to 2010), 3rd generation cephalosporins (1.13 to 1.46 DDD/100 bed-days in 2007 compared to 2010) and fluoroquinolones (8.60 to 11.08 DDD/100 bed-days in 2007 compared to 2010). Furthermore, the consumption of carbapenems increased from 2.53 DDD/100 bed-days in 2007 to 4.81 DDD/100 bed-days in 2010.

Discussion and conclusions: The hospital consumption of antimicrobial agents would be expected to significantly influence only those isolates that were nosocomial or hospital-associated. We do not know the exact number of hospital-associated or community-acquired isolates in our study. However, the interaction between consumption of antimicrobial agents inside and outside hospitals is complex. Ciprofloxacin is used in hospitals as well as in primary health care in Denmark (see Table 5.2), therefore the ciprofloxacin resistant *E. coli* could also be selected outside the hospitals; however, 2nd and 3rd generation cephalosporins and carbapenems are almost exclusively used in hospitals. Cephalosporinase-producing *E. coli* can be detected in meat and animals in Denmark [Textbox 7]; we do not know if the isolates in our study has an animal origin. Further studies are needed to quantify the risk of *E. coli* of animal origin in relation to human health.

The increased frequency of resistance in *E. coli* blood isolates parallels the increased consumption of broad spectrum antimicrobial agents. An increase in multi-resistant *E. coli* infections might explain part of the increased consumption of carbapenems. The increasing resistance in *E. coli* is not merely a Danish problem; an alarming increase in antimicrobial resistance in *E. coli* is observed in all of Europe [Gagliotti *et al.* 2011. Euro Surveill. 16: pii:19819]. Global efforts to reduce the use of broad spectrum antibiotics are warranted. A recent study has shown that bloodstream infections caused by 3rd generation cephalosporin resistant *E. coli* are associated with excess mortality and prolongation of hospital stay, posing a considerable burden on patients and health care systems [De Kraker *et al.* 2011. PloS Med. 8: e1001104].

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Figure 1. Data on antimicrobial resistance in *Escherichia coli* blood isolates and antimicrobial consumption in hospitals from nine Danish Departments of Clinical Microbiology. All data were obtained as part of the DANMAP programme. (a) The percentage of gentamicin, ciprofloxacin and 3rd generation cephalosporin resistant and multi-resistant (gentamicin, ciprofloxacin and 3rd generation cephalosporin) *E. coli* blood isolates (2008–2010) with 95% confidence intervals

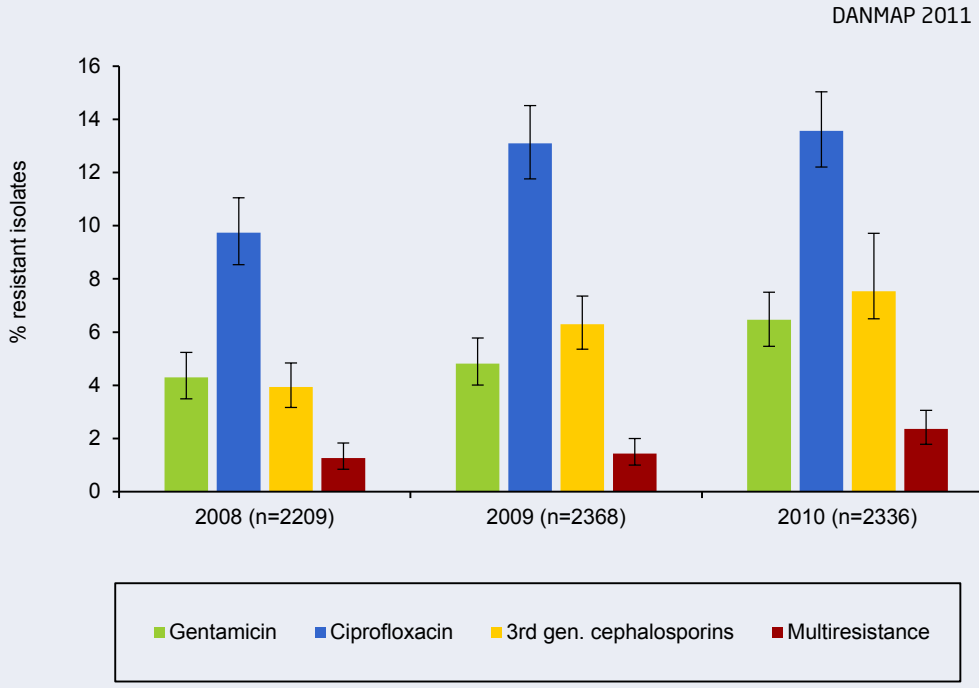
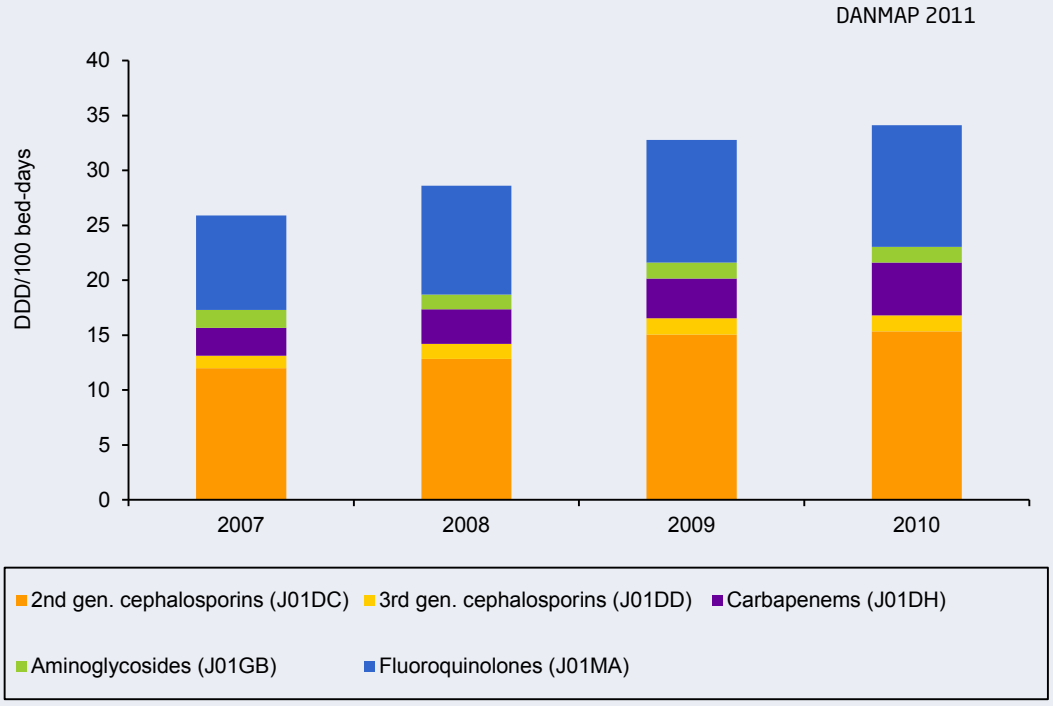


Figure 2. The consumption (DDD/100 bed-days) of 2nd generation cephalosporins, 3rd generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones in hospitals (2007–2010).



8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the intestinal flora in humans but is often the cause of extra-intestinal 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. Data on antimicrobial resistance in blood and urine isolates of *K. pneumoniae* in hospitals were obtained from 12 of the 13 Danish DCM working with hospital isolates; 11 of the 12 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *K. pneumoniae f*rom primary health care.

K. pneumoniae blood isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 900 *K. pneumoniae* isolates from blood was reported in 2011 (Table 8.2).

Until 2007, the occurrence of antimicrobial resistance in *K. pneumoniae* was low and at the same level as in the other Nordic countries (e.g. for 3rd generation cephalosporins <5%). However, since 2007 a steady increase in resistance has been observed until 2009. In general, the level of antimicrobial resistance in 2011 was similar to 2010.

In 2011, 3rd generation cephalosporin resistance (reported as resistance to ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) was 10% (min. 0%, max. 16%); this was at the same level as in 2010 (9%). 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 several other European countries in 2010 [EARS-Net 2010]. 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 2011. However, the occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 83 *K. pneumoniae* blood isolates from hospitalised patients (7%), as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. Thus, 3rd generation cephalosporin resistance among *K. pneumoniae* in Denmark is mostly due to ESBL-producing isolates and in the majority of the cases it was encoded by CTX-M-15.

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

DANMAP 2011

Substance	2008	2009	2010	2011[a]
	%	%	%	%
Gentamicin	7.6	9.0	6.0	5.8
Ciprofloxacin	16.1	18.1	11.3	11.7
Nalidixic acid	22.2	21.8	17.4	18.3
Cefuroxime	15.2	17.1	12.8	14.0
3rd gen. cephalosporins [b]	9.6	12.0	9.2	9.6
Meropenem	<1	0	0	<1
Max. number of isolates tested	788	886	799	908

a) Susceptibility to gentamicin was tested in 908 isolates, ciprofloxacin susceptibility in 888 isolates, nalidixic acid susceptibility in 367 isolates, cefuroxime susceptibility in 852 isolates, 3rd generation cephalosporin susceptibility in 906 isolates, and meropenem susceptibility in 589 isolates
b) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

The occurrence of fluoroquinolone resistance (ciprofloxacin 12%, nalidixic acid 18%) and aminoglycoside (gentamicin) resistance (6%) was above the level reported from the other Nordic countries and the same as reported to EARS-Net by other European countries in 2010 [EARS-Net 2010].

In 2011, one carbapenem (meropenem) resistant *K. pneumoniae* blood isolate was found. The mechanism behind the carbapenem resistance in this isolate is not known since the isolate was not sent to SSI for further investigation.

K. pneumoniae urine isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 5,700 *K. pneumoniae* isolates obtained from hospitalised patients with a urinary tract infection was reported in 2011 (Table 8.3).

The occurrence of 3rd generation cephalosporin resistance (reported as cefpodoxime or cefotaxime) decreased significantly from 12% in 2010 to 10% in 2011. The occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 650 *K. pneumoniae* urine isolates from hospitalised patients (10%), as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. The occurrence of 2nd generation cephalosporin (cefuroxime) resistance also decreased from 13% in 2010 to 11% in 2011.

Fluoroquinolone (ciprofloxacin) resistance decreased significantly from 14% in 2010 to 11% in 2011.

In 2011, carbapenem (meropenem) resistance was observed in five *K. pneumoniae* urine isolates from hospitalised patients. One of the five isolates was tested for the presence of genes encoding carbapenemases and it was positive for KPC-2. The origin of this KPC-2 producing *K. pneumoniae* was unknown [Textbox 11]. The mechanism behind the carbapenem resistance in the other four isolates was not tested. The presence of antimicrobial resistance in this species is not mandatory reportable and no calculation of the occurrence of carbapenem resistance could be made since only one DCM reported data on all isolates.

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

DANMAP 2011

Substance	2009 %	2010 %	2011[a] %
Mecillinam	13.3	14.3	11.9
Sulfonamide	27.0	28.6	33.4
Gentamicin		7.3	6.4
Ciprofloxacin	17.3	13.9	11.5
Nalidixic acid	21.9	19.6	18.8
Cefuroxime		12.8	10.9
3rd gen. cephalosporins [b]	12.8	12.0	9.6
Max. number of isolates tested	6394	5740	5746

a) Susceptibility to mecillinam was tested in 5746 isolates, sulfonamide susceptibility in 2683 isolates, gentamicin susceptibility in 3899 isolates, ciprofloxacin susceptibility in 4827 isolates, nalidixic acid susceptibility in 2065 isolates, cefuroxime susceptibility in 4720 isolates, and 3rd generation cephalosporin susceptibility in 4595 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

DANMAP 2011

Substance	2009 %	2010 %	2011[a] %
Mecillinam	15.1	16.3	12.5
Sulfonamide	30.2	33.9	34.9
Gentamicin		3.5	2.3
Ciprofloxacin	13.2	11.9	8.6
Nalidixic acid	19.9	19.7	14.1
Cefuroxime		8.6	6.8
3rd gen. cephalosporins [b]	8.1	7.0	5.3
Max. number of isolates tested	3200	3200	3489

a) Susceptibility to mecillinam was tested in 3489 isolates, sulfonamide susceptibility in 2510 isolates, gentamicin susceptibility in 1342 isolates, ciprofloxacin susceptibility in 3198 isolates, nalidixic acid susceptibility in 1338 isolates, cefuroxime susceptibility in 2183 isolates, and 3rd generation cephalosporin susceptibility in 2777 isolates
b) Tested 3rd generation cephalosporins were cefpodoxime and cefotaxime

Sulfonamide resistance increased from 29% in 2010 to 33% in 2011. Mecillinam resistance decreased from 14% in 2010 to 12% in 2011.

