

DANMAP 2014 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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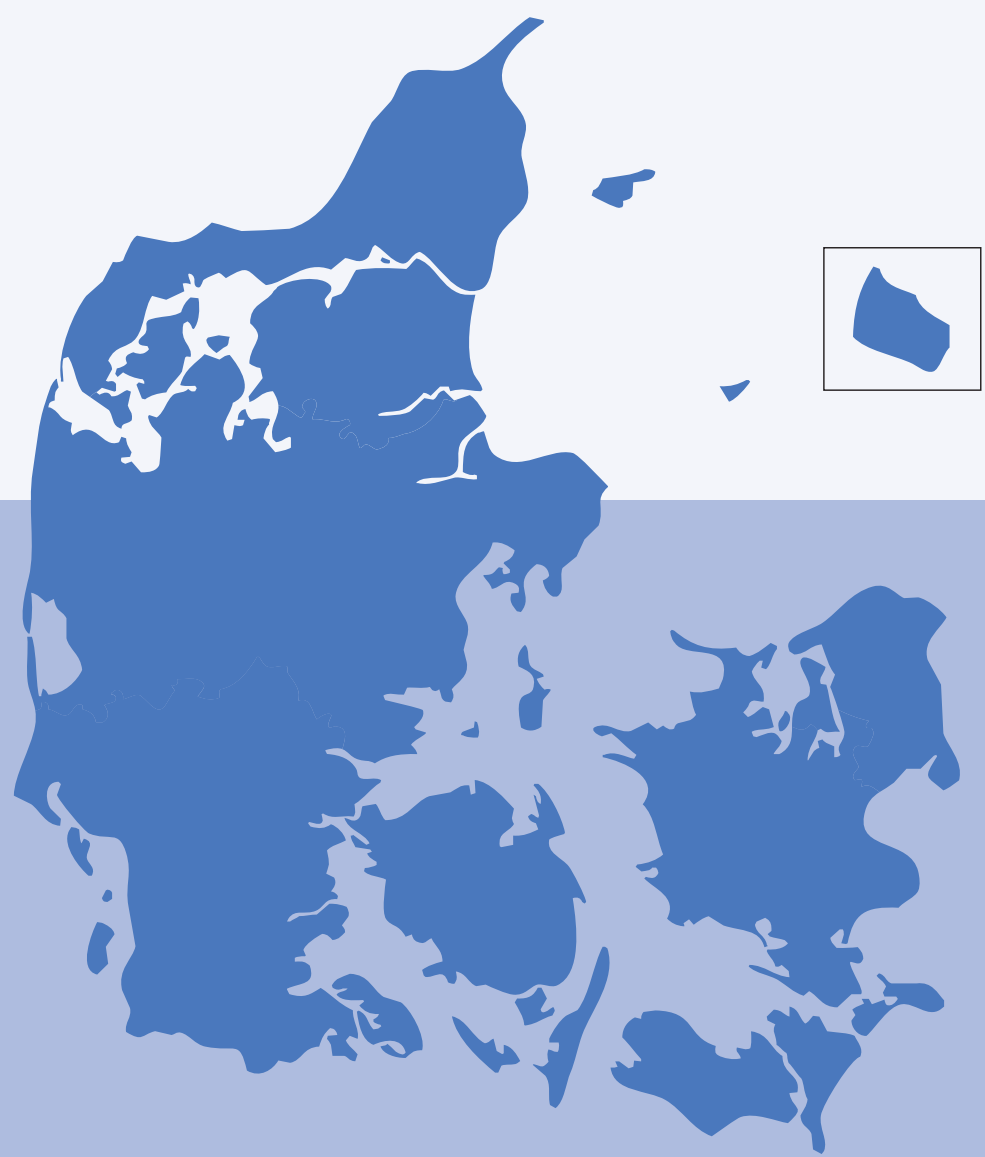
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DANMAP 2014

DANMAP 2014 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark



Statens Serum Institut
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DANMAP 2014

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DANMAP 2014

DANMAP 2014 - Use of antimicrobial agents and occurrence
of antimicrobial resistance in bacteria from food animals,
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Table of contents

1.	Introduction	6
1.1	About DANMAP	6
1.2	Acknowledgements	7
1.3	DANRES	8
2.	Summary	9
2.1	Sammendrag	10
2.2	Summary	16
3.	Background information	23
3.1	Populations	24
3.2	Marketed antimicrobial agents	25
4.	Antimicrobial consumption in animals	27
4.1	Introduction	28
4.2	Total antimicrobial consumption	30
4.3	Antimicrobial consumption by animal species	31
Textbox 4.1	National actions for prudent antimicrobial use in animals	36
Textbox 4.2	Use of vaccines in the Danish pig production 2004 - 2014	38
Textbox 4.3	Use of zink oxide in the Danish pig production 2005 - 2014	40
5.	Antimicrobial consumption in humans	41
5.1	Introduction	42
5.2	Total consumption in both primary healthcare and hospital care	42
5.3	Primary healthcare	45
5.4	Hospital care	48
Textbox 5.1	Antibiotic coverage of guidance for empirical antibiotic treatment of bacteraemia	53
Textbox 5.2	Consumption of antimicrobial agents and incidences of multi-resistant bacteria in Greenland	54
Textbox 5.3	Hospital-Acquired Infections Database (HAIBA). Monitoring hospital-acquired infections using existing data sources	56
6.	Resistance in zoonotic bacteria	57
6.1	<i>Salmonella</i>	58
6.2	<i>Campylobacter</i>	62

7.	Resistance in indicator bacteria	65
7.1	Enterococci	66
7.2	<i>Escherichia coli</i>	70
Textbox 7.1	Reduced occurrence of ESBL-producing <i>Escherichia coli</i> in meat from Danish retail and comparison to isolates from human bloodstream infections	74
8.	Resistance in human clinical bacteria	77
8.1	<i>Escherichia coli</i>	78
8.2	<i>Klebsiella pneumoniae</i>	81
8.3	<i>Pseudomonas aeruginosa</i>	83
Textbox 8.1	Characterisation of ESBL/AmpC-producing and carbapenemase-producing <i>Escherichia coli</i> from bloodstream infections, 2014 Denmark	84
Textbox 8.2	Carbapenemase-producing bacteria in Denmark, 2014	86
8.4	Streptococci	88
8.5	Enterococci	89
Textbox 8.3	Continued increase in occurrence of clinical vancomycin resistant enterococci in Danish hospitals in 2014	90
8.6	<i>Staphylococcus aureus</i>	91
Textbox 8.4	<i>Neisseria gonorrhoeae</i> 2014	94
Textbox 8.5	Livestock associated methicillin-resistant <i>Staphylococcus aureus</i> (LA-MRSA) among humans and in pig herds 2014	96
9.	Materials and Methods	97
9.1	General information	98
9.2	Data on antimicrobial consumption	98
Textbox 9.1	Revised method for calculating the ADD in Vetstat	100
9.3	Collection of bacterial isolates	101
9.4	Isolation and identification of bacteria	102
9.5	Susceptibility testing	103
9.6	Data handling	105
10.	Terminology	107
10.1	List of abbreviations	108
10.2	Glossary	109

1. Introduction

1.1 About DANMAP

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

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

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

The objectives of DANMAP are:

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

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

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

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

Public health risks

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

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

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

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

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

1.2 Acknowledgements

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- the meat inspection staff and the company personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful;
- the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup, and the DTU National Veterinary Institute for making isolates of animal pathogens available to the programme;
- the staff of the Regional Veterinary and Food Control Authorities for collecting samples, culturing and MIC testing the isolated bacteria.
- the Department of Medication Statistics and Research Support at SSI (formerly the Danish Medicines Agency) for

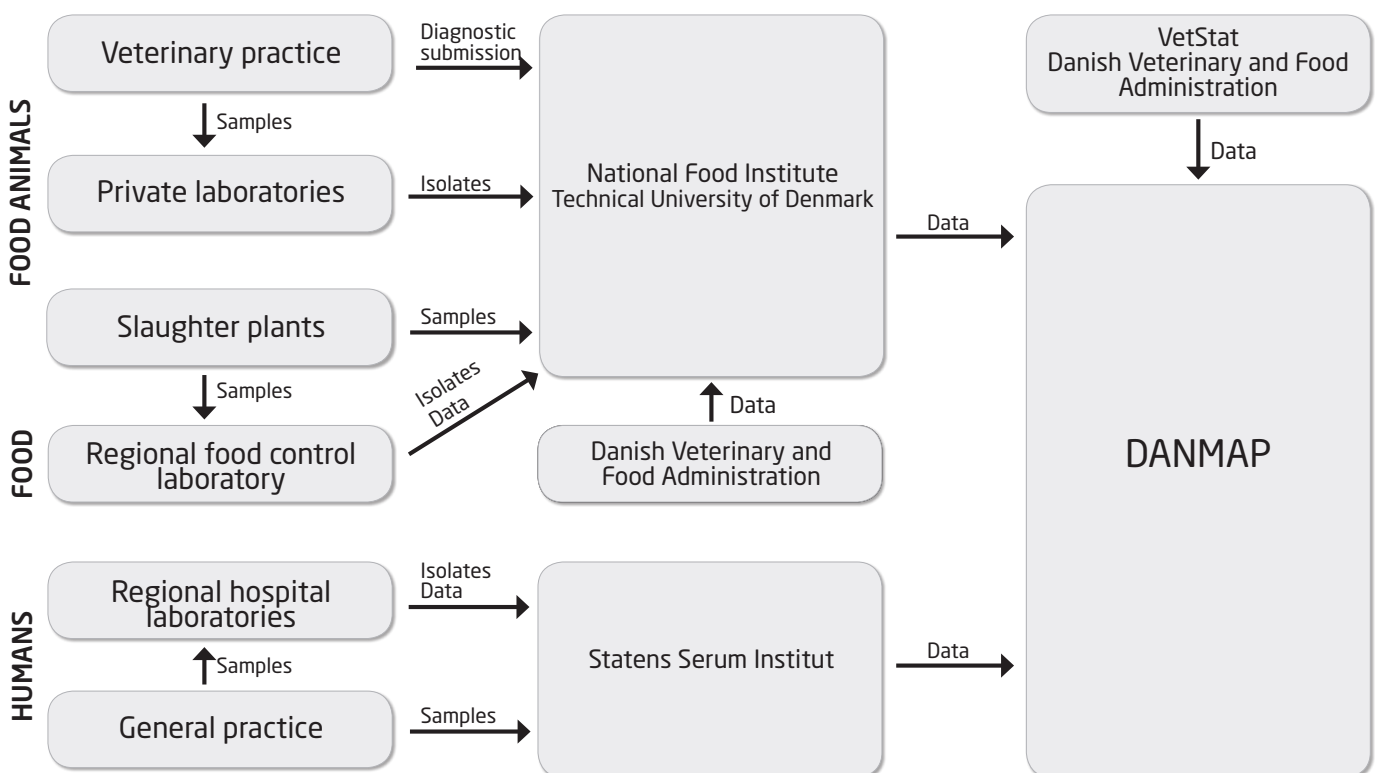
collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies;

- the Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, including statistics on consumption measured in tonnage; and
- the Danish Agriculture and Food Council for cooperation regarding the estimation of live biomass of production animals.

Statens Serum Institut would like to thank the following:

- the Departments of Clinical Microbiology and the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples;
- the staff of the Neisseria and Streptococcus Typing Unit at SSI;
- the staff of the Foodborne Pathogens Unit at SSI;
- the staff of the Staphylococcus Laboratory at SSI;
- the staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI;
- Erik Villadsen from the Department of Health Documentation at SSI for providing data on hospital activities.

Figure 1.1. Organisation of DANMAP 2014



1.3 DANRES

The Departments of Clinical Microbiology (DCM) and the Danish Study Group for Antimicrobial Resistance Surveillance (DANRES) in Denmark provide data and information on resistance in clinical isolates.