K. pneumoniae urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 3,400 *K. pneumoniae* isolates obtained from patients with a urinary tract infection from primary health care was reported in 2011 (Table 8.4).

The occurrence of 3rd generation cephalosporin resistance (reported as cefpodoxime or cefotaxime) decreased significantly from 7% in 2010 to 5% in 2011. The occurrence of 3rd generation cephalosporin resistance reported from the DCM corresponded to the prevalence of ESBL in 450 *K. pneumoniae* urine isolates from general practice patients (7%), as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2011 [Textbox 9]. Resistance to 3rd generation cephalosporins in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from both blood and urine from hospitalised patients.

The occurrence of fluoroquinolone resistance (ciprofloxacin 9%, nalidixic acid 14%) decreased significantly from the level observed in 2010 (ciprofloxacin 12%, nalidixic acid 20%). Ciprofloxacin and nalidixic acid resistance in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from urine from hospitalised patients.

In 2011, carbapenem (meropenem) resistance was observed in one *K. pneumoniae* urine isolate from primary health care. The carbapenem resistant isolate was not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since the DCM reported data on selected isolates only.

Sulfonamide resistance was 35% (min. 27%, max. 47%). Mecillinam resistance decreased significantly from 16% in 2010 to 12% in 2011.

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Extended spectrum beta-lactamase producing bacteria in patients, October 2011

Background: Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria are found in the intestinal flora of hospitalised patients and otherwise healthy persons in the community. Being an ESBL carrier is a risk factor for subsequent clinical infection with the same ESBL-producing bacterium. The bacteria are transmitted via the faecal-oral route and risk of infection is enhanced by the presence of catheters and other foreign bodies. In the community, ESBL-producing bacteria are often associated with urinary tract infections while in hospital settings they may also cause pneumonia and bloodstream infections. ESBL-producing enterobacteria, particularly *Klebsiella pneumoniae*, have a distinct tendency to cause nosocomial outbreaks. In Denmark, clonal spread has been detected at hospitals in Zealand [Lester *et al.* 2011. Int J Antimicrob Agents. 38: 180-2]. ESBL-producing *E. coli* are also detected in meat and faecal samples from animals [Textbox 7].

Materials and methods: In October 2011, 12 of the 13 Danish Departments of Clinical Microbiology screened all *E. coli* and *K. pneumoniae* urine and blood isolates for ESBL-production using a cephalosporin disc/tablet on the primary plate. Results were confirmed by demonstrating inhibition with clavulanic acid in combination with a 3rd or 4th generation cephalosporin using the disc-combination method or by gradient test. The majority of the isolates were sent to SSI for further confirmation and genotyping. Polymerase chain reaction (PCR) amplification and sequencing were performed with primers specifically to identify bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{OXA}, bla_{SHV} and bla_{AmpC} β-lactamase genes.

The prevalence of ESBL-producing bacteria was calculated from the total number of the same species tested at each site. These figures only include "true" ESBL-producing bacteria; i.e. excluding AmpC-producing bacteria. Several laboratories sent AmpC-producing isolates to SSI for genotyping, but it was not possible to calculate an AmpC-prevalence.

Results and discussion: The prevalence of ESBL-producing *Escherichia coli* and *K. pneumoniae* isolated from blood and urine, the latter divided into hospital- or community derived urine cultures, is shown in Table 1. The ESBL-prevalence obtained in 2011 was compared to data from similar studies performed in 2007 and 2009 [Table 1]. From October 2009 to October 2011, the prevalence of ESBL-producing *E. coli* increased significantly in urine samples from community and hospitals.

Table 1. Prevalence of Extended Spectrum Beta-Lactamase (ESBL)[a] in *Escherichia coli* and *Klebsiella pneumoniae* isolated from blood and urine cultures, September-October 2007, October 2009 and October 2011

DANMAP 2011

Sample type	Period	No. of tested cultures	No. of *E. coli*	ESBL *E. coli* (%)	No. of *K. pneumoniae*	ESBL *K. pneumoniae* (%)
Blood	2007	18259	625	4.2	160	5.0
	2009	11532	356	7.0	89	14.6
	2011	12891	368	7.3	83	7.2
Urine, hospitals	2007	NA[b]	6791	2.3	1078	6.6
	2009	16536	4004	3.8	675	11.1
	2011	19780	4068	4.7[c]	650	9.7
Urine, general practice	2007	NA[b]	4966	1.5	513	2.7
	2009	12574	3392	2.3	385	6.8
	2011	16984	4037	3.2[c]	450	7.1

a) AmpC producing isolates were not included
b) NA: Not applicable. For the study in 2007, no information was provided on the distribution of the in all 47,504 urine cultures performed in hospitals and general practices
c) A significant increase was detected from 2009 to 2011

As in previous years, CTX-M-15 dominated among ESBL-producing *E. coli* and *K. pneumoniae* isolates from urine and bloodstream infections from human patients, while CTX-M-1 and CTX-M-14 were present in a limited number of the human isolates only [Table 2]. CMY-2 was detected in some of the submitted AmpC producing *E. coli* isolates from both urine and blood, but it was not possible to calculate the prevalence of this enzyme since not all isolates were submitted to SSI. The most common ESBL-type among pig *E. coli* isolates was CTX-M-1 and only a small number of isolates were CTX-M-15 [Textbox 7]. Among the *E. coli* isolates from imported and Danish broiler meat, CMY-2 was most prevalent [Textbox 7]. The distribution of ESBL-types among animals and humans was therefore not consistent. However, CTX-M-15 producing *E. coli* from Danish pigs has been shown to belong to sequence types which have previously been detected in human patients [Hammerum *et al.* 2012. J Antimicrob Chemother. 67: 2049–51]. Further studies are needed to quantify this risk of ESBL-producing *E. coli* of animal origin in relation to human health.

Table 2. ESBL enzymes detected in the ESBL-producing *E. coli* and *K. pneumoniae* isolates from the ESBL-prevalence study, October 2011[a]

DANMAP 2011

ESBL enzyme	*E. coli* from urine (%)	*E. coli* from blood (%)	*K. pneumoniae* from urine (%)	*K. pneumoniae* from blood (%)
CTX-M-1	7.3	8.0	1.3	
CTX-M-14	16.7	4.0	10.0	
CTX-M-15	59.3	68.0	75.0	60.0
CTX-M-27	8.7	12.0	1.3	
Other types	8.0	8.0	11.3	40.0
Number of isolates	275	25	80	5

a) AmpC producing isolates (incl. CMY-2) were not included

Prior antimicrobial consumption is a risk factor for development of an infection with ESBL-producing bacteria. Consumption of ciprofloxacin and 2nd and 3rd generation cephalosporins has increased constantly in Danish hospitals for the last 10 years, further promoting the selection pressure for ESBL-producing bacteria in the hospitals [Textbox 8]. A reduction in antimicrobial consumption can lead to a reduction in resistant *K. pneumoniae* [Textbox 8, DANMAP 2010].

Infection with ESBL-producing bacteria frequently results in prolonged hospital stays with ensuing human and financial costs. Furthermore, empirical treatment on suspicion of septicaemia might be changed to a carbapenem (meropenem) [Textbox 8]. Carbapenems are the last resort antimicrobial agents for treatment of multi-resistant enterobacteria, and we should not expect new and more effective antibiotics to be introduced in the next decade. Furthermore, patients colonised or infected with carbapenem resistant enterobacteria have already been observed in Denmark [Textbox 11]. Consequently, infections may occur in Denmark for which no or limited treatment options exist.

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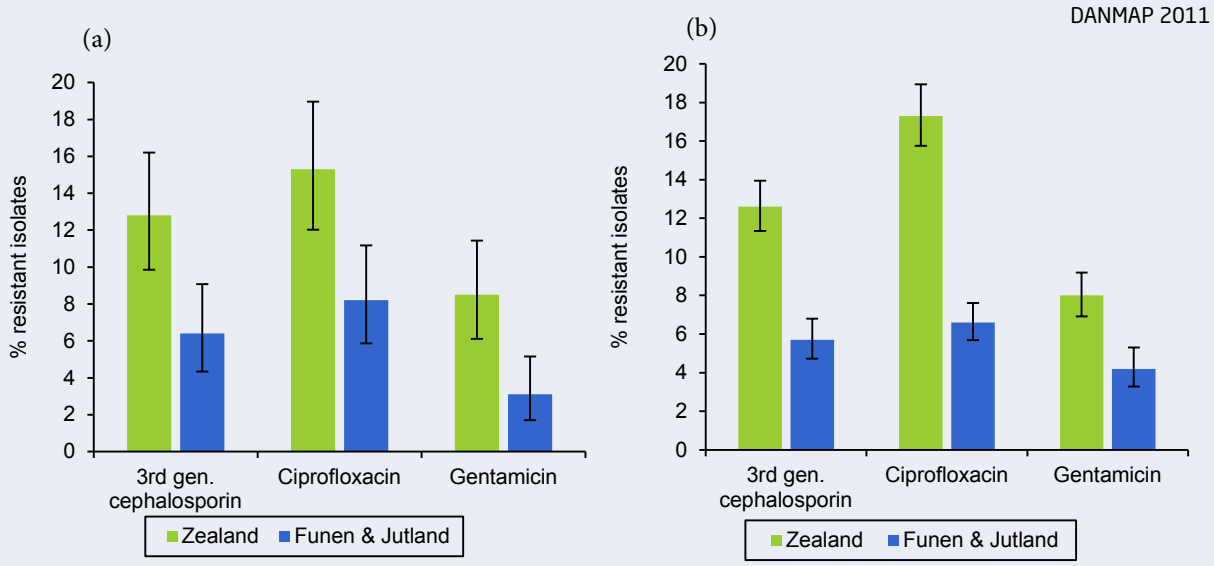
Klebsiella pneumoniae isolates from the Eastern part of Denmark (Zealand) is more resistant to 3rd generation cephalosporin, ciprofloxacin and gentamicin than isolates from the Western part (Funen and Jutland)

Background: Extended-spectrum-lactamase (ESBL)-producing *Klebsiella pneumoniae* has been increasingly detected in Europe, Africa, America and Asia. Before 2007, the occurrence of 3rd generation cephalosporin resistance was low among *K. pneumoniae* isolated from bloodstream infections in Danish patients [EARRS 2007]. In the following years, 3rd generation cephalosporin resistant *K. pneumoniae* has been increasingly seen in Denmark [Textbox 9]. In the present study, the resistance pattern of *K. pneumoniae* isolates from different regions in Denmark was investigated.

Methods: Data on gentamicin, ciprofloxacin and 3rd generation cephalosporin (ceftazidime, ceftriaxone, cefpodoxime and cefotaxime) resistance in *K. pneumoniae* bloodstream and urine isolates from hospitalised patients in 2011 were collected from 12 of 13 Departments of Clinical Microbiology (DCM) in Denmark; 11 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *K. pneumoniae* [8.2 *Klebsiella pneumoniae*].

Results: The level of resistance to 3rd generation cephalosporin, ciprofloxacin and gentamicin was significantly higher in *K. pneumoniae* isolates from bloodstream infections and urinary tract infections from hospitalised patients in the Eastern part of Denmark (Zealand) than in the Western part (Funen and Jutland) (Figure 1a and Figure 1b). The same tendency was observed in *K. pneumoniae* urine isolates from primary health care patients, where resistance to 3rd generation cephalosporins and fluoroquinolones (ciprofloxacin and nalidixic acid) was significantly higher in isolates from the Eastern part of Denmark (Zealand) than the Western part (Funen and Jutland).

Figure 1. The percentage of 3rd generation cephalosporin, ciprofloxacin and gentamicin resistance in *Klebsiella pneumoniae* blood (a) and urine (b) isolates from hospitalised patients at Zealand, and at Funen and in Jutland, respectively, in 2011 (with 95% confidence intervals).



Discussion: The 3rd generation cephalosporin resistant *K. pneumoniae* isolates from October 2011 were investigated for ESBL-encoding genes as a part of the ESBL-prevalence study [Textbox 9]. The majority of the isolates in the ESBL-prevalence study were CTX-M-15 producing, but none of the isolates in the present study was further typed by molecular methods [Textbox 9]. In an earlier study, *K. pneumoniae* bloodstream isolates from 2008 were investigated for the presence of ESBL-genes, multi-locus sequence typed and PFGE typed [Lester *et al.* 2011, Int J Antimicrob Agents. 38: 180-2]. In the study by Lester *et al.* it was shown that the spread of 3rd generation cephalosporin resistant *K. pneumoniae* from bloodstream infections was mostly due to spread of two clones (ST15 and ST16) producing the ESBL-enzyme CTX-M-15 among hospitals in Zealand. Both clones were co-resistant to ciprofloxacin and the majority of the isolates belonging to the ST15 clones were also gentamicin resistant. The ST16 clone was first detected in Zealand in 2006 and seems to persist for very long [Lester *et al.* 2011, Int J Antimicrob Agents. 38: 180-2]. Further studies are needed to investigate if the high occurrence of resistance in *K. pneumoniae* isolates from Zealand in 2011 is due to persistence of the same clones as in 2008. An intervention study at Bispebjerg Hospital in 2010 showed that a reduction in the number of resistant *K. pneumoniae* isolates is possible when the prescription of antimicrobial agents is changed [Textbox 8, DANMAP 2010].

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Textbox 11

Carbapenemase producing enterobacteria

Background: Carbapenems is one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant bacteria such as ESBL-producing *Klebsiella pneumoniae*. Frequently, none or only suboptimal antimicrobial agents are available for the treatment of infections with carbapenemase producing enterobacteria (CPE). Resistance is 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). Recently, major outbreaks were reported from Holland (OXA-48), Italy (KPC-2) and Poland (KPC-2). Additionally, VIM- and KPC-producing bacteria occur endemically in Greece. The occurrence of NDM is particularly high in Pakistan and India, but cases have been reported from nearly all parts of the world. CPE (and other multi-resistant enterobacteria) comprise a particular problem as they can be carried in the intestine without causing symptoms. Antimicrobial treatment may contribute to the sustaining of the carrier state and lead to multiplication and increased excretion. Particularly in hospitals and nursing homes this may cause problems. The ECDC (European Centre for Disease Prevention and Control) and the CDC (Centers for Disease Control and Prevention) in the USA recommend CPE-screening of patients transferred from hospitals abroad and patients suspected of forming part of an outbreak.

In recent years, Danish Departments of Clinical Microbiology (DCM) have, on a voluntary basis, submitted CPE isolates for verification and genotyping at Statens Serum Institut. We recently described 15 Danish CPE cases [EPI-NEWS No. 2012]. Data from this DANMAP report indicate that more cases might be present since several carbapenem resistant *E. coli* and *K. pneumoniae* isolates were detected in 2011. Currently, Denmark does not systematically screen for and monitor CPE. CPE are not reportable in Denmark.

Danish CPE cases (Table 1): The first case of VIM-producing *K. pneumoniae* was detected in Denmark in 2008 [Hasman *et al.* 2010. Int J Antimicrob Agents. 36: 468–9] and in 2010 the second case was seen. Both patients had previously been admitted to hospitals in Greece. In 2009, the first cases of KPC-2-producing *K. pneumoniae* in Denmark were observed [Hammerum *et al.* 2010. Int J Antimicrob Agents. 35: 610–2]. These patients had also been admitted to Greek hospitals. In 2010, the first Danish case of NDM-1 was seen [Hammerum *et al.* 2010. Lancet Infect Dis.10: 829–30]. The patient had been admitted to a hospital in Bosnia-Herzegovina and was one of the first NDM-1 cases in Europe to be linked to Balkan. In 2011, two cases of NDM-1 were found; one was linked to Egypt, the other to Pakistan [Nielsen *et al.* 2012. J Antimicrob Chemother. 67: 2049–51]. Furthermore, seven cases of OXA-48 in patients from Libya were observed in 2011. During the stay in Denmark, one patient developed sepsis and meningitis with the OXA-48-producing *K. pneumoniae* in the blood and spinal fluid. The patient was treated with colistin and fosfomycin with full resolution of symptoms and recovery [Hammerum *et al.* 2012. Int J Antimicrob Agents. 40: 191–2]. With the exception of one case, the known Danish CPE cases were all imported. There is no knowledge of secondary spreading at Danish hospitals or nursing homes.

Table 1. The 15 known Danish cases of carbapenemase-producing enterobacteria DANMAP 2011

Enzyme	Species	No. cases	Origin	Year
VIM	*K. pneumoniae*	1	Greece	2008
VIM	*K. pneumoniae*	1	Greece	2010
KPC-2	*K. pneumoniae*	2	Greece	2009
KPC-2	*K. pneumoniae*	1	Unknown origin	2011
NDM-1	*K. pneumoniae*	1	Bosnia-Herzegovina	2010
NDM-1	*K. pneumoniae*	1	Egypt	2011
NDM-1	*E. coli*	1	Pakistan	2011
OXA-48	*K. pneumoniae*	7	Libya	2011

Conclusion: Of the 15 CPE cases observed in Denmark 2008–2011, 10 were detected in 2011 and most of these were imported from Libya from patients sent to Denmark to receive medical assistance for injuries during the Libyan conflict. The increasing occurrence of CPE in Europe and now also in Denmark is worrying. Not only are these infections extremely difficult to treat with antimicrobial agents, the bacteria also have the potential to establish and spread endemically at hospitals and in the community. This is in line with what was previously seen for ESBL-producing bacteria. Due to the seriousness of this issue, the ECDC encourages systematic monitoring of these bacteria in all European countries. It is essential to be particularly aware of any occurrence of multi-resistant enterobacteria in patients transferred after admission to hospitals abroad.

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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*, this report includes data from 12 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. The antimicrobial susceptibility of approximately 400 *P. aeruginosa* isolates from blood was reported in 2011. Resistance to all the tested antimicrobial agents was at the same level as in 2010 (Figure 8.4). The occurrence of resistance was low compared to most of the other countries reporting to EARS-Net [EARS-Net 2010].

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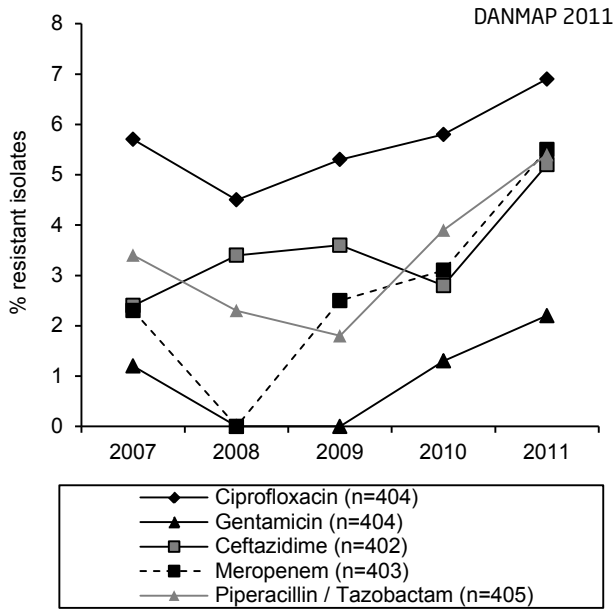
Figure 8.4. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark



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

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, bacteremia/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 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 2011, susceptibility testing was performed on 901 non-duplicate *S. pneumoniae* isolates from invasive infections (Figure 8.5).

Macrolide resistance in *S. pneumoniae* isolates from blood and cerebrospinal fluid was 4.9% (n = 44) in 2011 compared to 4.2% in 2010. As can be seen in Figure 8.5 macrolide resistance has been fluctuating but relatively stable around 6% from 2000 to 2008, then decreased significantly from 6.6% in 2008 to 3.6% in 2009 and now increasing again. The 44 macrolide resistant *S. pneumoniae* from 2011 belonged to 11 different serotypes and the most commonly found serotypes were type 19A (36.4%), 15A (20.5%) and 6C (15.9%).

The percentage of *S. pneumoniae* invasive isolates being non-susceptible (resistant and intermediary resistant) to penicillin was 4.8% (n = 43) in 2011 compared to 3.5% in 2010. The 43 penicillin non-susceptible *S. pneumoniae* from 2011 belonged to 10 different serotypes, and the most commonly found serotypes were type 19A (46.5%), 15A (16.3%) and 6C (9.3%). One of the 901 tested isolates (0.2%) was resistant to penicillin (MIC > 2 µg/ml).

The occurrence of resistance to erythromycin and penicillin was similar to the occurrence in other Scandinavian countries but much lower than reported in many of the other European countries reporting to EARS-Net [EARS-Net 2010].

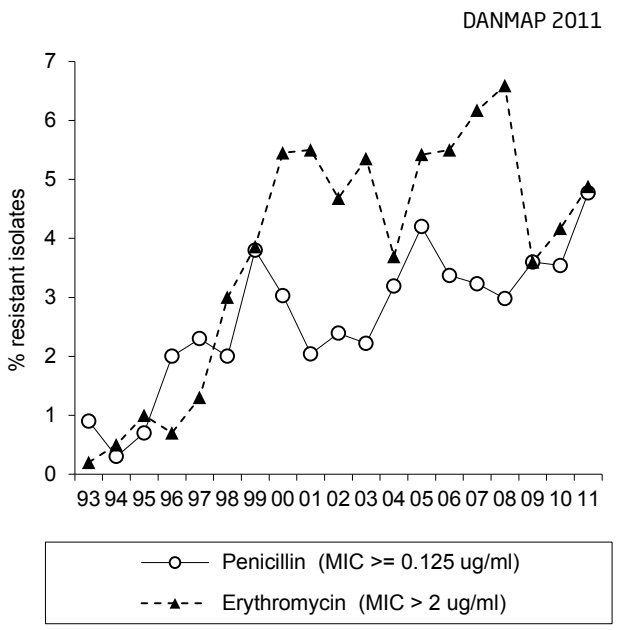
Group A streptococci

In 2011, 164 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 2011. Erythromycin resistance was detected in two isolates (1.2%) as compared to two of 155 (1.3%) in 2010.

Group B, C and G streptococci

As in previous years, no resistance to penicillin was reported in isolates from group B, C or G invasive infections in 2011. In 2011, 124 invasive group B

Figure 8.5. Resistance (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark



streptococci (*Streptococcus agalactiae*) isolates from invasive infections were tested. Erythromycin resistance was detected in 20 isolates (20.2%) compared to 12.7% in 2010.

Forty-nine isolates of invasive group C streptococci were tested in 2011. One isolate (2.0%) was resistant to erythromycin compared to one isolate (1.9%) in 2010.

Eleven (8.0%) of the tested 138 invasive group G streptococci were resistant to erythromycin compared to 12% in 2010.

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8.5 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but can 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

In 2011, 619 *E. faecium* isolates and 513 *E. faecalis* isolates from blood were tested for antimicrobial susceptibility.

As in previous years, most of the *E. faecium* isolates from bloodstream infections were ampicillin resistant. In 2011, 93% 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 past years. 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.

One of the DCM (Aalborg Hospital) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates at DCM Aalborg, 31% were HLGR, whereas 74% 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 2010 [EARS-Net 2010].

In 2011, vancomycin resistance was detected in 1.3% of the *E. faecium* isolates (n = 8), whereas none of the *E. faecalis* isolates from bloodstream infections were vancomycin resistant. Four of the vancomycin resistant *E. faecium* isolates were detected at DCM Aarhus and these isolates were part of an outbreak of 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 countries in Europe [EARS-Net 2010].

Since 2005, presumable vancomycin resistant enterococcal isolates from invasive and non-invasive infections have been sent from the DCM to SSI for national surveillance on vancomycin resistant enterococci. Besides the *vanA E. faecium* isolates from the outbreak at Aarhus University hospital, 17 *vanA E. faecium*, and 2 *vanB E. faecalis* isolates were received during 2011.

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.

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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, Methicillin Resistant *S. aureus* (MRSA) has been both laboratory and clinical notifiable since November 2006. In recent years, MRSA belonging to clonal complex 398 (CC398), has attracted special attention as this type has been closely connected to livestock animals, especially pigs, and increasingly affects people in direct contact with pigs.