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2

SAMMENDRAG / SUMMARY

2. Summary

2.1 Sammendrag

DANMAP (Danish Antimicrobial Resistance Monitoring and Research Program) har siden 1995 beskrevet det årlige forbrug af antibiotika og forekomsten af antibiotikaresistens hos dyr og mennesker i Danmark. Denne udgave beskriver udviklingen i 2014.

Antibiotikaforbrug til dyr

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

I 2014 blev der brugt 114 ton antibiotika (aktivt stof) til dyr i Danmark. Fordelt på dyrearter stod svin for ca. 76 % af antibiotikaforbruget, kvæg for 11 %, akvakultur for 4 %, pelsdyr for 4 %, fjerkræ for 1 % og kæledyr, heste og andre dyr for de resterende 4 %. Det totale forbrug (kg aktivt stof) til dyr var 2 % lavere i 2014 end i 2013.

De overordnede ændringer i antibiotikaforbruget til dyr styres primært af ændringer i forbrugsmønstret til svin. Svineproduktionen står for 86 % af den danske kødproduktion, men kun 43 % af den totale levende biomasse.

Svin. Det totale antibiotikaforbrug til svin i 2014 var på ca. 86 ton aktivt stof, hvilket i forhold til 2013 udgør et fald på næsten 5 ton. Dette svarer til et fald på 5 % når forbruget udregnes i DAPD og der justeres for eksport. Enheden DAPD anslår andelen af en population (i tusinder), som dagligt behandles med en standarddosis antibiotikum. Reduktionen i antibiotikaforbrug (DAPD) til svin skyldtes især et fald i forbruget af tetracykliner, men også i forbruget af pleuromutiliner og makrolider. Pleuromutiliner og tetracykliner tilsættes primært til foder eller vand i forbindelse med mave-tarm lidelser. I svin var forbruget af 3. og 4. generations cefalosporiner fortsat meget lavt (4 kg), hvilket er et resultat af, at svinebranchen i 2010 frivilligt indførte stop for brugen af disse stoffer. Forbruget af fluorkinoloner var også meget lavt (4 kg), hvilket det har været siden 2003, hvor lovgivning om begrænsning af fluorkinoloner forbruget trådte i kraft.

Kvæg. Forbruget til kvæg har været faldende i nogle år (14 % i perioden 2010 til 2013), men i 2014 steg forbruget med 8 % fra 12 ton i 2013 til 13 ton i 2014. Målt i standard doser (DADD) steg forbruget til golddyrbehandling med 12 %, hvorimod antallet af DADD til behandling af klinisk mastitis faldt med 2 % i forhold til 2013. Forbruget af beta-laktamase følsomme penicilliner udgjorde som tidligere år størstedelen af forbruget. Forbruget af fluorkinoloner, som er et af de kritisk vigtige antibiotika til human behandling, har siden 2003 været stort set nul. Sammenlignet med 2013 faldt forbruget af 3. og 4. generations cefalosporiner til systemisk behandling med 27%.

Fjerkræ. Antibiotikaforbruget til fjerkræ var cirka 1.548 kg aktivt stof i 2014, hvilket er 22 % højere end i 2013. Stigningen i antibiotikaforbrug skyldtes især en stigning i forbruget af tetracykliner, men også i forbruget af bredspektrede penicilliner. Der er flere forklaringer på stigningen i forbruget, herunder øget forekomst af diarre og ledinfektioner i slagtekyllingeproduktionen, og en fortsat høj forekomst af luftvejslidelser i kalkunflokke. Forbruget af fluorkinoloner i den danske fjerkræproduktion, inklusiv avl og opdræt, har været meget lavt siden 2006, og der er ikke rapporteret brug af cefalosporiner til fjerkræ i over 10 år.

Fisk. Det totale antibiotikaforbrug til fisk i akvakultur var på 5.116 kg aktivt stof i 2014, en generel stigning på 43 % i forhold til 2013. Som i 2013, var sommeren 2014 også præget af ekstraordinært høje vandtemperaturer, hvilket igen medførte øget forekomst af bakterielle infektioner i forhold til de foregående år. Branchen har fortsat fokus på udvikling af nye vacciner og forbedrede vaccinationsstrategier for at begrænse brugen af antibiotika.

Pelsdyr. I både 2013 og 2014 er forbruget af antibiotika til pelsdyr (mink) faldet. I 2014 blev der brugt 4.202 kg aktivt stof, hvilket var 14 % mindre end året før. Forbruget af fluorkinoloner og cephalosporiner har været tæt på nul i flere år. En mulig forklaring på faldet i antibiotikaforbrug kan være det øgede fokus branche, dyrlæger og producenter har haft på at forbedre foderkvalitet og dyrevelfærd samt at reducere antibiotikaforbruget.

Kæledyr og heste. Oplysningerne i VetStat om antibiotikaforbrug til kæledyr og heste er mindre detaljerede end oplysningerne vedrørende produktionsdyr. I 2014 var forbruget stort set på samme niveau som i 2013 (1.989 kg i 2013, 2.017 kg i 2014). Forbruget af kritisk vigtige antibiotika som cefalosporiner og fluorkinoloner faldt igen i 2014. Derimod steg forbruget af flere grupper af antibiotika, herunder aminoglycosider, makrolider, penicilliner (beta-laktamase følsomme), sulfonamider, trimethoprim og tetracykliner. Det let ændrede forbrugsmønstre til kæledyr kan være et resultat af den antibiotikavejledning for familiedyr, som blev udgivet af Dyrlægeforeningen i 2012. Forbruget af kritisk vigtige antibiotika er dog stadig relativt højt i kæledyr sammenlignet med forbruget i produktionsdyr og giver fortsat anledning til øget bevågenhed.

Antibiotikaforbruget til dyr faldt med 2 % i 2014. Det totale forbrug til svin, målt i kg aktivt stof såvel som beregnet i DAPD, faldt med 5 %. Brugen af kritisk vigtige antibiotika i den danske svineproduktion er fortsat lav. Brugen af især kritisk vigtige antibiotika til kæledyr er forholdsmæssigt højt sammenlignet med forbruget til produktionsdyr, dog er forbruget af disse stoffer til kæledyr faldet i både 2013 og 2014.

Antibiotikaforbrug til mennesker

Forbruget af receptordineret medicin på patientniveau er blevet overvåget siden begyndelsen af 1990'erne med etablering af Lægemiddelstyrelsens Lægemiddelstatistikregister i 1994.

I DANMAP rapporten 2014 er for første gang medtaget det perorale forbrug af metronidazol (P01AB01) og vancomycin (A07AA09). Totale forbrugstal samt forbrug i henholdsvis hospitals- og primærsektor er samtidig justeret fra 2005 og frem. Disse antibiotika har ikke tidligere været medtaget da forbruget ikke klassificeres i ATC-gruppen systemiske anti-infektiva (J01), men som henholdsvis antiparasitære (P01) og intestinale (A07) midler. Peroral metronidazol udgør en væsentlig del af den anaerobe antibiotiske behandling og af behandlingen af infektioner med *Clostridium difficile*. Peroral vancomycin anvendes ligeledes til behandlingen af infektioner med *Clostridium difficile*.

Totalforbrug. I 2014 faldt det totale humane forbrug af antibiotika til systemisk brug (primærsektoren og hospitalssektoren sammenlagt) med 3 % eller 0,5 DDD pr 1000 indbyggere pr dag (DID), 18,58 DID i 2014 sammenholdt med 19,10 DID i 2013. Primærsektoren udgjorde 90 % af forbruget og faldet i det totale forbrug skyldtes især et fald i forbruget af tetracykliner på 0,3 DID (se nedenfor). I 2011, hvor det højeste totale forbrug nogensinde blev observeret, var forbruget på 19,31 DID, en stigning fra 1997 på næsten 40 %.

Fra 2005 til 2014 er det totale forbrug af antibiotika i Danmark steget med 1,86 DID (11 %). Samlet set blev der i 2014 brugt 52.819 kg aktiv substans.

Primærsektor. Det totale antibiotikaforbrug i primærsektoren udgjorde i 2014 16,40 DID (16,95 DID i 2013). Det mest markante fald i forbrug var som anført blandt tetracyklinerne (fra 1,96 DID i 2013 til 1,66 DID i 2014); dette fald skyldes dog primært store ændringer i forbruget af doxycyklin, der havde en uventet top i 2013 men i 2014 faldt tilbage til tidligere niveau.

Som i de tidligere år udgjorde beta-laktamase følsomme penicilliner den største gruppe af antibiotika (27 %), efterfulgt af penicilliner med udvidet spektrum (22 %), makrolider (11 %), tetracykliner (10 %) og kombinationspenicilliner og beta-lakta-

mase resistente penicilliner (begge 8 %). Penicilliner udgjorde samlet 65 % af forbruget. Fluorkinoloner (primært ciprofloxacin) udgjorde 3 % af forbruget.

Fra 2013 til 2014 faldt forbruget af beta-laktamase sensitive penicilliner fra 4,65 DID til 4,38 DID (6 %). Forbruget af kombinationspenicilliner og beta-laktamase resistente penicilliner øgedes i samme periode, fra hhv. 1,22 DID til 1,30 DID (7 %) og fra 1,30 DID til 1,36 DID (5 %), hvilket ligger i tråd med det stigende forbrug, der er observeret over de sidste 10 år. Stigninger i forbruget af kombinationspenicilliner kan forklares med ændrede retningslinjer for behandling af lungeinfektioner hos patienter med kronisk obstruktiv lungesygdom (KOL), men der ses et samlet øget forbrug, også blandt andre patient- og aldersgrupper. Dette forsøges nærmere belyst over de næste år.

Forbruget af fluorquinoloner fortsatte den svagt faldende tendens, der er observeret over de sidste 3 år (fra 0,57 DID i 2011 til 0,50 DID i 2014).

Forbruget af trimethoprim, sulfonamider og nitrofurantoin forblev uændret.

I det seneste årti er forbruget af antibiotika i primærsektoren steget med 10 %, fra 14,96 DID i 2005 til 16,40 DID i 2014, dog kan denne udvikling opdeles i et støt stigende forbrug frem til 2011, hvorefter forbruget nu er stagneret. Stigningen skyldes sandsynligvis til dels et øget antal DDD (definerede dagsdoser) pr behandlet patient og et øget antal DDD pr udskrevet medicinpakning. Sidstnævnte kan afspejle ændrede retningslinjer for behandling af infektioner gående mod kortere behandlingstider med højere doser.

Hospitaller. På somatiske hospitaler steg antibiotikaforbruget når det blev opgjort i DDD pr 100 sengedage (DBD): fra 99,88 DBD i 2013 til 104,30 i 2014 (4 %). Fra 2005 til 2014 steg det totale antibiotikaforbrug med 57 % (fra 66,32 DBD). Denne stigning skyldes en kombination af stigning i DDD og et fald i antallet af sengedage.

Gruppen af penicilliner udgjorde 50 % af det samlede hospitalsforbrug, heraf penicilliner med udvidet spektrum 16 % og kombinationspenicilliner 15 %. 2.generations cefalosporiner og fluorkinoloner udgjorde hhv. 11 % og 9 %.

Fra 2013 til 2014 steg forbruget af kombinationspenicilliner fra 13,64 DBD til 16,04 DBD (18 %). Denne kraftige stigning kan forklares med at Piperacillin/tazobactam er blevet en væsentlig del af den empiriske sepsisbehandling på de fleste hospitaler. Penicilliner med udvidet spektrum øgedes fra 15,06 DBD til 16,40 DBD (9 %) og imidazol-derivater fra 4,08 DBD til 4,48 DBD (9 %), mens der sås et fald i forbruget af 2. generations cefalosporiner (cefuroxim) fra 12,31 DBD til 11,68 DBD (5 %). Forbruget af beta-laktamase følsomme penicilliner (totalt 10,31 DBD), beta-laktamase resistente penicilliner (9,57 DBD),

carbapenemer (totalt 4,09 DBD) og fluorkinoloner (totalt 9,88 DBD) ændredes ikke væsentligt.

De største ændringer over tid, der er observeret på hospitalerne er i forbruget af de forskellige typer penicilliner: Mens forbruget af beta-laktamase sensitive penicilliner er faldet markant siden 2005 fra 12,17 DBD til 10,31 DBD (15 %) er forbruget af kombinationspenicilliner mere end fordoblet, fra 1,16 DBD til 16,04 DBD. Kombinationspenicilliner udgør nu den andenstørste gruppe af antibiotika i hospitalssektoren svarende til 15 % af forbruget.

De væsentligste ændringer i antibiotikaforbruget på hospitalerne skyldes formentlig indførelse af nye lokale retningslinjer vedrørende antibiotisk behandling af sepsis, der på de fleste hospitaler har medført et skift fra cefalosporiner som førstevalgs præparat til et beta-laktam (evt i kombination med gentamicin). Disse retningslinjer følger Sundhedsstyrelsens anbefalinger om et reduceret forbrug af cefalosporiner. Derudover er der lanceret nye retningslinjer for behandling af KOL, hvor amoxicillin med clavulansyre nu er førstevalg.

I 2014 udgjorde det totale antibiotikaforbrug til mennesker 18,58 DID, et fald på 3 % sammenlignet med 2013. Dette er primært betinget af ændringer i forbruget af tetracykliner i primærsektoren. Siden 2005 er det totale antibiotikaforbrug steget med 11 %, den største stigning blev observeret frem til 2011 (hvor det totale forbrug var på 19,31 DID), siden er forbruget stagneret. De største ændringer i forbruget over tid er observeret i gruppen af penicilliner, hvor forbruget af kombinationspenicilliner i begge sektorer er steget markant over det sidste årti, mens forbruget af beta-laktamase følsomme penicilliner er faldet. Dette fald ses især i primærsektoren. Kombinationspenicilliner udgør nu den andenstørste gruppe af antibiotika på hospitalerne (15 %) og er blandt de største grupper i primær sektoren (8 %).

Resistens i zoonotiske bakterier

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

EU Harmonisering af resistensovervågningen

I 2014 blev antibiotikapanellerne, som anvendes til at undersøge for resistens, ændret i henhold til Europa Kommissionens afgørelse om gennemførelse af en koordineret kontrolplan for overvågning af antimikrobiel resistens hos zoonotiske agenser [Decision 2013/653/EU]. For *Salmonella*, *Campylobacter*, og indikator *E. coli* og *Enterococcus* betyder det, at flere af panelernes antibiotika blev skiftet ud. Dette skete for at prioritere resistensundersøgelser for de antibiotika, som anses for at have størst relevans for folkesundheden: Herunder screening for carbapenemase-enzymet i *Salmonella* og *E. coli*. Som konsekvens af ændringerne kan opgørelser af multiresistensresultater og fuldt følsomme isolater i 2014 ikke umiddelbart sammenlignes med resultater fra tidligere år.

***Salmonella* Typhimurium** er en af de mest almindeligt forekommende serotyper i danske svin, dansk svinekød såvel som i humane *Salmonella* infektioner. Blandt svin var 66 - 70 % af isolaterne resistente overfor ampicillin, sulfonamid og tetracyclin, og forekomsten af resistens overfor disse antibiotika er steget over de sidste fem år. Dette kan primært tilskrives den stigende forekomst af monofasiske varianter, som ofte er multiresistente. I 2014, var 56 % af de undersøgte *S. Typhimurium* isolater fra svin monofasiske. Generelt var forekomsten af multiresistens blandt *S. Typhimurium* isolater fra danske svin på 64 %. Dette er højere end i samlet set alle *Salmonella* typer (*Salmonella* spp.), hvor 32 % var multiresistente i 2014. Der blev fundet *Salmonella* i 22 % af raske svin, og det estimeres at 7 % af svinene havde multiresistent *Salmonella*. Også i prøver fra dansk svinekød var forekomsten af resistens blandt *S. Typhimurium* isolater høj. Som i de foregående år blev der ikke påvist resistens overfor cefalosporiner (ceftiofur og cefotaxim) eller kinoloner (ciprofloxacin og nalidixansyre) blandt *Salmonella* isolater fra svin eller dansk svinekød.

Ligesom for svin og svinekød, er der de seneste fem år sket en stigning i den relative forekomst af monofasiske *S. Typhimurium* varianter blandt isolater fra patienter, som havde erhvervet infektionen i Danmark (både sporadiske og udbrudstilfælde). I 2014 blev der påvist multiresistens i 48 % af *S. Typhimurium* isolater fra sporadiske tilfælde erhvervet i Danmark, og resistens niveauet var generelt på niveau med 2013. I tidligere år har forekomsten af resistens været højere i isolater fra patienter med rejserelaterede infektioner sammenlignet med infektioner erhvervet i Danmark. I 2014 derimod, var det kun forekomsten af kinolonresistens (ciprofloxacin og nalidixansyre) som var højere i de rejserelaterede infektioner.

I alt syv *S. Typhimurium* isolater (2 % af tilfældene) var resistente overfor 3. generations cefalosporiner (cefotaxime og/eller ceftazidime) og disse isolater var også resistente overfor ciprofloxacin. Co-resistens med ciprofloxacin og 3. generations cefalosporiner giver anledning til øget bevågenhed, da disse to antibiotika anses for kritisk vigtige for behandling af alvorlige *Salmonella* infektioner.

Ingen af de resistensbestemte *Salmonella* isolater fra dyr, kød eller mennesker udviste carbapenemase aktivitet (meropenem resistens).

Forekomsten af monofasiske *Salmonella Typhimurium*, som ofte er multiresistente, er steget i svin, svinekød og blandt humane infektioner de seneste fem år. Resistensforekomsten har, blandt de humane infektioner erhvervet herhjemme, ligget stabilt højt de seneste fire år (50 - 60 %). I 2014 var det udelukkende forekomst af kinolon-resistens som var højere blandt de humane infektioner erhvervet i udlandet.

I 2014 var resistensforekomsten i *Campylobacter jejuni* isolater fra kyllinger og kvæg på samme niveau som de seneste fem år. Der ses generelt lav til moderat forekomst af resistens og størstedelen (74 - 81 %) af isolaterne var fuldt følsomme overfor alle seks antibiotika i testpanelet. Set i en europæisk sammenhæng er resistensforekomsten i *C. jejuni* fra danske slagtekyllinger og kyllingekød samt *C. coli* fra svin blandt Europas laveste.

I flere år har forekomsten af fluorkinolon resistens i *C. jejuni* været højere blandt isolater fra importeret kyllingekød (82 %) end fra dansk kyllingekød (15 %), og forskellen på det importerede og det danske kød er blevet mere udtalt.

Som i de foregående år var forekomsten af fluorkinolon resistens i *C. jejuni* isolater fra patienter med rejse-relaterede infektioner (81 %) højere end i isolater fra patienter, hvor infektionen var erhvervet i Danmark (35 %).

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

Resistens i indikatorbakterier

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

Blandt *Enterococcus faecalis* isolater fra danske slagtekyllinger og kyllingekød blev der fundet moderate til høje forekomster af tetracyclin resistens (henholdsvis 49 % og 35 %) og erythromycin resistens (henholdsvis 27 % og 19 %). Derimod var der meget lidt resistens for de øvrige antibiotika i testpanelet. I *E. faecium* isolater fra importeret kyllingekød, var resistensen generelt højere end i det danske kyllingekød.

Der blev fundet højere forekomst af resistens blandt isolater fra svin end fra slagtekyllinger, hvilket sandsynligvis afspejler antibiotikaforbrugsmønstrene til disse dyrearter. Blandt *E. faecalis* isolater fra svin var der højest forekomst af resistens for tetracyclin (83 %) og erythromycin (49 %).

Tetracykliner er og har været det mest anvendte antimikrobielle stof til danske svin i en årrække og har primært været brugt til behandling af *E. coli* infektioner. Forekomst af resistens var højere i importeret end i dansk produceret svinekød.

Generelt blev der fundet mindre resistens i *E. faecium* end i *E. faecalis* og forekomsten af resistens overfor kritisk vigtige antibiotika var lav.

Resistens blandt **Indikator *Escherichia coli*** fra slagtekyllinger faldt for en lang række antibiotika; tetracyclin, ampicillin, trimethoprim og sulfonamid, uagtet at der har været en stigning af forbruget til slagtekyllinger de seneste år. Der blev påvist fluorkinolon (ciprofloxacin) resistens i 12 % af isolaterne. Resistensforekomsten i *E. coli* fra slagtekyllinger og dansk kyllingekød var sammenlignelige. Resistens overfor 3. generations cefalosporiner (ceftiofur) blev fundet i et isolat fra danske slagtekyllinger, men ikke i kyllingekød. Resistensforekomsten i isolater fra kvæg og dansk oksekød var som det foregåede år ganske lav. Resistens i *E. coli* fra svin var den højeste blandt produktionsdyrene for ampicillin, sulfonamid og tetracyclin (33 - 37 %) og forblev på samme høje niveau som i 2012 og 2013.

Blandt isolaterne fra kød, var der generelt højere resistensforekomst blandt isolaterne fra importeret kyllingekød, også når gjaldt de kritisk vigtige antibiotika. Sammenlignet med dansk kyllingekød havde *E. coli* isolater fra importeret kyllingekød højere resistens overfor 6 af de 13 testede antibiotika. I *E. coli* isolater fra dansk svinekød var resistensforekomsten lavere overfor kinoloner (ciprofloxacin og nalidixin syre) end i isolater fra importeret svinekød.

ESBL-producerende bakterier er et af de hurtigst voksende resistensproblemer verden over. Flere nyere undersøgelser finder de samme ESBL gener, plasmider og kloner i *E. coli* isolater fra både dyr og mennesker, hvilket tyder på et zoonotisk link. Forekomsten af enterobakterier, der er resistente overfor car-

bapenemer, er også en voksende trussel, idet carbapenemer er blandt de få antibiotika, der kan behandle infektioner forårsaget af multiresistente Gram-negative bakterier i mennesker.

ESBL (ceftriaxon) resistens blev fundet i 9 % af *E. coli* isolater fra dansk kyllingekød og i 25 % af isolater fra importeret kyllingekød. Som i tidligere år er forekomsten generelt lav (< 1 %) i dansk og importeret okse- og svinekød.

Faldet i forekomsten af ESBL-producerende *E. coli* i dansk kyllingekød fortsatte i 2014. For første gang blev der også set et signifikant fald i importeret kyllingekød. Dette skyldes sandsynligvis et frivilligt stop af brug af 3. generations cefalosporiner i toppen af avlspyramiden i udlandet, hvilket resulterer i at der overføres færre ESBL-producerende *E. coli* fra importerede forældredyr til de danske slagtekyllinger. I lighed med 2012 og 2013 var der i 2014 stadig ingen fund af carbapenemase-producerende *E. coli*.

I 2014 var der et signifikant fald i forekomsten af ESBL-producerende *E. coli* i dansk kyllingekød, hvilket også ses i kyllingekød importeret fra udlandet (hovedsageligt fra EU lande) og sandsynligvis skyldes et frivilligt stop i brug af cefalosporiner i toppen af avlspyramiden i udlandet.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Rapporteringen af antibiotikaresistens i kliniske isolater fra mennesker er baseret på frivillig indrapportering af data fra de Klinisk Mikrobiologiske Afdelinger (KMA) i Danmark. Undtagelser omfatter methicillin-resistente *Staphylococcus aureus* (MRSA) og invasive *Streptococcus pneumoniae*, som er anmeldtepligtige. Data vedr. disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt ***Escherichia coli* isolater fra blod** var forekomsten af 3. generations cefalosporin resistens 7 % i 2014, hvilket har været stort set uændret siden 2010. I de andre nordiske lande var resistensen 5 - 6 % (EARS-Net 2013). Ciprofloxacin resistens var 12 %, det samme som i 2013. Den eneste signifikante stigning, der blev observeret, var for piperacillin/tazobactam, hvor resistensen steg fra 4 - 5 %. Mecillinam resistens var 8 %. I løbet af det seneste årti steg resistensen for gentamicin (fra 2 - 7 %), men steg ikke signifikant fra 2012 til 2014, mens resistens for cefalosporiner og ciprofloxacin steg frem til hhv. 2009 og 2011, men siden har været faldende. Resistens for meropenem er fortsat < 1 %.