Surveillance of bacteraemia

In 2011, 1,525 *S. aureus* bacteraemia cases corresponding to 24.6 per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. Twenty-one (1.4%) of the cases were caused by MRSA. This is at the same level as in previous years and very low compared to most of the other countries participating in EARS-Net [EARS-Net 2010]. Antimicrobial resistance in *S. aureus* bacteraemia isolates from 2006–2011 are presented in Table 8.5. The highest frequency of resistance other than to penicillins was observed for fusidic acid (13.2%), erythromycin (6.5%), clindamycin (5.8%) and norfloxacin (3.6%). Susceptibility to all tested antimicrobials was at the same level as in 2010. Resistance to at least 1, 2 or 3 other antimicrobials in addition to penicillin was demonstrated in 15%, 6% and 1% of the cases, respectively.

Surveillance of Methicillin Resistant *S. aureus*

In 2011, 1,292 new MRSA cases were detected (23.3 per 100,000 inhabitants). This is the highest number of cases observed in over 25 years (Figure 8.6). 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 2011, the number of MRSA increased by 18% and thereby continued the large increase seen from 2009 to 2010 (34%). In 2011, eleven persons were found with their second case of MRSA (i.e. MRSA of a new subtype) and one person with a third case. At the time of diagnosis, 681 (53%) of the new cases were found due to infection, this proportion was lower than in 2010 (646 cases (59%)). The proportion of bloodstream infections with MRSA was 1.4% in 2011 (see surveillance of *S. aureus* bacteraemia). The incidence rate of new MRSA cases per year for each DCM in the last four years is shown in Table 8.6. The incidence varied from 13.5 per 100,000 inhabitants in Århus to 38.1 per 100,000 inhabitants in the greater Copenhagen area (Greater Copenhagen was served by three DCM, and is shown as one).

Table 8.5. Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark DANMAP 2011

Antimicrobial agent	2006 %	2007 %	2008 %	2009 %	2010 %	2011 %
Methicillin	1.4	0.6	1.3	1.6	1.4	1.4
Penicillin	80	78	77	77	75	77
Erythromycin	5	4	5	7	5	7
Clindamycin	4	3	4	6	4	6
Tetracycline	3	2	3	2	3	2
Fusidic acid	10	9	9	9	13	13
Rifampicin	<1	<1	<1	<1	<1	<1
Norfloxacin	2	1	2	2	3	4
Kanamycin	1	<1	1	1	1	<1
Linezolid	0	0	0	0	0	0
Mupirocin	0	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	nt	nt	<1
Number of isolates	1329	1345	1344	1480	1418	1525

nt = not tested

Figure 8.6. Number of MRSA cases, with a three years moving average, Denmark

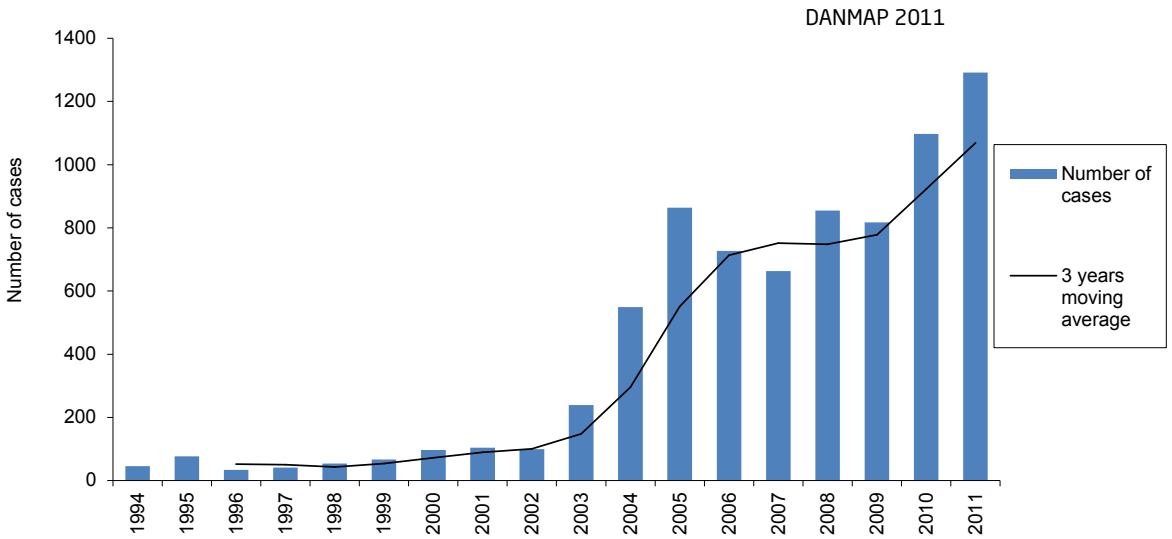


Table 8.6. Incidence rate of new MRSA cases per 100,000 inhabitants per Department of Clinical Microbiology, Denmark

DANMAP 2011

Department of Clinical Microbiology	2008	2009	2010	2011
Greater Copenhagen[a]	23.6	26.9	30.9	38.1
Hillerød	21.8	16.5	27.0	18.7
Statens Serum Institut[b]	17.6	17.6	–	–
Slagelse[c]	19.3	15.1	17.9	28.6
Næstved	12.1	8.8	19.8	18.1
Odense	12.9	8.9	16.3	14.0
Sønderborg	11.1	12.8	25.8	23.8
Esbjerg	8.8	6.2	11.8	18.0
Vejle	20.0	11.6	10.6	16.3
Herning	7.7	7.3	15.1	13.7
Århus	6.0	10.1	11.6	13.5
Viborg	18.5	9.1	11.0	17.3
Aalborg	10.7	7.6	16.6	21.0
Denmark total	15.5	14.7	19.8	23.3

a) Rigshospitalet (national referral hospital), Hvidovre and Herlev
b) Statens Serum Institut is no longer serving former Roskilde County
c) Including isolates from former Roskilde County

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.7. Most of the cases (80%) were acquired in Denmark. The epidemiological classification of MRSA infections 2006–2011 is shown in Figure 8.7. Only 10 of the HACO infections could be associated with a known exposition, 4 from hospitals, 4 from nursing homes and the remaining 2 from other social institutions. The remaining 122 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, 69 cases were with an association to hospitals and 37 cases with an association to nursing homes and private home care. The remaining 16 cases were associated to other social institutions. The number of infections classified as community-acquired (CA) was 365 in 2011 (Figure 8.7). The proportion of CA-infections with known exposure was at the same level (30%) as in 2010.

Molecular typing of the MRSA strains

In total, *spa* typing revealed 193 different strain types. The number of isolates belonging to the 10 dominating *spa* types isolated in 2011 is shown in Table 8.8. They constituted 57% of the total number of MRSA isolates. Seven *spa* types constituted 49% of the 681 clinical infections with MRSA (out of 154 different *spa* types associated with clinical infection). Most prevalent *spa* types causing clinical infections at time of presentation were t008, t019, t002, t034, t024, t044 and t127. Of the 438 strains isolated from asymptomatic carriers, t034 was the most prevalent *spa* type (n = 62), followed by t002 (n = 48), t008 (n = 36), t304 (27) and t019 (n = 25). The PVL gene was demonstrated in 42% of the infections and in 25% of the asymptomatic carriers.

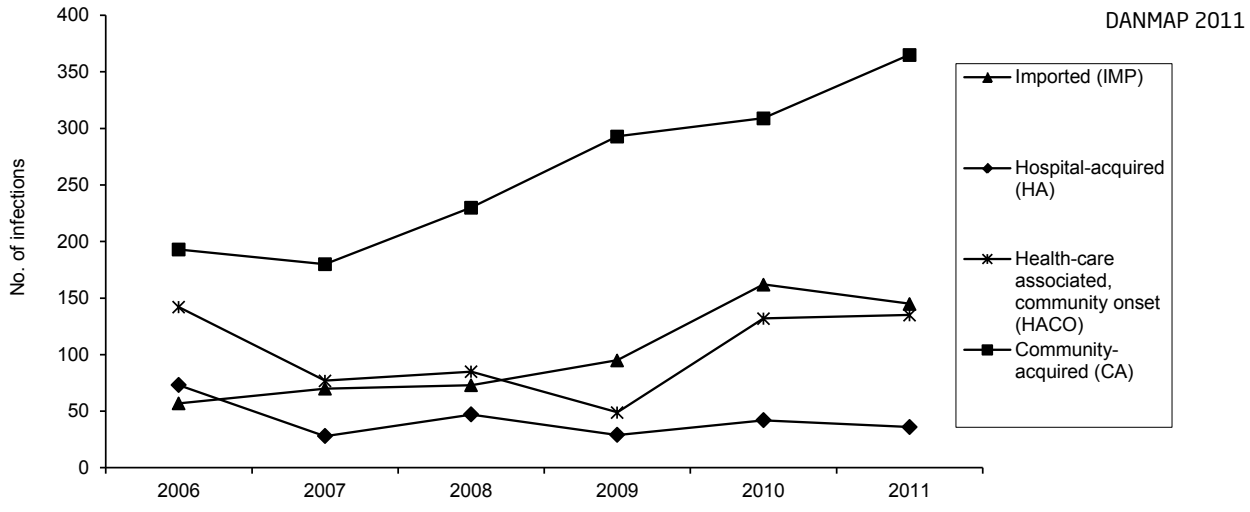
Table 8.7. Epidemiological classification of new MRSA cases, Denmark

DANMAP 2011

Epidemiologic classification	Exposure	2010		2011	
		No. of cases[a]	No. (%) of cases with infections	No. of cases	No. (%) of cases with infections
Imported (IMP)		**247**	163 (66)	**253**	145 (57)
Hospital-acquired (HA)		**62**	34 (55)	**61**	32 (53)
Health-care associated, community onset (HACO)	with health care risk	**169**		**187**	
	with known exposure	40	16 (40)	44	10 (23)
	without known exposure	129	116 (90)	143	122 (85)
Health care worker		**35**	8 (23)	**40**	7 (18)
Community-acquired (CA)	without health care risk	**578**		**751**	
	with known exposure	331	100 (30)	454	111 (24)
	without known exposure	247	210 (85)	297	254 (86)
Unclassified		**0**	0 (0)	**0**	0 (0)

Note: Numbers shown in bold are totals
a) Epidemiological classification missing for 6 cases

Figure 8.7. Number of MRSA infections according to epidemiological classification, Denmark



Live-stock associated MRSA

There was no targeted screening for CC398 in 2011. The total number of CC398 isolates increased to 164 (42 in 2009, 111 in 2010). The most frequent *spa* type related to CC398 was type t034 (n = 130). Forty-nine t034 cases presented with infections (Table 8.8). MRSA isolates carrying the new *mecA* homologue *mecAlga251*, now proposed called *mecC*, were demonstrated in 37 cases (9 in 2009, 21 in 2010). The preliminary data from 2012, however, indicate that the number is stabilizing/declining. The livestock association for *mecC* MRSA may not be as strong as for CC398 MRSA but two of the cases were considered associated with livestock contact. In addition, screening of animals in close contact with *mecC* positive patients in Region Zealand confirmed a possible bovine and ovine association in two cases.

Eleven (0.7%) of the bacteraemia cases belonged to CC398. None of these were MRSA and association to pig farming is not known. The corresponding numbers were eleven in 2010, ten in 2009, six in 2008 and five in 2007.

Resistance among MRSA isolates

The occurrence of resistance to erythromycin and clindamycin decreased when comparing all MRSA isolates in 2011 with all MRSA isolates in 2010 (Table 8.9). The resistance pattern varied considerably between *spa* types (Table 8.9). In 2011, 100% of CC398 *spa* type t034 isolates were resistant to tetracycline and 93% of CC8 *spa* type t008 were resistant to erythromycin and clindamycin. In contrast, the majority of t019, a primarily community-acquired *spa* type, continued to be susceptible to all tested antimicrobial agents except for beta-lactams. 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 69%, 53% and 34% of the cases, respectively. The most common resistance patterns and any frequent *spa* types are shown in Table 8.10.

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Table 8.8. The ten most prevalent *spa* types demonstrated in MRSA cases, Denmark 2011

DANMAP 2011

spa type	CC group[a]	No. of cases	No. causing infections (%)
t034	CC398	130	49 (38)
t008	CC8	122	74 (61)
t002	CC5	119	56 (47)
t019	CC30	97	59 (61)
t024	CC8	68	36 (53)
t044	CC80	58	34 (59)
t127	CC1	46	27 (59)
t304	CC8	37	8 (22)
t032	CC22	32	15 (47)
t223	CC22	29	14 (48)

a) CC = Clonal complex

Table 8.9. Resistance (%) in the six most prevalent *spa* types demonstrated in MRSA cases compared with all MRSA cases, Denmark 2011

DANMAP 2011

spa type	t034	t008	t002	t019	t024	t044	All cases
Clonal complex	CC398	CC8	CC5	CC30	CC8	CC80	
	%	%	%	%	%	%	%
Erythromycin	35	60	41	0	93	3	37
Clindamycin	82	16	32	0	93	3	34
Tetracycline	100	4	16	1	3	60	28
Fusidic acid	0.8	7	25	0	32	90	13
Rifampicin	0	0	2	1	1	0	1
Norfloxacin	34	59	34	2	43	5	28
Kanamycin	3	62	24	0	1	93	28
Linezolid	0.8	0	0	0	0	0	<<1
Mupirocin	0	2	0.8	0	0	0	0.4
Trimethoprim-sulfamethoxazole	0	0	0	0	0	0	1
Number of isolates	130	122	119	97	68	58	1292

Table 8.10. Resistance markers in addition to cefoxitin demonstrated in MRSA cases, Denmark 2011

DANMAP 2011

No. of markers	No. of cases	Frequent patterns (no of isolates)	Any frequent *spa* type (no of isolates)
0	403	–	–
1	208	N(57)	t032(17)
		T(55)	t034(18)
		F(45)	t002(13), t008(8)
		K(43)	t355(11)
2	239	E,C(83)	t024(31)
		C,T(35)	t034(32)
		N,K(27)	t008(9)
3	286	E,N,K(65)	t008(36), t657(19)
		E,C,T(64)	t034(30)
		E,C,N(47)	t002(13), t032(13)
		T,F,K(33)	t044(29)
4	128	E,C,T,K(35)	t127(19), t437(7)
		E,C,N,K(29)	t008(12)
		E,C,F,N(21)	t024(17)
5	22	E,C,T,N,K(12)	t008(3), t045 (3)
6	4	E,C,T,R,N,S(2)	t4866(2)
7	2	E,C,T,F,R,N,K,S(2)	t037(2)

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

Adaptive evolution of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398

Background: Recent reports of methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 carriage in livestock, particularly pigs, and carriage/infections among people with close and frequent livestock contact has provided evidence of the existence of a true MRSA reservoir in livestock. This livestock-associated MRSA (LA-MRSA) CC398 clone is efficiently transmitted to people with close and frequent livestock contact but is a poor long-term colonizer of humans and is significantly less transmissible to and between people with no exposure to livestock. In Denmark, ~90% of LA-MRSA CC398 carriage/infections in humans can be linked to livestock production, whereas ~10% of the cases have no direct or indirect contact to livestock.

Methicillin-susceptible *S. aureus* (MSSA) CC398 has also been identified in people with no prior exposure to livestock, including communities in New York, Caribbean islands, and the Amazonian forest of French Guiana.

These observations suggest that distinct *S. aureus* CC398 strains circulate in livestock and humans and that adaptation to different hosts has occurred.

To test this hypothesis, we investigated the evolutionary relationships among 90 *S. aureus* CC398 isolates recovered from livestock and humans around the world.

Materials and methods: A detailed phylogenetic (family) tree of the 90 isolates was constructed based on whole-genome sequence data, and potential genetic markers of adaptation were mapped onto the tree.

Results and discussion: The 90 *S. aureus* CC398 isolates clustered into two major groups: one consisting of mainly MSSA isolates from humans and the other comprising MRSA and MSSA isolates from both humans and livestock. The phylogeny supports that livestock-associated *S. aureus* CC398 strains are the descendants of a human-to-animal host jump by an MSSA CC398 strain. In the livestock reservoir, MSSA CC398 underwent rapid diversification accompanied by loss of several genes that encode modulators of human innate immunity (e.g., *scn*) and acquisition of the tetracycline resistance gene *tet*(M). Importantly, the phylogeny suggests that the methicillin-resistant phenotype was acquired after adaptation to livestock through local acquisition of the *mecA* gene by geographically distinct MSSA CC398 strains. The different stages in the adaptive evolution of *S. aureus* CC398 are illustrated in Figure 1.

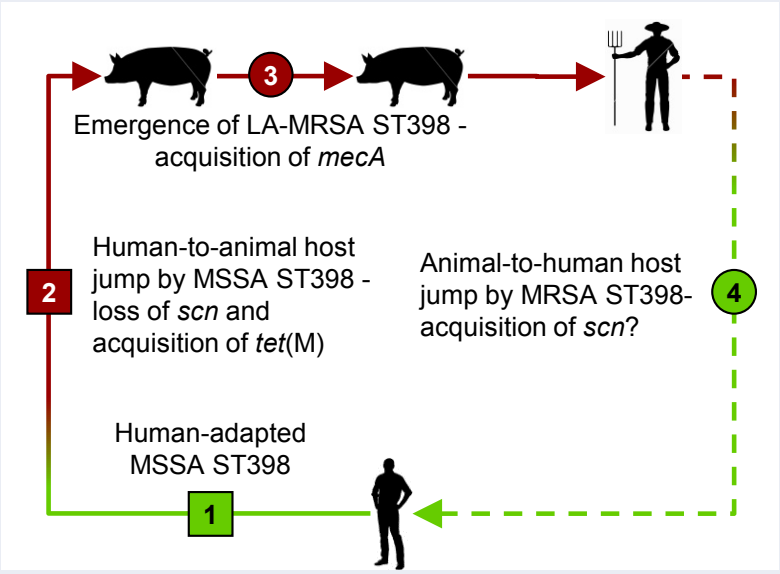

Figure 1. Stages in the adaptive evolution of *S. aureus* CC398. The original MSSA CC398 strains were, and still are, circulating in the human population (stage 1). The human-to-animal host jump by an MSSA strain was accompanied by loss of *scn* and acquisition of *tet*(M) (stage 2). In the livestock reservoir, geographically distinct MRSA CC398 strains have emerged through acquisition of the methicillin resistance gene *mecA* (stage 3). LA-MRSA CC398 strains are able to reacquire the *scn* gene, which may be a first step in the adaption back to humans (stage 4).

The loss of several functions associated with human adaptation may explain why LA-MRSA CC398 has lost part of its colonization and transmission ability in humans. On the other hand, our findings show that LA-MRSA CC398 is able to reacquire the same genes (e.g., *scn*), probably as a result of lateral gene transfer from a bacterial member of the human microbiome. Additional studies accessing the risk for spread of *scn*-positive LA-MRSA CC398 strains from the livestock reservoir into the community are currently under way.

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Methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pig herds, in pigs and cattle at slaughter, and in Danish and imported retail meat

Background: Methicillin resistant *Staphylococcus aureus* (MRSA), especially belonging to the clonal complex CC398, has since 2003 emerged in livestock worldwide. In 2009, the prevalence in Danish pigs at slaughter was 13% [DANMAP 2009]. As MRSA can be transmitted between animals during transportation and prior to slaughter, the prevalence found in the slaughter house may not be equivalent to the prevalence of MRSA positive farms [Broens *et al.*, Vet. J. 2011. 189: 302–5]. Therefore, the prevalence in the pig herds was investigated in 2010 and a prevalence of 16% was found. A *mecA* homologue LGA_{251} has been found in cattle and sheep in Denmark [García-Álvarez *et al.*, 2011. Lancet Infect Dis.11: 595–603] but the prevalence among these species are unknown.

In 2011, the prevalence of MRSA was investigated in pig herds, pigs and cattle at slaughter and in retail meat. The aim was to investigate the prevalence of MRSA at the pig farm level and compare to previous years. The prevalence in cattle at slaughter was also investigated. Meat samples were collected to follow changes in prevalence when compared to data from previous years.

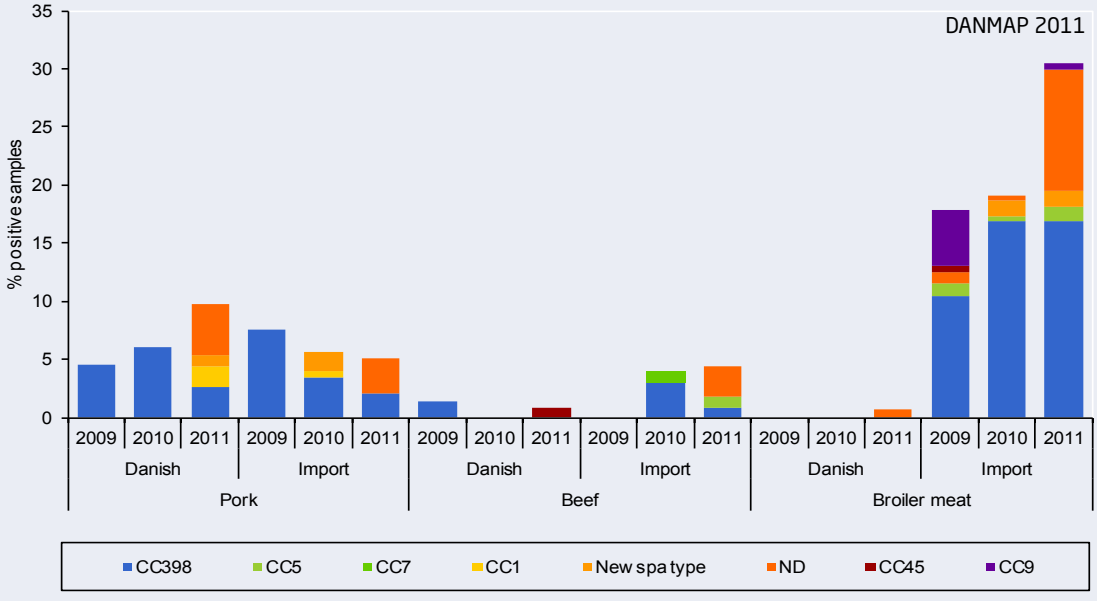
Materials and methods: During February through October 2011, pools of five nasal swab samples (n = 79) were taken from five slaughter pigs in five different pens in 79 farms in Denmark and nasal swabs were collected from 777 pigs at slaughter, as previously described [DANMAP 2009 and DANMAP 2010]. Cattle, mainly young bulls (n = 179), were tested at slaughter by taking skin swabs between leg and udder/testis, representing at least 160 different farms as previously described [DANMAP 2010]. From January through November 2011, samples of broiler meat (133 Danish, 147 imported), beef (122 Danish, 114 imported) and pork (226 Danish, 103 imported) meat samples were collected randomly in retail stores and outlets in all regions of Denmark.

The samples were randomly selected; for pig farms, each farm was sampled only once. For cattle and pigs sampled at slaughter, one animal represented one farm and no herds were sampled more than once in the same month.

MRSA was isolated from each pool of nasal swabs, single swabs, skin swabs or from 25 g of meat after pre-enrichment in Mueller-Hinton medium with 6.5% NaCl followed by selective enrichment in tryptone soya broth supplemented with 4 mg/L cefoxitin and 75 mg/L aztreonam. Ten µl were transferred to brilliance MRSA agar and colonies with *S. aureus* morphology were verified by PCR and selected isolates were *spa* typed.

Results and discussion: Thirteen (16%) of the pig farms were positive for MRSA, this was at the same level as in 2010. Six isolates were *spa* typed and all except one isolate had *spa* types corresponding to CC398. Surprisingly, the prevalence of MRSA in pigs at slaughter (44%) was significant higher than in 2009 (13%) and also significant higher than in the pig herds. One hundred and sixty-eight isolates from pigs at slaughter were *spa* typed and most isolates belonged to CC398 (94%) as found previously.

Figure 1. Occurrence (%) of MRSA in meat, Denmark



Note: ND (Not determined) indicate that the isolates were not *spa* typed
a) Each year, approximately 1,000 samples are collected evenly distributed between the six categories of meat

No MRSA was found among the 179 cattle at slaughter including the *mecA* homologue LGA_{251}. The absence of MRSA in cattle may be due to the sampling method or that the cattle at slaughter mainly being young bulls as described in DANMAP 2010. The prevalence was highest in imported broiler meat (31%) and had increased significantly when compared to previous years, followed by Danish pork (10%), imported pork (5%), imported beef (4%) and Danish beef (0.8%). For the first time MRSA was detected in Danish broiler meat, the isolate could originate from broilers, but an investigation of MRSA in broilers in 2010 did not detect any [DANMAP 2010]. Forty-six of the isolates from meat were *spa* typed (Figure 1). Even though *spa* types corresponding to CC398 were most prevalent, other *spa* types corresponding to other clonal complexes were observed such as CC127 in Danish pork a clonal complex often found in pork in Italy but also found in Danish pigs at slaughter in 2009 [Battisti *et al*. 2010. Vet Microbiol. 142: 361–6].