Blandt ***E. coli* isolater fra urin** fra patienter på hospital var forekomsten af ciprofloxacin resistens 11 % i 2014 mod 12 % i 2013. I 2005 var 5 % resistente. Resistensen for aminoglykosid (gentamicin) og mecillinam forblev uændret (henholdsvis 5 % og 7 %).

Blandt ***E. coli* isolater fra urin** fra patienter i **almen praksis** var forekomsten af ciprofloxacin resistens i 2014 9 %.

Hos 2.283 hospitalspatienter blev der i 2014 påvist en 3. generations cefalosporin resistent *E. coli* i urinen. Dette svarer til 6 %. Resistens mod 3. generations cefalosporiner i urin-isolater har siden den første registrering i 2008 svinget mellem 5 og 6 %. Blandt *E. coli* urin-isolater fra patienter i praksis steg forekomsten af 3. generations cefalosporin resistens fra 3 % i de foregående fem år til 4 % i 2014.

I 2014 blev der iværksat en frivillig **overvågning af ESBL/AmpC producerende *E. coli*** fra blodinfektioner. I alt blev 261 *E. coli* isolater hel-genom sekventeret. I 245 isolater blev der fundet gener der kodede for ESBL produktion og/eller carbapenemase produktion. For 240 isolater var der oplysning om demografi; 53 % af patienterne var mænd og gennemsnitsalderen var 70 år. Syvogtyve (11 %) af patienterne døde indenfor 30 dage efter diagnosen.

Blandt de 245 isolater, blev der påvist 21 forskellige ESBL (inkluderende plasmid AmpC) gener og carbapenemaser. CTX-M-15 (49 %) var den hyppigste enzymtype, efterfulgt af CTX-M-14, CTX-M-27 og CTX-M-101. Forskellige varianter af CMY blev påvist i 7 % af isolaterne. Der blev påvist OXA-48 i et isolat og OXA-181 i to isolater.

De 245 isolater tilhørte 51 forskellige MLST typer, hvor ST131 var den mest dominerende type (51 %), (**Tekstboks 8.1**).

Blandt ***Klebsiella pneumoniae* isolater fra blod** var forekomsten af 3. generations cefalosporin resistens 8 % i 2014 mod 9 % i 2013 og aminoglykosid (gentamicin) 5 % i 2014 mod 4 % i 2013. Ciprofloxacin resistens faldt fra 9 % i 2013 til 7 % i 2014. Dette var på niveau med de andre nordiske lande (EARS-Net 2013).

Blandt ***K. pneumoniae* isolater fra urin** var forekomsten af 3. generations cefalosporin resistens 7 % i isolater fra hospitaler og 5 % i isolater fra praksis, hvilket er på samme niveau som i 2013. Ciprofloxacin resistens var uændret 8 % i urin-isolater fra hospitaler og 7 % i urin-isolater fra praksis. Mecillinam resistens var på 10 % i urin-isolater fra hospitaler og 9 % i urin-isolater fra praksis. Sulfonamid resistens var faldende på andet år i træk til hhv. 19 % i hospitals-isolater og 17 % i praksis-isolater. I 2009 var resistens mod sulfonamid på 27 % i urin-isolater fra hospitaler og 30 % i urin-isolater fra praksis.

Blandt ***Pseudomonas aeruginosa* isolater fra blod** var niveauet af resistens for alle testede antibiotika det samme som i 2013, med undtagelse af gentamicin som faldt fra 2013 til 2014 (fra 5 % til 2 %).

I 2014 blev der ikke fundet stigende forekomst af resistens blandt indberettede data fra de klinisk mikrobiologiske afdelinger. I 4.500 *E.coli* fra blodisolater var resistensen for 3.generations cefalosporiner 7 % og for ciprofloxacin 12 % . Begge var uændret fra 2013. For 943 *K.pneumoniae* var resistensen for 3.generations cefalosporiner 8 % (mod 9 % i 2013) og for ciprofloxacin 7 % (9 % i 2013). Generelt har resistensen været stigende i årene frem mod 2011, men har siden været let faldende eller stagneret. Sammenlignet med de andre nordiske lande har Danmark i flere år haft en lidt højere forekomst af cefalosporinresistens, men tallene for 2014 tyder på at Danmark nu nærmer sig sine nordiske naboer (EARS-Net fra 2013 viste 5-6 % cefalosporinresistens i *E.coli*).

CPO overvågning. I 2014 modtog Antibiotika Reference Laboratoriet 55 carbapenemase-producerende bakterier fra 48 patienter som en del af den frivillige overvågning (**Textbox 8.2**). Der indgik mere end et isolat per patient, hvis isolaterne tilhørte forskellige bakteriearter og/eller hvis isolaterne indeholdt forskellige carbapenemaser. Tretten af patienterne (28 %) døde indenfor 30 dage efter diagnosen. I mange tilfælde var kilden til de carbapenemase-producerende bakterier ukendt, eller de var relateret til spredning imellem patienter i Danmark. Dette er anderledes i forhold til de tidligere år, hvor de fleste tilfælde var relateret til rejser.

I 2014 blev der påvist 35 carbapenemase-producerende enterobakterier (CPE) fra 29 patienter sammenlignet med 19 i 2013 og 20 i perioden 2008-2012. NDM-1 *C. freundii* udbrudt, der startede i Nordjylland i 2012 fortsatte i 2014. Der var udbrud af NDM-5 producerende *K. pneumoniae* i Region Hovedstaden.

Der blev i 2014 påvist 12 carbapenemase-producerende *Acinetobacter sp.*, hvilket var på samme niveau som i 2013. Der blev påvist otte carbapenemase-producerende *P. aeruginosa*.

I 2014 var forekomsten af resistens for penicillin og erythromycin stadig lav blandt *Streptococcus pneumoniae* i isolater fra blod og spinalvæske (henholdsvis 5 % og 7 %). I gruppen af hæmolytiske streptokokker var resistensen for penicillin < 1 %, mens den varierede for erythromycin: 1 % i gruppe A, 4 % i gruppe C, 10 % i gruppe G og 22 % i gruppe B.

Forekomsten af ampicillin resistens i *Enterococcus faecium* isolater fra blod var 94 % i 2014. Vancomycin resistens var 4 % i *E. faecium* og < 1 % i *Enterococcus faecalis* blod-isolater. I 2014, sås en stigning i antallet af vancomycin resistente enterokokker der blev indsendt frivilligt til Antibiotika Reference Laboratoriet. I 2014 var der 297 *vanA E. faecium* fra kliniske prøver sammenlignet med 231 i 2013 (**Tekstboks 8.3**).

I 2014 var der en stor stigning i antallet af patienter, som fik infektioner med multiresistente bakterier som vancomycin resistente enterokokker (VRE) og carbapenemase-producerende enterokokker (CPE) på de danske hospitaler. I 2014 blev der rapporteret 303 tilfælde hvor VRE var påvist, hvilket er en stigning på 30 % i forhold til 2013 (235). Antallet af blodforgiftninger med VRE steg i 2014 til 33 sammenlignet med 23 i 2013. I 2014 blev der fundet 35 prøver med carbapenemase-producerende enterobakterier (CPE bakterier) hos 29 patienter.

Ciprofloxacin resistens i *Neisseria gonorrhoeae* faldt i 2014 til 46 % (55,8 % i 2013). I perioden fra 2005 til 2009 var resistensen øgedes støt fra 30 % til 75 %. Penicillinase produktion blandt Gonococci isolater fluktuerede mellem 24 % i 2005 og 11 % i 2014. Som i de foregående år blev der ikke rapporteret om ceftriaxon resistente isolater eller tilfælde med behandlingssvigt overfor ceftriaxon. Azithromycin og cefixime resistens lå på henholdsvis 36% og 6%. (**Textbox 8.4**).

Der blev i 2014 indberettet 1.964 tilfælde af *Staphylococcus aureus* bakteræmi, svarende til en incidens på 34,9 tilfælde per 100.000 indbyggere. Antallet af methicillin-resistente *S. aureus* (MRSA) var 57 (2,9 %) sammenlignet med 30 i 2013. Trods den betydelige stigning er antallet af MRSA bakteræmier stadig meget lavt sammenlignet med de andre europæiske lande. De højeste niveauer af resistens ud over penicillin var fusidinsyre (15 %), erythromycin (8 %), clindamycin (8 %) og norfloxacin (6 %). Prævalensen af resistens over for de enkelte antibiotika var på samme niveau som i 2013.

Antallet af nye MRSA tilfælde steg i 2014 til 2.965 (infektioner og bærertilstand), til sammenligning var antallet i 2013 på 2.092. Stigningen sås primært blandt husdyrs associeret MRSA LA-MRSA (først og fremmest CC398), hvor der i 2014 var 1.277 tilfælde, sammenlignet med 643 i 2013. Det svarede til en samlet andel på 43 % af alle tilfælde. Størstedelen (89 %) af personerne, der var inficeret med eller bærer af CC398, havde tæt kontakt til husdyr (70 %), eller boede i samme husstand med personer, der havde kontakt til husdyr (17 %). Der var fortsat ingen tegn på væsentlig spredning til byområder, hvilket indikerer, at fødevarer ikke har en nævneværdig betydning for spredningen.

Andelen af tilfælde hvor der var infektion var lavere i 2014 end i 2013 (henholdsvis 38 % og 45 %). Det skyldes den store stigning og andel af CC398, der overvejende findes ved screening. Antallet af hospitalserhvervede tilfælde var fortsat lavt og udgjorde kun 3 % af samtlige antal MRSA tilfælde i 2014.

Den mest almindelige spa type relateret til CC398 var type t034 (n = 185), hvoraf 75 af t034- tilfældene havde en egentlig MRSA infektion. MRSA isolater med den nye *mecA* homolog *mecC* blev fundet i 24 tilfælde.

Der blev i 2014 foretaget en undersøgelse blandt 70 avlsbesætninger og 205 tilfældigt udvalgte produktionsbesætninger for at estimere prævalensen af LA-MRSA. Der blev konstateret MRSA i 63 % af avlsbesætningerne og 68 % af produktionsbesætningerne. Dette er en kraftig stigning i forhold til foregående undersøgelser, hvor 16 % af besætningerne blev fundet positive (DANMAP 2010 og DANMAP 2011, Tekstboks 8.5).

2.2 Summary

DANMAP (Danish Antimicrobial Resistance Monitoring and Research Program) has monitored antimicrobial resistance and consumption of antimicrobial agents in food animals and in humans in Denmark since 1995. This report describes trends and changes in 2014.

Antimicrobial consumption in animals

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

The total consumption of antimicrobial agents in 2014 amounted to 114 tonnes of active compound, a decrease of 2% compared with 2013. Pigs accounted for 76%, cattle for 11%, fur animals for 4%, aquaculture for 4%, and poultry for 1% of the total veterinary consumption of antimicrobials measured in kg active compound. The remaining 4% was used in pets, horses and others.

The overall changes in veterinary consumption were generally driven by changes in consumption in the pig production. Pigs account for approximately 86% of the meat production in Denmark, but only for about 43% of the total live biomass.