MRSA CC398 was found in 164 human cases in 2011, the majority in persons with close contact to pigs or being a household member. In 24 cases no such direct contact could be established even when specifically asked. The majority of the 24 cases were in persons living in rural areas with known occurrence of MRSA CC398 in pigs. There are still no sign of spread of CC398 to urban areas.

Conclusions: The prevalence of MRSA in Danish pig farms (16%) was at the same level as in 2010, but the level in pigs at slaughter had dramatically increased which could reflect a higher in herd prevalence increasing the transmission between pigs during transportation and before slaughter. This was further supported by an increasing occurrence in Danish pork suggesting the pork production chain being more contaminated compared to previous years. In meat, MRSA in imported broiler meat was found in a significantly higher prevalence compared to 2010, but so far meat is not considered a source for human infection. The relatively frequent occurrence of MRSA in meat combined with very few cases in urban areas makes it safe to conclude that there is very little if any risk for meat being a risk for contracting MRSA CC398.

Pigs still seem to be the most important reservoir for MRSA CC398 and the in herd prevalence may have increased.

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RESISTANCE IN DIAGNOSTIC SUBMISSIONS FROM ANIMALS 9

Resistance in diagnostic submissions from animals

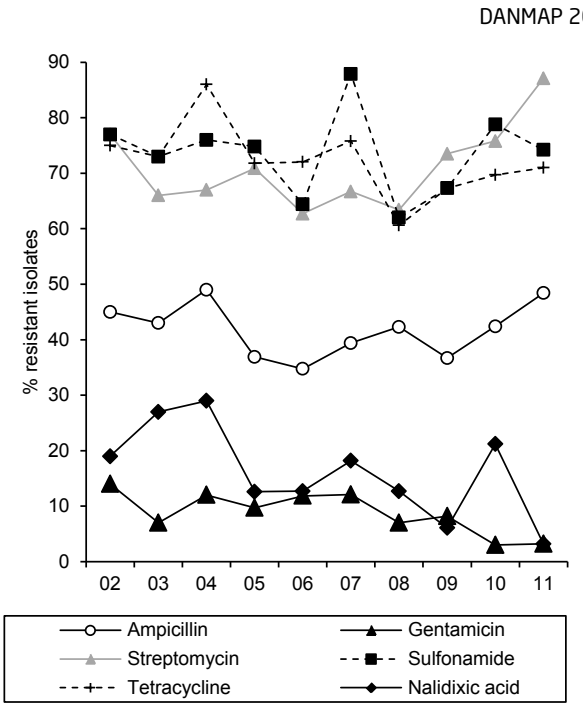
The DANMAP programme monitors antimicrobial susceptibility in *Escherichia coli* O149 and *Staphylococcus hyicus* from diagnostic submissions from pigs, as well as in *E. coli* F5 (K99) from diagnostic submissions from cattle. *E. coli* was isolated from faecal samples, typically from pigs or calves with diarrhoea, and *Staphylococcus hyicus* was isolated from clinical submissions from skin infections in pigs.

Most isolates from diagnostic submissions originated from animals in antimicrobial therapy or with a history of recent antimicrobial therapy. A higher frequency of resistance is therefore expected in bacteria from these diagnostic submissions compared to bacteria originating from healthy animals sampled at slaughter.

In 2011, 31 *E. coli* O149 isolates from pigs were collected. Since relatively few *E. coli* F5 (K99) isolates from cattle and *Staphylococcus hyicus* isolates from pigs are available each year, the overall results of the susceptibility testing are presented for two and three year periods, respectively. During 2010–2011, 25 *E. coli* F5 (K99) isolates from cattle were collected, and 30 *Staphylococcus hyicus* isolates from pigs were collected during the period 2009–2011.

E. coli isolates resistant to three or more of the ten different antimicrobial classes included in the test panel were considered multi-resistant (see definition of multi-resistance and the included antimicrobial classes in section 10.5).

Figure 9.1. Resistance (%) in *Escherichia coli* O149 from diagnostic submissions from pigs, Denmark



The distribution of MICs among *E. coli* O149 and *Staphylococcus hyicus* from pigs and *E. coli* F5 (K99) from cattle are presented in the web annex (Table A9.1, A9.2 and A9.3).

9.1 *Escherichia coli*

Pigs

Trends in resistance to the selected antimicrobial agents in *E. coli* O149 isolates from pigs are presented in Figure 9.1. The isolates were mainly from weaners with diarrhea (>7.5 to 30 kg).

In 2011, only one isolate (3%) was fully susceptible, where as 96% of the isolates were multi-resistant. As in previous years, high levels of resistance were found to tetracycline (71%), sulfonamide (74%) and streptomycin (87%) (Figure 9.1). Sulfonamide and streptomycin are not used for weaning pig diarrhoea, and the consumption in weaners has been stable at a low level (Figure 4.4). However, the resistance to these agents may be co-selected with tetracycline resistance, and tetracyclines are the most common antimicrobial agents used for weaning pigs (Figure 4.4).

The consumption of quinolones has been at a very low level in the pig production since 2003 (web annex, Table A4.2), and from 2010 to 2011 resistance towards nalidixic acid decreased significantly from 21% to 3% (Figure 9.1). In contrast, a significant increase in the proportion of isolates resistant to trimethoprim (51% to 77%) was observed from 2010 to 2011 (data not presented).

In 2011, none of the 31 *E. coli* O149 isolates were resistant to cefotaxime and ceftiofur.

Cattle

In cattle, *E. coli* F5 (K99) was almost entirely isolated from diagnostic submissions from calves with diarrhea.

In 2010–2011, only one isolate (<1%) was fully susceptible to all antimicrobial agents included in the test panel, where as 72% of the isolates were multi-resistant.

There were no significant changes in the level of resistance to any of the antimicrobial agents included in the panel among isolates from 2010–2011 compared to the isolates from 2009. As in previous years, high levels of resistance were found to tetracycline (80%) and ampicillin (96%) but also resistance to sulfonamide (52%) and streptomycin (60%) were common (Table 9.2). The most important antimicrobial agents used for systemic treatment in calves were tetracyclines and macrolides (Figure 4.7).

The usage of quinolones to calves has remained at a very low level since 2003 (web annex, Table AP4.3). The level of quinolone resistance decreased from 2009 (31%), to 2010–2011, where 20% of the diagnostic *E. coli* isolates from cattle were resistant to nalidixic acid and ciprofloxacin. A similar decrease in quinolone resistance was observed in the diagnostic *E. coli* O149 isolates from pigs.

During 2010–2011, none of the *E. coli* F5 (K99) isolates were resistant to ceftiofur, whereas two isolates (4%) were resistant to cefotaxime (Table 9.2).

Table 9.2. Resistance (%) among *Escherichia coli* F5 (K99) from diagnostic submissions from cattle, Denmark

DANMAP 2011

Antimicrobial agent	2009 %	2010–2011 %
Tetracycline	83	80
Chloramphenicol	17	24
Florfenicol	2	0
Ampicillin	92	96
Ceftiofur	0	0
Cefotaxime	2	4
Trimethoprim	33	20
Sulfonamide	52	52
Streptomycin	52	60
Gentamicin	0	4
Neomycin	12	20
Apramycin	0	4
Ciprofloxacin	31	20
Nalidixic acid	31	20
Colistin	0	0
Spectinomycin	17	28
Number of isolates	48	25

Table 9.1. Resistance (%) among *Staphylococcus hyicus* from diagnostic submissions from pigs, Denmark

DANMAP 2011

Antimicrobial agent	2008 %	2009–2011 %
Tetracycline	29	43
Chloramphenicol	3	0
Florfenicol	3	0
Penicillin	68	60
Cefoxitin	0	0
Trimethoprim	48	50
Sulfonamide	0	0
Erythromycin	35	33
Streptomycin	48	40
Gentamicin	0	0
Ciprofloxacin	0	0
Spectinomycin	29	37
Tiamulin	42	47
Number of isolates	31	30

9.2 *Staphylococcus hyicus* from pigs

During the period 2009–2011, *Staphylococcus hyicus* was isolated from 30 clinical submissions from skin infections in pigs. The level of resistance in the pooled data from 2009 to 2011 is comparable to the occurrence observed in the isolates collected in 2008 (Table 9.1). Overall, for the period 2009–2011, only 10% of the isolates were fully susceptible to all antimicrobial agents included in the test panel and among the resistant isolates most (18/27) were resistant to three or more of the antimicrobial agents included in the panel. Resistance to penicillin (60%), trimethoprim (50%) and tiamulin (47%) was most common, but also resistance to tetracycline (43%), erythromycin (33%), streptomycin (40%) and spectinomycin (37%) frequently occurred (Table 9.1).

Since 2008, none of the 61 *Staphylococcus hyicus* isolates were resistant to cefoxitin, sulfonamide, gentamicin or ciprofloxacin.

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3%
11%
9%
10%
7
10%
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10%
140
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10. Materials and methods

10.1 General information

For the DANMAP 2011 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 humans, the epidemiological unit was defined as the individual patient and the first isolate per patient per year was included. For food, the epidemiological unit was defined as the individual meat sample.

10.2 Data on antimicrobial consumption

Antimicrobial agents used for humans and animals in Denmark are presented in Table 3.

10.2.1 Antimicrobial consumption in animals

Since 2001, consumption data presented in this report were obtained from the national monitoring program VetStat, a database hosted by the Danish Veterinary and Food Administration. Prior to 2001, data were based on national sales figures from the pharmaceutical industry (web annex, Table 4.1).

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 some instances medicines prescribed on special license (ie. medicines not approved for marketing in Denmark). In addition, data on consumption of coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (no use since 2000) are collected by VetStat. Data on coccidiostatic agents are presented in the web annex (Table A4.5).

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) can now, on certain conditions (identical to the pharmacies), sell prescribed veterinary medical products for animals. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration cost 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. Hence, in 2010, only 10% of the antimicrobial agents used for animals were used or distributed by veterinarians.

In 2011, the animal owners and veterinarians purchased the antimicrobial agents almost equally from the pharmacies (43%) and the veterinary drug trading companies (55%), while only 2% was purchased from the feed mills.
In 2011, sales from feed mills comprised antimicrobial agents for aquaculture and zinc chloride for the pig production, both 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. For the DANMAP report, the amounts of antimicrobial agents reported by the veterinary practitioners are validated against pharmacy data on the total sales of therapeutic drugs for use in practice. The electronic registration of the sales at the pharmacies is linked to the billing process, 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 (ADD). The ADD system is a national veterinary equivalent to the international Defined Daily Doses (DDD) system applied in the human field [www.whocc.no]. See further description of the ADD system in the DANMAP 2009 report.

Denominators
The consumption is reported in relation to the size of the population sizes of the different animal species or the volume of produced meat. Three different denominators used in the DANMAP report: 1) production in kg meat, 2) number of animals produced or 3) standing live biomass.

Due to a relative high number of pigs exported around 30 kg (26% of pigs produced in 2011), 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) =
[ADDs * (Nf/Nw) + ADDw * (Nf/Nw) + ADDf] / Nf,

where ADDs = amount of antimicrobial agents used in sows (ADDkg), ADDw = amount of antimicrobial agents used in weaners (ADDkg), ADDf = amount of antimicrobial agents used in finishers (ADDkg), Nw = number of pigs produced to 30 kg bodyweight, including pigs exported at 15-50 kg (mostly at 30 kg), Nf = number of pigs produced to slaughter, whether exported domestically or exported.

The estimation of standing live biomass depends on the available data sources for each species:

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, 2012] after an estimated average life span of 30 days. The estimated mean live biomass per broiler is assumed to be half of the weight at slaughter. In addition, there are an estimated 1 million parental animals in Denmark, with average live weight at slaughter of 2.3 kg [Statistics Denmark, 2012]. Rearing comprise an average of 1.06 million hens and 0.2 million cocks, with an average weight of 1.2 kg bodyweight. In the egg production chain, parent flocks comprise 1.06 million hens and 0.25 million cocks, with an average weight of 3.5 kg body weight. Overall in 2011, the estimated standing live biomass within the Danish broiler and layer production were 24.5 mill kg and 4.5 mill kg, respectively.

Turkey production. The live biomass is estimated based on number of turkeys produced (Table 3.1) and an average live weight at slaughter of 19 kg for male turkeys and 11 kg for hens [Statistics Denmark, 2012] after an estimated average life span of 20 weeks and 17 weeks, respectively [Danish Agro]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter, and the overall estimated standing live biomass within the Danish turkey production in 2011 were 2.8 mill kg.

Pig production. The live biomass is of the cattle population is estimated from census data [Statistics Denmark, 2012] and the average live weight of the different age groups. The average live weight was estimated for: sows and gilt in first gestation (210 kg); boars and sows for slaughter (228 kg); piglets before weaning (4 kg); weaned pigs up to 50 kg (28 kg) and finishers and gilts from 50 kg to gestation (80 kg). Overall in 2011, the estimated standing live biomass within the Danish pig production was 677 mill kg.

Cattle production. The live biomass of the cattle population is estimated from census data [Statistics Denmark, 2012] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Fresian, but also other breeds such as Jersey, and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calvs (young beef) of dairy origin. The average live weight was estimated for: cows and heifers, >2 years (600 kg); heifers, bull calves and steers, 1-2 years (400 kg); bulls and steer calves, ½-1 years (300 kg); heifer calves, ½-1 years (230 kg); bull and steer calves, 0-½ years (135 kg) and heifer calves, 0-½ years of age (100 kg). These average body weights are estimated as the average weight in the respective age class of the large dairy breeds, and might represent a slight overestimation. Overall in 2011, the estimated standing live biomass within the Danish cattle production was 685 mill kg.

Fur Animals. The live biomass of mink is estimated from production data [Statistics Denmark, 2010] and the average weight at pelting was 2.45 kg [Danish Fur]. The progeny live for approximately 7 months. Taking into account the longer lifespan of the parental animals, an average lifespan of 8 months is used for the estimation. Overall in 2011, the estimated standing live biomass within the Danish mink production was 24.5 mill 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 relativ stable during the last ten yesrs [Danish Dog register, 2012] including approximately 550.000 dogs . The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data). Overall in 2011, the estimated standing live biomass of Danish cats and dogs was 13.6 mill kg.

Aquaculture. Generally, the average live biomass of fresh water fish in aquaculture is around 40% of the total production, corresponding to approximately 14 ton in 2011 [NH Henriksen, Danish Aquaculture]. The live weight of saltwater fish, increase from zero in January up to around 10 ton in October-November. Assuming an average weight increase of 1 ton per month, the average annual standing live biomass of saltwater fish was 4.6 tonnes (across 12 months).

Figure 4.2, presents the live biomass (mill kg) and therapeutic antimicrobial consumption in the main animal species in Denmark in 2011.

10.2.2 Antimicrobial consumption in humans

Data on consumption of antibacterial agents in humans were obtained from Statens Serum Institut (formerly the Danish Medicines Agency). The DMA 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 the DMA. 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. This year, data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres 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 (DDD), 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 the DMA in an electronic format.
Information on the indication for the prescription is not yet available, but due to legislative changes, more informative indication data will be available from 2012 onwards.

The present report includes data on the consumption of antibacterial agents for systemic use, or group J01, of the 2011 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 and per day (DDD/1,000 inhabitant-days). 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 to primary health care and DBD; the number of DDDs per 100 occupied bed-days and per day (DDD/100 occupied bed-days). 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

In the DANMAP program, samples are collected from randomly selected healthy production animals at slaughter. The following bacteria were isolated from pigs: *Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Campylobacter coli, Campylobacter jejuni* and *Salmonella* spp.; from cattle: *E. coli*, *C. coli* and *C. jejuni*, and from broilers: *E. coli*, *C. coli*, *C. jejuni*, *E. faecalis* and *E. faecium*. In addition, *Salmonella* isolates were collected from subclinical infections as well as from cases of clinical salmonellosis, and 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 at slaughter for the DANMAP program, by meat inspection staff or company personnel, and sent for examination at the National Food Institute. For broilers, cloacal swab samples were collected weekly from April through September, representing 94% of all broiler farms in Denmark. A typical Danish broiler farm often houses several broiler flocks (e.g. 2–12 flocks), but even though more than one flock was sampled, only one isolate per farm of each bacterial species was finally included in the report.

For pigs and cattle, the slaughter plants included in the DANMAP programme accounted for 98% and 94%, respectively, of the total number of animals slaughtered in Denmark during 2011. The number of pig and cattle samples taken at each slaughter plant was proportional to the number of animals slaughtered at the plant per year. Samples were collected once a month from January through November as ceacum samples from pigs and rectum samples from cattle. Only one isolate per farm of each bacterial species was included in the DANMAP report. Accordingly, the bacterial isolates from the 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, the number of isolates obtained and the number of MIC-determinations for pigs, cattle and broilers is presented in Table 10.1. For *Campylobacter,* the isolation rates of *C. jejuni* from pigs and of *C. coli* from cattle and broilers were low and MIC-determinations therefore not performed.

Isolates from diagnostic submissions were collected specifically for the DANMAP programme at the Laboratory of Swine Diseases, the Danish Agriculture & Food Council, Kjellerup (*E. coli* O149 from diarrhoeic pigs

Table 10.1. Number of DANMAP samples, number of isolates and MIC-tests from healthy production animals at slaughter, Denmark

DANMAP 2011

		E. coli	*E. faecium*	*E. faecalis*	*C. jejuni*	*C. coli*
Pigs[a]	No. of samples analysed (1 per farm)	311	757	757	311	311
	No. of isolates obtained	269	130	161	6	162
	No. of isolates MIC-tested/reported	157	116	117	0	102
Cattle	No. of samples analysed (1 per farm)	131	0	0	187	187
	No. of isolates obtained	121	0	0	113	10
	No. of isolates MIC-tested/reported	93	0	0	95	0
Broilers	No. of samples analysed (no. of flocks)	160	201	201	231	231
	No. of farms represented	150	150	150	150	150
	No. of isolates MIC-tested/reported	134	107	110	43	0

Note: Data in this table should not be used for reportation of prevalences of the bacterial species
a) From 2011, the DANMAP samples from pigs were also part of the surveillance programme for *Salmonella*

and *S. hyicus* from skin infections) and at the National Veterinary Institute (*E. coli* F5 (K99) from diarrhoeic cattle). Due to a low number of isolates of *E. coli* F5 (K99) and *S. hyicus*, MIC-data for isolates collected over the last two and three years, respectively, were presented in this report with only one isolate per farm included.

***Salmonella*.** The National Food Institute is the national reference laboratory for *Salmonella* in animals, feeding stuffs and food and therefore receives all isolates for typing. Among all serotyped isolates, 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 the antigenic formulas *S.* 4,5,12:i:- and *S.* 4,12:i:-.

The *Salmonella* isolates from pigs (202 isolates in 2011) primarily originated from the national surveillance programs: The results of a serological *Salmonella* surveillance at the slaughterhouses and in all breeding herds, appointed risk herds to be further examined by analysing pen-faecal samples (n = 107). In addition, a yearly surveillance of 100 farms, where pen-faecal samples were taken at medicine- and welfare control visits, was launched in 2011 (n = 12) together with the onset of additional analysis for *Salmonella* among the random sampling of healthy animals at slaughter (i.e. the ceacum samples described for isolation of *Campylobacter*, enterococci and *E. coli* isolates) (n = 50). Finally, *Salmonella* in samples from pig herds investigated due to clinical disease (not necessarily salmonellosis) were included (n = 33).

No *Salmonella* data for broilers or cattle is presented in the DANMAP 2011 due to a low number of isolates observed per serotype (<15). For broilers, all flocks (including flocks intended for export) were sampled twice before slaughter as part of the *Salmonella* surveillance program. For cattle, the herds examined were based on clinical indications.

Further details on the sampling procedures and the findings of the Danish *Salmonella* surveillance programs are presented in Textbox 4, and in the Annual Report on Zoonoses in Denmark, 2011 [www.dtu.food.dk].

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 carried out by the authorities or on specific request from the Danish Veterinary and Food Administration (DVFA) for the DANMAP programme. The collected material consisted of both Danish and imported meat. Only one isolate per bacterial species per meat sample was selected for DANMAP. Further details on sampling and findings are presented in Textbox 4.

***Salmonella*.** The *Salmonella* isolates from Danish pork and beef originated from the national *Salmonella* surveillance programme, comprising swab samples from pork and beef carcasses taken at the slaughterhouses after cooling. *Salmonella* isolates from imported poultry meat and other imported fresh meats originated from a case-by-case risk assessment control programme (DFVA). Further details on sampling and findings are presented in Textbox 4. One isolates of *S.* Typhimurium and *S.* Enteritidis per phage type from a positive batch of meat were included in the DANMAP report. Isolates of *S.* Typhimurium include the monophasic variants, antigenic formula *S.* 4,5,12:i:- and *S.* 4,12:i:-.

10.3.3 Humans

***Salmonella enterica* serovars Typhimurium and Enteritidis and *Campylobacter 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 these 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 section 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 since then it has been mandatory to send 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 determination 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 *Enterococcus faecium* and invasive *Enterococcus faecalis. Data were provided on all isolates recorded from either blood samples (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. faecium* and *E. faecalis*) or urine samples (*E. coli, Klebsiella pneumoniae*) submitted for susceptibility testing to the participating DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev, Hillerød, Region Sealand, 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 processed at the National Food Institute was done 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 plate with Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium was inoculated with 0.1 ml of BPW deposited as 3 drops. After incubation o/n at 41.5°C, material from MSRV swarming zones were inoculated onto Brilliant Green Agar. Incubation o/n at 37°C was followed by serotyping of suspect colonies by slide agglutination. Isolates submitted for typing to the National Food Institute from other laboratories, were isolated according to the standard methods at the submitting laboratory, and upon reception, inoculated onto Brilliant Green Agar at the National Food Institute followed by serotyping. All isolates received for typing are stored at -80°C.

Campylobacter. The material was inoculated directly onto mCCD agar (Oxoid, Denmark) and incubated in micro-aerophilic atmosphere for 2-3 days at 41.5°C. For 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* suspect 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] and all isolates of *C. jejuni* and *C. coli* were stored at -80°C. All analyses were performed at the National Food Institute.

I**ndicator *E. coli***. Material from healthy animals were 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 identified as *E. coli* after o/n incubation at 37°C. All analyses were performed at the National Food Institute and all isolates were stored at -80°C.

Indicator enterococci. One drop of material suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz-Bartley agar and incubated two days at 42°C. Up to four colonies with a typical morphology of *E. faecalis* or *E. faecium* were sub-cultivated on blood agar. Colonies were identified by the following criteria: Colour, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose and raffinose. All analyses were performed at the National Food Institute and 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 & Food Council, Kjellerup (for *E. coli* O149 and *S. hyicus*) and at the National Veterinary Institute (for *E. coli* F5(K99)). All isolates were stored at -80°C at the National Food Institute.

10.4.2 Meat

Salmonella was isolated by the regional laboratories within the DVFA according to the open reference methods issued by NMKL (NMKL No. 187, 2007 or NMKL No. 71, 1999) or the ISO 6579:2002, or alternative methods validated against the reference method according to ISO 16140:2001. Sero- and phage-typing was performed at the 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 within the DVFA. All isolates of *C. jejuni, C. coli* and *C. lari* were sent to the National Food Institute for MIC-testing and stored at -80°C.

Indicator *E. coli* was isolated by the regional laboratories within the DVFA, by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulfphate-broth, which was incubated o/n at 44°C, and subsequently streaked onto violet red bile agar and incubated for 24h 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. The *E. coli* isolates were sent to the National Food Institute for MIC-testing and stored at -80°C.

Indicator enterococci were isolated by the regional laboratories within the DVFA, by adding 5 g of the sample to 45 ml azide dextrose broth, which was incubated o/n at 44°C and subsequently streaked on Slanetz-Bartley agar. After incubation at 44°C for 48 hours, colonies typical of *E. faecium* and *E. faecalis* were identified by a real-time PCR assay. All isolates of *E. faecium* and *E. faecalis* were sent to the National Food Institute for MIC-testing and stored at -80°C.

10.4.3 Humans

Salmonella isolates were serotyped 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 used both for species conformation and typing purposes. Any *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) was performed [Enright *et al.* 2000. J Clin Microbiol. 38: 1008–1015] and 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 the *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

Animal and meat isolates

Antimicrobial susceptibility testing of *Salmonella, Campylobacter,* indicator *E. coli, Enterococcus* and the veterinary pathogens was performed as microbroth dilution MIC using 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 following quality control strains were used: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.