Pigs. The total consumption of veterinary antimicrobial agents in the Danish pig production was approximately 86 tonnes. Measured in DAPD, this represented a 5% decrease from 2013 to 2014 (adjusted for export of live animals). DAPD is a measure that estimates the proportions of animals (treated daily) with a standard dose of an antimicrobial agent. The decrease was attributed mainly to a decrease in tetracyclines and to a lesser extent, pleuromutilins and macrolides. Pleuromutilins and tetracyclines are mainly used in feed or water medication for gastrointestinal disease. The use of 3rd and 4th generation cephalosporins in pigs was 4 kg in 2014 (3 kg in 2013), and thus remained at a low level as a result of a voluntary ban on cephalosporins introduced by the Danish pig industry in 2010. Legal restrictions on the use of fluoroquinolones were enforced in 2003. Since the clinical trials, for which fluoroquinolones have largely been used for over the past years, were discontinued in 2014 the consumption of fluoroquinolones was reduced to 4 kg in 2014.

Cattle. Following an overall 14% decrease in consumption from 2010 to 2013, there was an 8% increase from 12 to 13 tonnes in 2014. Measured in standard doses (DADD), the use of drying-off treatment increased by 12%, whereas the number of DADDs for treatment of clinical mastitis decreased by 2% compared with 2013. The beta-lactamase sensitive penicillins accounted for the majority of the consumption. For critically important antimicrobials, the use of fluoroquinolones has been close to zero since 2003. The use of 3rd and 4th generation cephalosporins for systemic treatment decreased by 27% compared with 2013.

Poultry. In 2014, the overall consumption of antimicrobial agents in poultry was approximately 1,548 kg active compound, which represents a 22% increase compared with 2013. The increase was mainly driven by an increase in the use of tetracyclines and to a lesser extent broad spectrum penicillins. There seem to be several reasons for this continued increase in consumption for poultry; increased occurrence of bacterial arthritis and diarrhoea in the broiler production along with widespread problems with respiratory disease in turkey flocks produced in early 2014. The reported use of fluoroquinolones in poultry has been low since 2006, and they were not used in the poultry production in 2014. Furthermore, use of cephalosporins has not been reported in Danish poultry production for more than a decade.

Aquaculture. The antimicrobial consumption in aquaculture increased by 43% to 5,116 kg in 2014. This is the highest level of antimicrobial consumption in aquaculture in a decade. As in 2013, the relatively large increase in consumption in 2014 was caused by extraordinary high temperatures during the summer, leading to higher water temperatures and increased occurrence of bacteriological infections. The aquaculture industry continues to focus on developing improved vaccines and vaccination strategies to reduce the occurrence of diseases that require antimicrobial treatment.

Fur animals. As in 2013, the antimicrobial consumption for fur animals continued to decrease in 2014. The overall use was 4,202 kg active compound, which represented a 14% reduction compared with 2013. Use of fluoroquinolones and cephalosporins has been close to zero for several years. In recent years, the industry, farmers and veterinarians have had increased focus on the consumption of antimicrobial agents, quality of feed and animal welfare in the mink production, and this may explain the reduced consumption.

Pets and horses. The information available on antimicrobial consumption in pet animals and horses is less detailed than for production animals. The overall antimicrobial consumption for pets remained almost at the same level in 2014 as in 2013 (1,989 kg in 2013 to 2,017 kg in 2014). An increase was seen for several antimicrobial classes; beta-lactamase sensitive penicillins, sulphonamides, trimethoprim, as well as for tetracyclines and aminoglycosides. The consumption of antimicrobials critical for human treatment such as cephalosporins and fluoroquinolones decreased in both 2013 and 2014. This slight shift in consumption of the different antimicrobial classes may be an effect of the treatment guidelines published by the Danish Veterinary Association in 2012. Nonetheless, consumption of broad-spectrum antimicrobials in pet animals and the use of antimicrobial agents critical for treatment of human infections remains high compared with production animals and is a continued matter of concern.

In 2014 the overall antimicrobial consumption in animals decreased by 2%. In the pig production - the main driver of veterinary consumption - the total consumption decreased by 5%. Use of critically important antimicrobials in the pig production remained low. However, the use of critically important antimicrobials in pets remained relatively high compared with other species, but decreased in both 2013 and 2014.

Antimicrobial consumption in humans

In Denmark all antimicrobial agents are sold only by prescription and the sale is recorded through the Register of Medicinal Product Statistics at Statens Serum Institut. Detailed monitoring has been performed since 1994.

The DANMAP report covers consumption of all antimicrobials under the ATC code J01. This report also covers the consumption of the per oral and rectal products of metronidazole (P01AB01) and vancomycin (A07AA09) for both hospital and primary sector. Data have been updated 10 years back. Metronidazole is an important drug in the treatment of different anaerobic infections, and both metronidazole and vancomycin are first line treatment of infections with *Clostridium difficile* which has been an emerging problem in Danish hospitals since 2006.

Total consumption. In 2014 the total consumption of antimicrobials was 18.58 DDD per inhabitant per day (DID), which is a decrease of 3% or 0.5 DID compared to 2013 (19.10 DID). The decrease in consumption was primarily driven by a marked decrease (15%) in the consumption of tetracyclines in the primary sector (see below). The consumption in primary health care accounts for 90% of the total antimicrobial consumption and thus significant changes in prescription here will markedly affect the total consumption.

During the last decade, the total consumption increased by 11%; since 1997 the increase has been almost 40 % until 2011 (19.31 DID) where after it has stagnated.

The total consumption in 2014 corresponds to 52,819 kg active substance.

Primary health care. Primary health care accounted for 16.40 DID of the total consumption (16.95 DID in 2013). Penicillins were the most used group with a consumption of 10,57 DID accounting for 65% of the total consumption; of these beta-lactamase sensitive penicillins accounted for 27%, penicillins with extended spectrum for 22% and the beta-lactamase resistant penicillins and combination penicillins for each 8%. Macrolides and tetracyclines accounted for 11% and 10%, respectively.

The most marked changes were observed for tetracyclines, which decreased with 15% from 1.96 DID in 2013 to 1.66 DID in 2014. However, this is primarily due to marked changes in consumption of doxycycline, showing an unexplained increase from 2012 to 2013 but returning to the 2012 level in 2014.

For the penicillin group, beta-lactamase sensitive penicillins decreased from 4.65 DID to 4.38 DID (6%), whereas combination penicillins increased from 1.22 DID to 1.30 DID (7%) and beta-lactamase resistant penicillins from 1.30 DID to 1.36 DID (5%). The increased consumption of combination penicillins partly owed to changes in recommendations for the treatment of pneumonia in patients with chronic lung disease (COPD), but increased consumption was also observed for other age and patient populations. These changes will be investigated further in the future.

Fluoroquinolones (primarily ciprofloxacin) accounted for 3% of the consumption (0.50 DID). The consumption of ciprofloxacin increased from 2005 to 2011 (from 0.33 to 0.57 DID) and has since shown a decreasing trend.

No significant changes were shown for trimethoprim and sulfonamides or nitrofurantoin.

During the last decade the total consumption in the primary sector increased by almost 10%, from 14.96 DID in 2005 to 16.40 DID in 2014. The increase reflects an increase in the number of DDD per treated patient as well as an increased number of DDD per package. These might reflect changed recommendations in the treatment of patients with higher doses and shorter treatment periods.

Hospitals. At somatic hospitals, the consumption of antimicrobial agents increased when calculated in DDD per 100 bed days (DBD): from 99.88 DBD in 2013 to 104.30 DBD in 2014. During the last decade the consumption increased with 57% (from 66.32 DBD in 2005).

Penicillins accounted for 50% of the total consumption. The biggest group of these were the penicillins with extended spectrum (primarily ampicillin and mecillinam), constituting 17% of the total consumption at hospitals, which has not changed significantly for the last decade. Notably decreasing changes since 2005 were observed for the beta-lactamase sensitive penicillins: While a decade ago these constituted the second biggest group (with 12.17 DBD in 2005, corresponding to 20% of the total consumption), the consumption in 2014 had decreased to 10.31 DBD, corresponding to 11% of the total consumption at hospitals.

From 2013 to 2014 the consumption of the combination penicillins increased from 13.64 to 16.04 DBD (18%). The group of combination penicillins covers two drugs: amoxicillin with clavulanic acid and piperacillin with tazobactam. Consumption of both has increased following changed recommendations

on treatment of septic and severely ill patients, as well as the mentioned changes in recommendations for treatment of COPD patients suffering from pneumonia.

Penicillins with extended spectrum increased from 15.06 to 16.40 DBD (8%) and imidazole-derivatives from 4.08 to 4.48 DBD (9%), while decreases were shown for 2nd generation cephalosporins (cefuroxime) from 12.31 to 11.68 DBD (5%).

No marked changes were observed for the beta-lactamase sensitive penicillins (10.31 DBD), beta-lactamase resistant penicillins (9.57 DBD), carbapenems (4.09 DBD) and fluoroquinolones (9.88 DBD). The latter two remained unchanged despite recommendations on reduction of consumption

When presented in DID (for comparison with the consumption in the primary sector) the hospital sector accounted for 2.18 DID (2.15 in 2013). A continuing increase has been observed for this sector over the last decade, from 1.76 DID in 2005.

The steady increases in consumption at hospitals are primarily caused by an increase in DDDs and a decrease in the number of hospital bed-days.

From 2005 to 2014, the total consumption of antimicrobial agents by humans in Denmark increased by 11%, since 1997 the increase has been 40%.

In humans, the overall consumption of antimicrobial agents for systemic use in 2014 was 18.58 DID, which is a 3% decrease from 2013. Most of this decrease owes to notable changes in the consumption of tetracyclines in the primary sector, where especially consumption of doxycycline had shown an unexplained peak in consumption from 2012 to 2013 but in 2014 returned to a lower level. For the last decade most prominent changes in consumption in both primary sector and hospital sector have been observed for the different groups of penicillins. While the consumption of beta-lactamase sensitive penicillins continues to fall the consumption of combination penicillins increases. Combination penicillins now constitute the second biggest antimicrobial group at hospitals accounting for 15% and one of the biggest groups consumed in the primary sector accounting for 8%.

Resistance in zoonotic bacteria

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

Harmonised monitoring of antimicrobial resistance

In 2014, the panel of test antimicrobials was changed to accommodate the European Commission's decision on harmonising the monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. For *Salmonella*, *Campylobacter* as well as indicator *E. coli* and *Enterococcus*, several compounds were replaced in order to prioritise testing for resistance to antimicrobial agents most relevant from a public health perspective. For *Salmonella* and indicator *E. coli* isolates, screening for carbapenemase enzymes was initiated. As a consequence, trends in proportions of fully sensitive and multi-resistant isolates, as well as occurrence of selected resistance profiles, cannot be directly compared with results from previous years.

***Salmonella* Typhimurium** is one of the most common reservoirs in Danish pigs and pork as well as in human infections. Among *S. Typhimurium* from healthy pigs, 66 - 71% of the isolates were resistant to ampicillin, sulfonamide, and tetracycline; and the occurrences of resistance to these antimicrobial agents have increased over the last five years. This can mainly be attributed to an increasing prevalence of monophasic *S. Typhimurium* that has a strong tendency to be multi-resistant. In 2014, 56% of the *S. Typhimurium* isolates from pigs were of the monophasic S 4,[5],12:i; variants. High levels of resistance in *S. Typhimurium* were also found among isolates from Danish pork. In general, we found higher levels of multi-resistance among *S. Typhimurium* (including the monophasic variants) isolates from healthy Danish pigs (64%) than on average among all *Salmonella* serotypes isolated from pigs (32%) in 2014. *Salmonella* was recovered from 22% of the healthy pigs tested, and it was estimated that overall 7% of Danish pigs were positive with multi-resistant *Salmonella*. As in previous years, resistance to cephalosporins (cefotaxime, ceftazidime or ceftiofur) or quinolones (ciprofloxacin or nalidixic acid) was not detected among *Salmonella* from Danish pigs or pork.