Isolates from animals and meat were tested at the National Food Institute, and the *Salmonella* and *Campylobacter* isolates of human origin were tested at SSI. MIC-testing at the National Food Institute is accredited by DANAK (the Danish 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 of 100 to 150 isolates was appointed to MIC. Due to low number of isolates, *C. jejuni* from pigs and *C. coli* from cattle and broilers were not susceptibility tested.

Table 10.2 presents the interpretation of MIC-values used for all combinations of bacteria and antimicrobial agents. Since 2007, data were interpreted by EUCAST epidemiological cut-off values (ECOFFs) with a few exceptions (see footnotes in Table 10.2). In the figures, data from before 2007 have been interpreted using the 2007 ECOFFs, otherwise interpretation is based on the ECOFFs from the year in question. The corresponding EUCAST clinical breakpoints are also presented in both Table 10.2 and the MIC-distribution tables to visualize the impact of the use of ECOFFs contra clinical breakpoints.

The MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the number of isolates tested, the applied interpretation of the MIC-values and the estimated level of resistance together with the corresponding confidence intervals.

Multi-resistance was defined as resistance to 3 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 antimicrobials were tested: Erythromycin, clindamycin, kanamycin, rifampicin, penicillin, cefoxitin, fusidic acid, norfloxacin, linezolid, tetracycline, trimethoprim-sulfametoxazole 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 ≥ 8 mg/L for vancomycin and teicoplanin or an MIC ≥12 mg/L for teicoplanin, population analysis profile against vancomycin was performed [Wootton *et al.* 2001. J Antimicrob Chemother. 47: 399–403].

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 2011

Antimicrobial agent	*Salmonella*		*E. coli*		*E. faecium*		*E. faecalis*		*C. jejuni*		*C. coli*		*S. hyicus*[d]	
	ECOFF µg/ml	Clinical break-point µg/ml	ECOFF **µg/ml**	Clinical break-point µg/ml	**ECOFF µg/ml**	Clinical break-point µg/ml	**ECOFF µg/ml**	Clinical break-point µg/ml	**ECOFF µg/ml**	Clinical break-point µg/ml	**ECOFF µg/ml**	Clinical break-point µg/ml	**ECOFF µg/ml**	Clinical break-point µg/ml
Ampicillin	**>8***	>8*	**>8***	>8*	**>4***	>8*	**>4***	>8*						
Apramycin	**>16**		**>16**											
Cefotaxime	**>0.5***	>2*	**>0.25***	>2*										
Cefoxitin													**>4***	
Ceftiofur	**>2***		**>1***											
Chloram-phenicol	**>16***	>8*	**>16***	>8*	**>32***		**>32***		**>16***		**>16***		**>16***	
Ciprofloxacin	**>0.06 ***	>1*	**>0.06***	>1*	**>16**[b]		**>8**[b]		**>1***	>1*	**>1***	>1*	**>1***	>1*
Colistin	**>2*/ >8**[c]	>2*	**>2***	>2*										
Erythromycin					**>4***		**>4***		**>4***	>4*	**>16***		**>1**	>2*
Florfenicol	**>16***		**>16***										**>8***	
Gentamicin	**>2***	>4*	**>2***	>4*	**>32***		**>32***		**>1***		**>2***		**>0.5***	>1*
Kanamycin					**>1,024**		**>1,024**							
Linezolid					**>4***	>4*	**>4***	>4*						
Nalidixic acid	**>16***		**>16***						**>16***		**>32***			
Neomycin	**>4***		**>8***											
Penicillin					**>16***		**>16***						**>0.125***	>0.125*
Quinupristin/ dalfopristin					**>4**[a]	>4*								
Salinomycin					**>4**		**>4**							
Spectinomycin	**>64**		**>64***										**>128***	
Streptomycin	**>16***		**>16***		**>128***		**>512***		**>2***		**>4***		**>16***	
Sulfonamide	**>256**		**>64***										**>128***	
Teicoplanin					**>2***	>2*	**>2***	>2*						
Tetracycline	**>8***		**>8***		**>4***		**>4***		**>2***		**>2***		**>1***	>2*
Tiamulin													**>2***	
Tigecycline					**>0.25***	>0.5*	**>0.25***	>0.5*						
Trimethoprim	**>2***	>4*	**>2***	>4*									**>2***	>4*
Vancomycin					**>4***	>4*	**>4***	>4*						

* EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints. Orange fields mark changes in ECOFF values since DANMAP 2010

a) The EUCAST ECOFF (>1) was not applied for quinopristin/dalfopristin according to investigations presented in DANMAP 2006 (trade name synercid)

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 Antimicob Agents;35:119-125)

c) The EUCAST ECOFF (>2) for colistin was applied for *S.* Typhimurium, but for *S.* Enteritidis the ECOFF >8 was applied as recommended by Agersøe et al, 2011 [Textbox 6]

d) *S. aureus* ECOFFs were applied for *S. hyicus*, except for erythromycin where a specific *S. hyicus* ECOFF was available

Table 10.3. Definitions of antimicrobial classes for calculation of multi-resistance (MR) in zoonotic and indicator bacteria in DANMAP 2011

DANMAP 2011

Antimicrobial classes	*Salmonella* and *E. coli*[a]	*Campylobacter*[b]	*Enterococcus*[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
Polymycins	Colistin		
Macrolides		Erythromycin	Erythromycin
Glycopeptids			Vancomycin and/or teicoplanin
Ionophores			Salinomycin
Oxazolidinones			Linezolid
Glycylcyclines			Tigecycline

Note: An isolate is considered fully sensitive if susceptable to all antimicrobial agensts included in the panel for the selected bacterial species
a) An isolates is considered multi-resistant if resistant to three or more of the ten antimicrobial classes
b) An isolates is considered multi-resistant if resistant to three or more of the six antimicrobial classes

Invasive *Streptococcus pneumoniae* from humans
Screening for penicillin-resistant *S. pneumoniae* was performed using a 1 µg oxacillin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on Müller-Hinton agar (Mueller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostika), and for erythromycin-resistant *S. pneumoniae* using a 15 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on Müller-Hinton agar (SSI Diagnostika). The breakpoints used are defined by the EUCAST. Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, United Kingdom) as recommended by the manufacturer. The breakpoints used are defined by EUCAST. Resistant isolates were defined as both fully and intermediary resistant isolates.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans
Screening for penicillin-resistant streptococci was performed using a 1 µg oxacillin disk (Oxoid, Greve, Denmark) on Müller-Hinton Agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant streptococci using a 15 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on Müller-Hinton Agar (SSI Diagnostika). Erythromycin resistant streptococci were tested with a 15 µg erythromycin disk (Oxoid) and a 2 µg clindamycin disk (Oxoid) on Mueller-Hinton Agar. Erythromycin MICs were determined using the Etest® (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar incubated at 36°C, 5% CO2. The breakpoints used are defined by the EUCAST. Resistant isolates were defined as both fully and intermediary resistant isolates.

***E. coli, K. pneumoniae,* invasive *P. aeruginosa*, invasive *E. faecium* and *E. faecalis* from humans**
The DCMs performed either disk (Oxoid, Basingstoke, UK) or tablet (Neo-Sensitabs®, A/S Rosco) diffusion susceptibility testing on a number of media using breakpoints defined by either CLSI or EUCAST (DCM at Hvidovre Hospital, Herlev Hospital, Slagelse Hospital/ Region Sealand (per May 2011), Odense University Hospital (per November 2011), Herning and Viborg Hospitals/Midt-Vest (Viborg per September 2011, and Herning), Aarhus Hospital, and Aalborg Hospital).

Data on antimicrobial resistance from private hospitals and clinics and 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).

10.6 Data handling

10.6.1 Animal

The results from the primary examination of samples from slaughterhouses and primary production for the bacteria of interest - positive as well as negative findings - and of the susceptibility testing were stored in an Oracle Database 9i Enterprise Edition® at the National Food Institute. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant ECOFF. Each isolate was identified by the bacterial species, by 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 was 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 Danish Veterinary and Food Administration, except for the data on *Salmonella*, which were reported to and extracted from the laboratory database at the 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 RFCA that collected and processed the sample, and an identification number, which makes it possible to obtain further information about the isolate from the 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). In this database, patients were registered, regardless of whether it was colonization 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 colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 12 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations 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 at 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. Thirteen DCM provided data on resistance levels in *E. coli, Klebsiella pneumoniae,* invasive *Pseudomonas 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 Calculation of confidence limits

timation of exact 95% (two-sided) confidence intervals for proportions were based on binomial probability distributions as described in Armitage & Berry [Statistical Methods in Medical Research, 4th ed. 2001, Oxford: Blackwell Scientific Publications].

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. When appropriate, significance of temporal trends are tested using linear logistic regression using Proc LOGISTC procedure in SAS (Wald test). In the text, significant differences imply statistically significant differences where $p<0.05$ using Chi-square, or Fisher's Exact Test when the number of samples is low (<25).

When comparing proportions between years, the EUCAST epidemiological cut-off values for 2011 were also used for interpretation of previous years MIC's.

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Terminology 11

List of abbreviations

ACD	Defined Animal Course Dose
ADD	Defined Animal Daily Dose
ADD_{kg}	Defined Animal Daily Dose per kg animal
AGP	Antimicrobial Growth Promoter
ATC	Anatomical Therapeutic Chemical Classification System
CHR	Central Husbandry Register
CI	Confidence Interval
CNS	Central Nervous System
CPR	Danish Civil Registry
DAD	Defined Daily Doses per 100 admissions
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DID	Defined Daily Doses per 1,000 inhabitants per day
DDD	Defined Daily Dose
DMA	Danish Medicines Agency
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ESBL	Extended Spectrum Beta-Lactamase
GI	Gastrointestinal
GP	General Practitioner
HLGR	High-level gentamicin resistance
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
PMWS	Postweaning multisystemic wasting syndrome
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
WT	Wild type

List of words

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) (http://www.whocc.no/atcddd/indexdatabase/). The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (http://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 numerous information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined Daily Dose (DDD). This is the assumed average maintenance dose per day 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 (http://www.whocc.no/atcddd/indexdatabase/). DDD/1,000 inhabitant-days is called DID.

Defined Animal Daily Dose (ADD and ADDkg). This is a national veterinary equivalent to the DDD. This is an assumed average daily dose per animal, defined as the daily maintenance dose for a drug used for its main indication in a specified species. The dose is defined for a 'standard animal', i.e. an animal with an estimated average weight within a specified age group. In VetStat, ADDs are calculated for each age group. Otherwise, the general principles for standardisation of dosage for animals are similar to that used by the WHO Collaborating Centre for Drug Statistics and Methodology to calculate Defined Daily Dose (DDD) in humans [Jensen *et al*. 2004. Prev Vet Med. 64: 201-215]. The ADDkg is the ADD per kg animal. Consumption calculated in ADDkg allows summation of consumption across different age groups and animal species.

Defined Animal Course Dose (ACD and ACDkg). The duration of the treatment related to one application may vary substantially between antimicrobial drugs. To correct for this, total course dose has been introduced as unit of measurement for antimicrobial usage. As a standard, the length of the course is here defined as 6 days, if nothing else is stated. Course doses are assigned per kilogram (live weight) of the animal species (ACDkg) or age group of the relevant species (ADCxx) and are based on the corresponding ADDkg or ADDxx, respectively, for the relevant animal species and drug formulations.

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 kilogram live weight to time of slaughter at app. 100 kilogram live weight.

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.

Heifer. A young female cow before first calving.

Intramammaria. Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Intramammary syringe. A one dose applicator for use in the udder.

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 resistant to three or more antimicrobial classes. The number of antimicrobial classes and antimicrobial agent included therein depend on the test panel for each bacteria. See table 10.3 in section 10.5 for a detailed description.

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 not included.

Piglet. The newborn pig is called at 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 kilogram.

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.

Significant. When written in the text, significant differences imply statistically significant differences where $p<0.05$. Pairwise comparisons are made using Chi-square or Fisher's Exact Test when the number of samples is low (<25). When appropriate, linear logistic regression is used to analyse for significant trends over several years.

Sow. Any breeding female pig that has been served and is on the farm.

Steer. Castrated male cattle, usually young animal.

Weaner. Any pig, 7–30 kilogram live weight.

Wild type. The typical form of an organism, strain, gene, or characteristic as it occurs in nature.

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