As in isolates from pigs and pork, the occurrence of monophasic variants of *S. Typhimurium* in humans increased in prevalence among both domestic sporadic cases and outbreaks over the last five years. In 2014, multi-resistant isolates were recovered from 48% of all cases, and generally the levels of resistance were comparable to 2013. The tendency seen in previous years of higher levels of resistance among travel-associated isolates compared to domestic infections is not distinct in 2014; only resistance to ciprofloxacin and nalidixic acid resistance were higher in isolates from travel-related cases compared with isolates from domestic cases.

Resistance to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) was found in seven *S. Typhimurium* isolates (1.6% of cases); however these seven isolates were also resistant to ciprofloxacin. Co-resistance to ciprofloxacin and 3rd generation cephalosporins are of major concern as these two antimicrobial agents are considered the most important for treatment of severe salmonellosis.

None of the susceptibility tested *Salmonella* isolates from animals, meat or human cases displayed carbapenemase activity (meropenem resistance).

The occurrence of monophasic *Salmonella* Typhimurium, which are often multi-resistant, has increased in pigs, pork and among isolates from human infections over the past five years. Among isolates from human infections acquired in Denmark, resistance to ampicillin, sulfonamide and tetracycline have stabilised at a fairly high level in the past four years (50 - 60%). In 2014 only resistance to quinolone were higher among isolates from the travel related human cases.

In 2014, resistance in ***Campylobacter jejuni*** isolates from Danish cattle, broilers and Danish broiler meat were generally similar to the levels observed over the last five years. Low to moderate levels of resistance were observed, and the majority (74 - 81%) of isolates were fully sensitive to the six antibiotic agents included in the test panel. In a European context, Denmark reports relatively low levels of antimicrobial resistance among *C. jejuni* from cattle, broilers and broiler meat.

For several years, the level of fluoroquinolone resistance in *C. jejuni* has been higher among isolates from imported broiler meat (82%), compared with isolates from Danish broiler meat (15%); a difference that has been increasingly pronounced.

As observed in previous years, the *C. jejuni* isolates from the travel-associated human cases continued to have a significantly higher level of fluoroquinolone resistance (81%) compared with domestic cases (35%).

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

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 in meat.

In *Enterococcus faecalis* from broilers and Danish broiler meat, resistance to tetracycline (49% and 35%, respectively) and erythromycin (27% and 19%, respectively) was moderate to high, but low or absent for the other compounds in the test panel. Resistance to tetracycline in *E. faecalis* from broilers has been increasing over the last five years. In *E. faecium* from imported broiler meat resistance was generally higher compared with broiler meat produced in Denmark.

Higher levels of antimicrobial resistance were observed among *E. faecalis* isolated from pigs compared to poultry, which reflects the differences in usage pattern of antimicrobials in these animal species. Among *E. faecalis* from pigs, the highest occurrence of resistance was to tetracycline (83%) and erythromycin (49%). Tetracycline has been the most widely used antimicrobial agent in the Danish pig production for more than a decade and has primarily been used for treatment of *E. coli* infections in piglets. Tetracycline resistance in *E. faecalis* was higher in imported pork when compared with domestic-produced pork.

Generally lower levels of resistance are observed in *E. faecium* compared with *E. faecalis*, and resistance to antimicrobial agents of critical importance for human treatment was low.

Among Indicator *Escherichia coli* from broilers, resistance to tetracycline, ampicillin, trimethoprim and sulfonamide decreased compared with 2012 and 2013, despite an increased consumption of these compounds in the broiler production in 2014. Resistance to fluoroquinolones was observed in 12% of the isolates from broilers. Resistance in *E. coli* from Danish broiler meat was similar to the findings in broilers, and resistance to ceftiofur (3rd generation cephalosporin) was observed in one *E. coli* isolate from Danish broiler meat; but not among isolates from broilers. The highest occurrence of resistance, including resistance to critically important antimicrobial agents, was found in imported broiler meat. Compared with Danish broiler meat, resistance was higher for 6 of 13 tested antimicrobial agents from imported broiler meat.

As in previous years, the highest levels of resistance for ampicillin, sulphonamides, trimethoprim and tetracyclines among *E. coli* from production animals were observed in pigs (33 - 37%), whereas resistance in isolates from cattle was generally low - at levels comparable to 2013. Also resistance levels in Danish and imported pork and beef were comparable in 2014.

ESBL-producing bacteria is one of the fastest emerging resistance problems worldwide. Lately, several studies have found the same ESBL genes, plasmids and clones in *E. coli*

isolates originating from animals and isolates involved in human infections, suggesting a zoonotic link. The occurrence of Enterobacteriaceae resistant to carbapenems is a growing threat in human medicine because carbapenems are the last resort for treatment of infections caused by multidrug resistant Gram-negative bacteria in humans.

ESBL (ceftriaxone) resistant *E. coli* was found in 9% of Danish broiler meat and 25% of imported broiler meat samples. As in previous years, the occurrences were generally low (< 1%) in the Danish and imported beef and pork. The decreasing occurrence of ESBL-producing *E. coli* in Danish broiler meat continued in 2014. For the first time a significant reduction was also observed in imported broiler meat. This is most likely due to a voluntary stop in the usage of 3rd generation cephalosporins in the top of the breeding pyramid abroad resulting in a reduced transmission of ESBL-producing *E. coli* from imported parent animals to the Danish broilers. As in 2012 and 2013 no carbapenemase producing *E. coli* were found.

We observed a significant decrease in the occurrence of ESBL-producing *E. coli* in Danish broiler meat as well as in broiler meat produced abroad (mainly in EU countries). This is most likely due to a voluntary stop in usage of cephalosporins at the top of the breeding pyramid abroad.

Resistance in human clinical bacteria

The Departments of Clinical Microbiology (DCM) in Denmark reported data on antimicrobial resistance in human clinical blood and urine isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Enterococcus faecium*. For methicillin-resistant *Staphylococcus aureus* (MRSA) and invasive *Streptococcus pneumoniae* data were obtained from the reference laboratories at SSI where these bacteria are received for surveillance.

Data on the antimicrobial susceptibility of approximately 4,500 ***Escherichia coli* blood isolates** were referred. The resistance to ampicillin was 45%, ciprofloxacin 12%, mecillinam 8%, gentamicin 7% and 3rd generation cephalosporins 7%. There were no significant changes compared to 2013 except for piperacillin/tazobactam, which increased from 4 - 5%. Compared with EARS-Net data from 2013 the resistance in Danish isolates was slightly higher than in the other Scandinavian countries, but lower than in most other European countries.

In ***E. coli* urine isolates from hospital patients**, ampicillin resistance was 42%, sulfonamide resistance was 33% and aminoglycoside (gentamicin) resistance was 5%, which are at the same levels as in the previous three years. Resistance to 3rd generation cephalosporins was 6% - the same level as in

2013. The occurrence of ciprofloxacin resistance (11%) was slightly lower than in 2013 (12%), but a steady increase has been seen in ciprofloxacin resistance from 3% in 2005.

In ***E. coli* urine isolates from primary health care** the occurrence of ampicillin resistance was 39% and sulfonamide resistance was 32%. No significant changes in resistance since 2013 were observed but both decreased since 2009 (from 42% and 38% respectively). For 3rd generation cephalosporins, resistance was 4%, unchanged since 2012. Ciprofloxacin resistance was 9%. Resistance has increased steadily from the beginning of the century (1% in 2000) and reached its highest peak in 2011 with 11%.

Data on 943 ***Klebsiella pneumoniae* blood isolates** were received. Resistance to ciprofloxacin was 7%, mecillinam 8%, piperacillin/tazobactam 8%, 3rd generation cephalosporins 8% and gentamicin 5%. There were no significant changes compared with 2013.

In ***K. pneumoniae* urine isolates from hospital patients** resistance to mecillinam (10%), sulfonamide (19%), gentamicin (4%), 3rd generation cephalosporins (7%) and ciprofloxacin (8%) all remained at the same level as in 2013. For all of these drugs, marked decreases have been observed since 2009 (decreasing from 14%, 33%, 7%, 13% and 17%, respectively).

In ***K. pneumoniae* urine isolates from primary health care**, resistance to mecillinam (9%), 3rd generation cephalosporins (5%) and ciprofloxacin (7%) remained at the same level as in 2013. Sulfonamide resistance decreased from 22% in 2013 to 17% in 2014.

In 2014, the first data from the Surveillance of **ESBL/AmpC producing *E. coli* from bloodstream infections** were reported in the DANMAP report. In total, 261 *E. coli* isolates were whole-genome sequenced. In 245 isolates genes encoding ESBL/AmpC or carbapemase production were detected. For 240 isolates demographic were available; 53% of the patients were men and the mean age was 70 years. Twenty-seven (11%) of the patients died within 30 days after the diagnosis.

Among the 245 isolates, 21 genes encoding ESBL/AmpC or Carbapemase production were detected. CTX-M-15 was most common (49%), followed by CTX-M-14, CTX-M-27 and CTX-M-101. Different variants of CMY were detected in 7% of the isolates. OXA-48 was detected in one isolate and OXA-181 in two other isolates. The 245 isolates belonged to 51 different MLST types, ST131 was most common (51%).

In ***Pseudomonas aeruginosa* isolates from blood**, resistance to all tested antimicrobial agents was at the same level as in 2013, except for gentamicin, which decreased from 2013 to 2014.

For 2014 no increases in resistance were observed for data on blood isolates received from the Departments of Clinical Microbiology. In 4,500 *E. coli* resistance to 3rd generation cephalosporins was 7% and for ciprofloxacin 12%. Both were unchanged from 2013. In 943 *K. pneumoniae* resistance to 3rd generation cephalosporins was 8% and to ciprofloxacin 7% (compared to 9% for both in 2013). In general resistance in both bacteria has been increasing during the last decade but since 2011 stagnation or even decreases were observed. Compared to the other Nordic countries Denmark has for some years shown higher resistance towards cephalosporins but the results from 2014 point towards a positive change (EARS-Net 2013 showed 5 - 6% resistance to cephalosporins in *E. coli* for the other Nordic countries).

In 2014, 35 **carbapenemase producing enterobacteria (CPE)** were detected from 29 patients, whereas 19 CPE were detected in 2013 and 20 in the period 2008-2012. The NDM-1 producing *C. freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2013 and 2014. During 2014, NDM-5 producing *K. pneumoniae* were detected from three patients at a hospital in the Capital Region of Denmark.

In 2014, 12 **carbapenemase producing *Acinetobacter* spp** were detected, which was at the same level as in 2013. In 2014, 8 **carbapenemase producing *P. aeruginosa*** were detected.

In 2014, resistance to ampicillin was 93 % in ***Enterococcus faecium* isolates from blood**. Vancomycin resistance was 4% in *E. faecium* and 0.2% in ***Enterococcus faecalis* isolates from blood**. During 2013 and 2014, an increasing number of **vancomycin resistant enterococci** were referred to SSI. In 2014, 297 *vanA E. faecium* from clinical samples were obtained compared with 231 in 2013 (Textbox 8.3).

In 2014, there was a large increase in the number of patients with infections caused by vancomycin resistant Enterococci (VRE) and carbapenemase-producing Enterococci (CPE) in Danish hospitals. In 2014 303 clinical isolates with VRE (vancomycin resistant enterococci) were referred to the reference laboratory at Statens Serum Institut, 297 of these were VanA-positive *E. faecium*. This is an increase of 30% from 2013 (235 isolates). VRE was found in 33 bacteremias (compared to 23 in 2013). In 2014, carbapenemase-producing Enterococci were found on 35 samples from 29 patients.

The number of new cases of MRSA (both infected and colonized persons) increased in 2014 to 2,965 compared with 2,092 in 2013. The increase was primarily seen in livestock associated MRSA, belonging to clonal complex 398 (CC398), with 1,277 cases in 2014 compared with 643 cases in 2013. In 2014, CC398 constituted 43% of all new MRSA cases in Denmark. The majority (89%) of persons infected or colonized with CC398 had close contact to pigs or were household members to persons, who had pig contact. There were, however, no signs of significant spread of CC398 to urban areas, which indicates that food does not constitute an important transmission route.

Resistance to penicillin and erythromycin in *Streptococcus pneumoniae* and in group A, B, C and G streptococci remained low in 2014.

Ciprofloxacin resistance in *Neisseria gonorrhoeae* increased steadily from 30% in 2005 to 75% in 2009, followed by a decrease to 46% in 2014. Penicillinase production among gonococcus isolates fluctuated between 24% in 2005 and 11% in 2014. As in previous years no ceftriaxone resistant isolates or cases of ceftriaxone treatment failure were reported. Azithromycin and cefixime resistance was 36% and 6%, respectively (Textbox 8.4).

In 2014, 1,964 cases of *Staphylococcus aureus* bacteraemia were reported, corresponding to 34.9 cases per 100,000 inhabitants. The number of methicillin-resistant *S. aureus* (MRSA) from bacteraemia was 57 (2.9%). This is a steep increase compared with the levels in previous years but still among the lowest incidences recorded in Europe.

The highest frequency of resistance in addition to penicillin was observed for fusidic acid (15%), erythromycin (8%), clindamycin (8%) and norfloxacin (6%). Susceptibility to the tested antimicrobial agents was at the same level as in 2013.

Among all MRSA cases, the proportion of cases presenting infection was lower in 2014 compared with 2013 (38% and 45%, respectively). The number of hospital-acquired (HA) cases continued to be low and constituted only 3% of the total number of MRSA cases in 2014.

A national survey was carried out in 2014 to estimate the prevalence of LA-MRSA in 70 breeding herds and 205 randomly selected slaughter pig herds. The prevalence of MRSA was 63% in breeding herds and 68% in slaughter pig herds, which is a marked increase compared to previous surveys, where only 16% of the herds were found positive for MRSA, (Textbox 8.5)



3

BACKGROUND INFORMATION

3. Background information

The following sections present general information about the human population in Denmark in 2014, as well as the production of food animals and the amount of meat available for human consumption in Denmark over the past decade. It also provides an overview of the antimicrobial agents for systemic and intramammary therapeutic use in humans and animals in 2014.

3.1 Populations

The distribution of the Danish human population, which could potentially have received antimicrobial treatment in 2014, is displayed in Figure 3.1, together with the five healthcare regions and the 11 Departments of Clinical Microbiology (DCM) in Denmark.

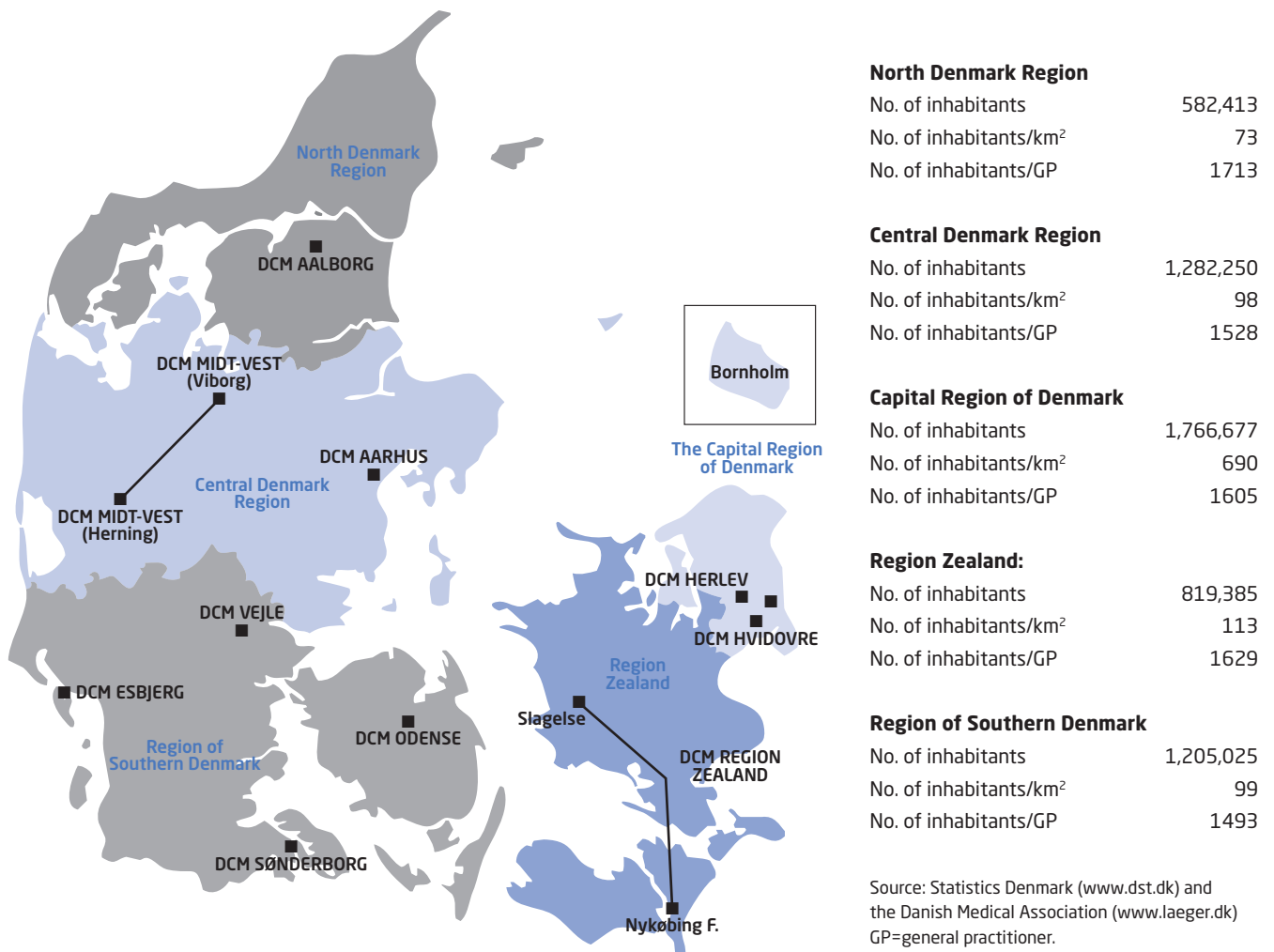
The production of food animals and the production of meat and milk are presented in Table 3.1.

While the number of pigs produced was approximately 3% higher than in 2013, the number of fattening pigs (15-50 kg) exported increased by 13%, and the export has increased by more than six-fold since 2005. As in the previous years, the amount of milk produced increased (2%).

The number of broilers produced decreased by approximately 2% from 2013 to 2014, and approximately 11% of the broilers produced in 2014 were exported for slaughter. The annual production of turkeys has fluctuated considerably over the last decade, and decreased with 14% from 2013 to 2014. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus almost all turkey meat available in Denmark is listed as imported.

Figure 3.1 The five health care regions and 11 Departments of Clinical Microbiology (DCM) in Denmark

DANMAP 2014



3.2 Marketed antimicrobial agents

Table 3.2 shows the antimicrobial agents that are registered to treat bacterial infections in humans and animals. Some of these are considered to be critically important for treating bacterial infections in humans. An antimicrobial agent is considered critically important if it is the only compound, or one of a limited number compounds, available to treat serious human disease. Fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides and glycopeptides are among these critically important antimicrobial agents [AGISAR, 3.revision, WHO 2011]. Critically important antimicrobial agents are also used

to treat diseases in food animals and pets, and bacteria that are resistant to critically important agents may be transmitted to humans. Also, bacteria that cause human disease may acquire resistance genes from bacteria of animal origin.

Growth promoters, which are no longer used for animals in Denmark, are shown in parentheses in Table 3.2. Most of these had effects on Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance to growth promoters.

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

DANMAP 2014

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish ^(a)	
	1,000 heads	mill. kg ^(b)	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	Export 1,000 heads ^(c)	mill. kg	Fresh water	Marine
1990	94560	116	571	2.5	789	219	753	4542	16425	-	1260	-	-
1992	107188	137	761	5.4	862	236	712	4405	18442	-	1442	35	7
1994	116036	152	1091	8.6	813	210	700	4442	20651	-	1604	35	7
1996	107895	149	961	9.3	789	198	701	4494	20424	-	1592	32	8
1998	126063	168	1124	11.6	732	179	669	4468	22738	-	1770	32	7
2000	133987	181	1042	10.3	691	171	636	4520	22414	-	1748	32	7
2001	136603	192	1086	13.2	653	169	623	4418	23199	-	1836	31	8
2002	136350	190	1073	12.8	668	169	611	4455	24203	-	1892	32	8
2003	129861	197	777	11.2	625	161	596	4540	24434	-	1898	34	8
2004	130674	198	1086	19.6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	183	1237	17.4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	161	785	11.3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	163	1009	14.4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	163	1068	12.3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	165	1175	11.1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	178	1184	14	519	142	574	4830	28505	7074	1974	28	10
2011	115454	175	960	9.4	551	145	575	4801	29399	7632	2008	28	11
2012	111080	168	1103	12.4	539	138	580	4928	29047	8794	1902	28	13
2013	117315	177	692	8.3	551	140	574	5025	28996	9318	1896	30	13
2014	115497	174	595	8.8	556	143	563	5113	29926	10517	1924	28	12
Increase(d)	-2%	-2%	-14%	1%	1%	2%	-2%	2%	3%	13%	1%	-5%	-9%

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Live animals exported prior to slaughter are included in number of animals and amount of meat produced. Export data for 15-50 kg live pigs from the Danish Agriculture and Food Council.

a) The numbers for 2014 are not final. The production of farmed fish includes fish transferred from one production facility to another.

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

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

d) Increase from 2013 to 2014.

Table 3.2. Antimicrobial agents marketed for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2014

DANMAP 2014

ATC / ATCvet codes ^(a)	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, oxytetracycline, tetracycline, tigecycline
J01BA / QJ01BA	Amphenicols	Florfenicol	Chloramphenicol
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/clavulanic acid, 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, doripenem
J01DI	Fifth-generation cephalosporins		Ceftaroline
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb. of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxazol/trimethoprim	
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
J01FG / QJ01XX ^(b)	Streptogramins	(Virginiamycin)	
J01G / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin, amikacin, netilmicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ ^(b)	Quinoxalines	(Carbadox, olaquinox)	
J01XA,A07AA / Not in ATCvet ^(b,c)	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
J01XB / QA07AA ^(b)	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB ^(c)	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, tedizolide
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet ^(b)	Oligosaccharides	(Avilamycin)	
Not in ATCvet ^(b)	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes starts with a Q

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

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



4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

4.1 Introduction

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

Overall, the antimicrobial consumption for both humans and animals has increased since the late 1990s. While the consumption for humans has gradually increased throughout the period, the consumption in animals has fluctuated notably. Increases in veterinary consumption can partly be explained by the increase in pork production, which constitutes approximately 86% of the meat production in Denmark (Table 3.1). Figure 4.1 shows the total antimicrobial consumption in animals and humans since 1994 and 1997, respectively.

The prescription pattern has been clearly influenced by implemented legislation. For example, the decrease in antimicrobial consumption after 1994 was likely the result of the following important actions: 1) limitation of veterinarians profit from sales of medicine 2) implementation of preventive veterinary strategies with Veterinary Advisory Service contracts (Textbox 4.1) and regular monthly visit from the veterinarian in order to promote preventive veterinary strategies and optimize antimicrobial use, and 3) enforcement of the so called "cascade rule" [Order (DK) 142/1993], which limits the use of (cheaper) extemporaneously produced medicines - this particularly affected the use of tetracyclines from 1994. Another important intervention was the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003. Furthermore, in July 2010, the pig industry imposed a voluntary ban on the use of cephalosporins, concerns regarding the emergence of extended beta-lactamase resistance (ESBL) in Gram-negative bacteria, followed by a similar initiative by dairy cattle farmers in July 2014.

From 2010 to 2011, consumption decreased following the introduction of threshold values for antimicrobial consumption adopted within the "yellow card initiative". This enforces legal actions on pig farmers with high antimicrobial agent use per pig [DANMAP 2010]. Effects from other parts of the legislation may be less obvious, but are important to keep in mind, when interpreting the veterinary prescription patterns.

Official guidelines for veterinarians, regarding the selection of antimicrobial agents for pigs and cattle, have been available

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

4.1.1 Data sources

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

Consumption data for 2014 - for use in DANMAP - were extracted from VetStat by the Danish Veterinary and Food Administration (DVFA) in May 2015. National Food Institute DTU has carried out no further validation of the received data.

4.1.2 Methods

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

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

In DANMAP 2012, we introduced two new metrics to follow trends in antimicrobial consumption to ensure the robustness of the analyses over time and to facilitate comparisons between animal species, as well as comparisons between the veterinary and human sectors. The new metrics are defined

below, and for more information on methodology, please refer to Chapter 9 and the web annex [www.Danmap.org].

DADD (Defined animal daily dose)

DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species.

The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species; and when appropriate, also age group. DADD has been specifically defined for use in DANMAP and does not always completely match the prescribed daily dose or the recommended dosage in the Summaries of Product Characteristics (SPC) or in the VetStat database.

Since 2012, the DADD has replaced the ADD (as defined in VetStat), which had been used since DANMAP 2003. For more details, see Chapter 9, Materials and Methods. The DADDs used in DANMAP 2014 are presented in the web annex.

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

Trends in veterinary consumption, both within and across species, are presented in DAPD, allowing for comparison between sectors and adjusting for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight on a given day. This may also be referred to as the ‘standard-animals-at-risk’

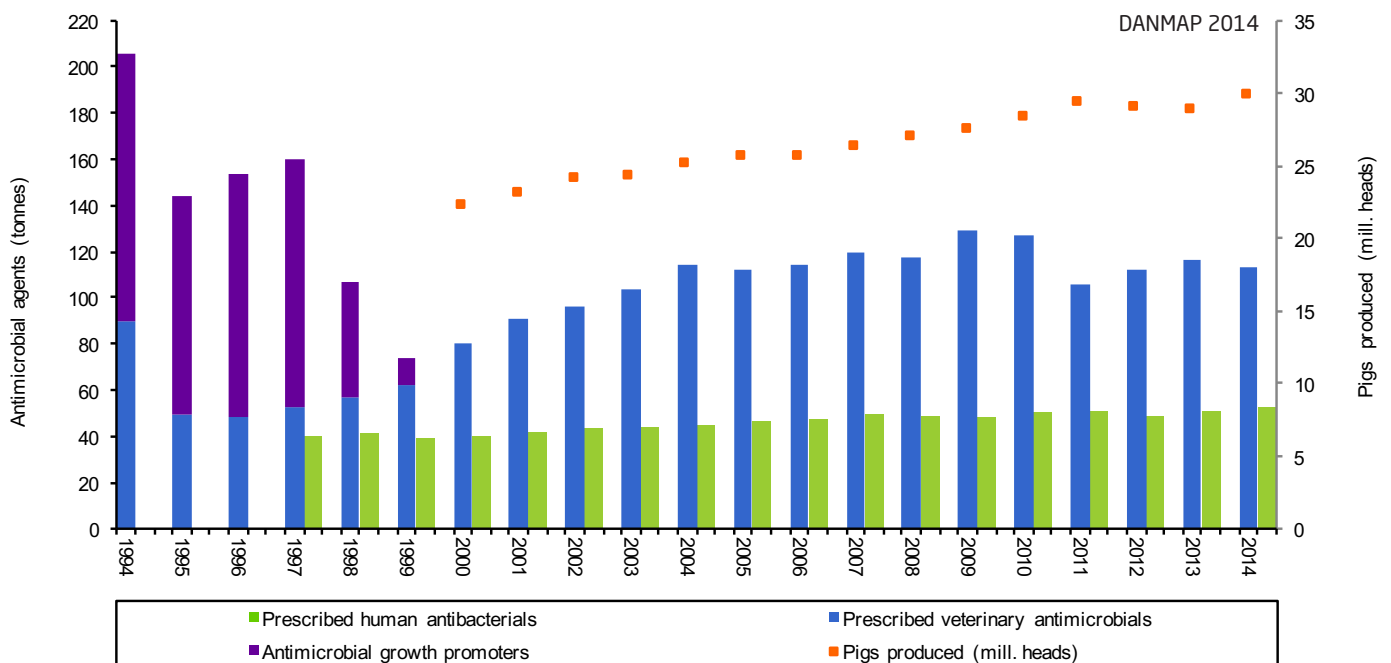
and takes into account species differences in body-mass and life-span.

DAPD or estimated treatment proportion is a statistical measure, providing a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the pig population, on average, receives a certain treatment on a given day (Section 4.3 and Chapter 9, Materials and Methods). Furthermore, presenting the veterinary consumption in DAPD allows comparisons with the antimicrobial consumption in the human sector as expressed in defined daily dose per 1,000 inhabitants per day (DID), see Chapter 10, Terminology, for a description of DID.

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

In the context of DANMAP, we base our comparison on dosages in order to keep in focus the newer, potent antimicrobials

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



Sources: Human therapeutics: The Danish Medicines Agency. Veterinary consumption: Until 2001, data are based on reports from the pharmaceutical industry of total annual sales from the Federation of Danish pig producers and slaughterhouses (1994-1995) and Danish Medicines Agency and Danish Plant Directorate (1996-2000). Data from 2001-2014 originate from VetStat.

such as fluoroquinolones and cephalosporins that are critically important in the treatment of human infections. Furthermore, the biomass of the live population is used as denominator to allow for comparisons of selection pressure between animal populations.

4.2 Total antimicrobial consumption

In 2014, the total veterinary consumption of antimicrobial agents, including agents used for companion animals, amounted to 114 tonnes active compound (Table 4.1), representing a 2% decrease compared with 2013.

The decrease was mainly attributed to a 5% decrease in the

amount used in pigs. The two major species, cattle and pigs, comprise equal proportions of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have a very low consumption of antimicrobial agents compared with growing animals. In 2014, the antimicrobial consumption in pigs, cattle and poultry comprised 76%, ~11%, and ~1% of the total veterinary consumption, respectively (Figure 4.2).

Historically, the overall consumption - measured as kg active compound - was 44% lower in 2014 compared with 1994 - while the total meat production increased by 15% during this period (Table 3.1 and Figure 4.1). A major part of the decrease in consumption can be explained by the discontinued use of growth promoters (1994-1999).

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

DANMAP 2014

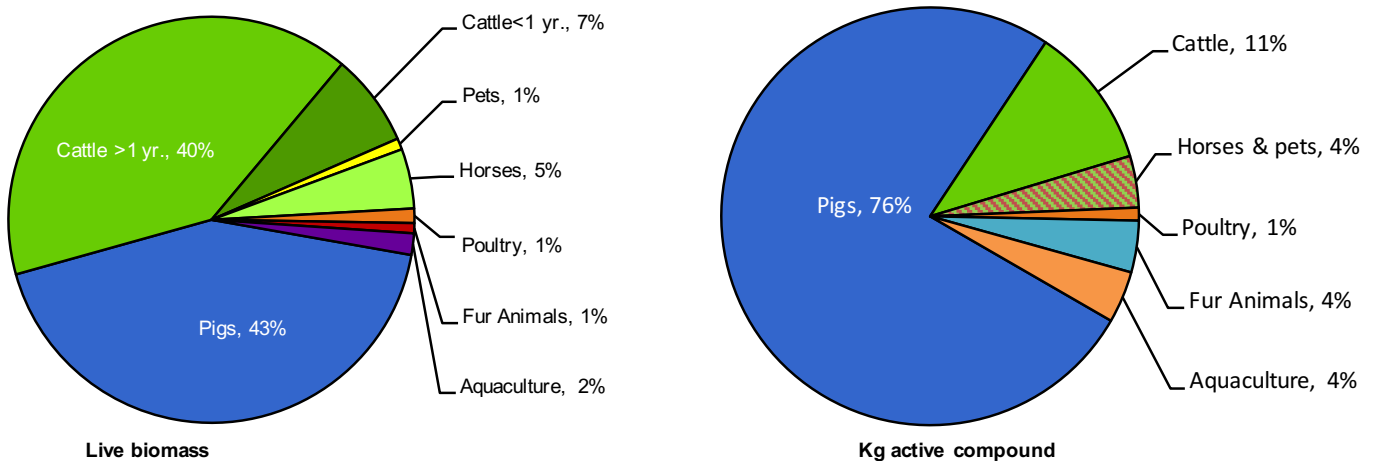
ATCvet code	QJ01B	QJ01G	QJ01D	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XQ	QJ01CE	QJ01CA	QJ01E	QJ01AA		
Therapeutic group	Amphenicols	Aminoglycosides	Cephalosporins	Fluoroquinolones	Other quinolones	Lincosamides	Macrolides	Pleuromutlins	Penicillins, beta-lactamase sensitive	Penicillins, others(a)	Sulfonamides and trimethoprim	Tetracyclines	Others	Total
<i>Pigs, total</i>	244	4,494	4	4	0	2,067	11,049	8,120	16,675	7,784	8,074	26,850	655	86,020
Sows and piglets	206	1,826	2	4	0	480	652	602	8,883	3,529	5,631	2,239	86	24,140
Weaners	32	2,446	2	0	0	729	6,899	3,123	1,735	3,250	2,149	15,207	559	36,131
Finishers	7	222	0	0	0	858	3,499	4,395	6,056	1,005	294	9,403	10	25,749
<i>Cattle, total</i>	539	492	101	0	0	6	193	0	7,979	948	1,048	1,720	22	13,048
Intramammaries	-	25	72	-	-	2	-	-	226	164	6	-	2	497
Cows and bulls	11	259	27	0	0	2	129	0	7,306	581	888	1,174	1	10,377
Calves < 12 mdr	525	191	2	0	0	2	62	0	363	195	143	506	19	2,008
Heifers and steers	3	18	0	0	0	0	3	0	84	8	10	40	0	166
<i>Poultry, total</i>	9	21	0	0	0	10	359	0	120	326	83	617	2	1,548
Poultry incl. broilers, layers and other poultry	2	3	0	0	0	1	137	0	81	187	83	184	2	679
Turkeys	7	18	0	0	0	9	222	0	39	139	0	434	1	869
<i>Other production animal species</i>	297	247	0	0	1,678	123	514	0	1	1,574	4,264	613	6	9,318
Fur animals	0	247	-	0	-	123	514	-	1	1,565	1,132	613	6	4,202
Aquaculture	297	-	-	-	1,678	-	-	-	-	9	3,132	-	-	5,116
<i>Companion animals</i>	0	164	216	15	1	74	37	0	853	838	1,670	186	34	4,088
Horses	0	7	4	6	0	0	0	0	810	150	1,065	28	0	2,071
Pets ^(b)	0	156	211	9	1	74	37	0	43	687	605	158	34	2,017
<i>Total</i>	1,089	5,418	321	19	1,679	2,281	12,153	8,121	25,628	11,469	15,138	29,987	720	114,023

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

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

b) Antimicrobials used for companion animals: DVFA has allocated kg active compound to the appropriate target species (horses/pets) based on knowledge of which products are used for the particular species.

Figure 4.2. Live biomass (mill. kg) and antimicrobial consumption (kg) in main animal species, Denmark DANMAP 2014



Note: The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals, aquaculture). For poultry: the figures comprise only the biomass for the main production types (turkey and broiler production). The live biomass estimates for cattle, broiler, turkey, fur animals, aquaculture and pet animals based on 2012 data. The estimation procedures are described in Chapter 9.

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

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

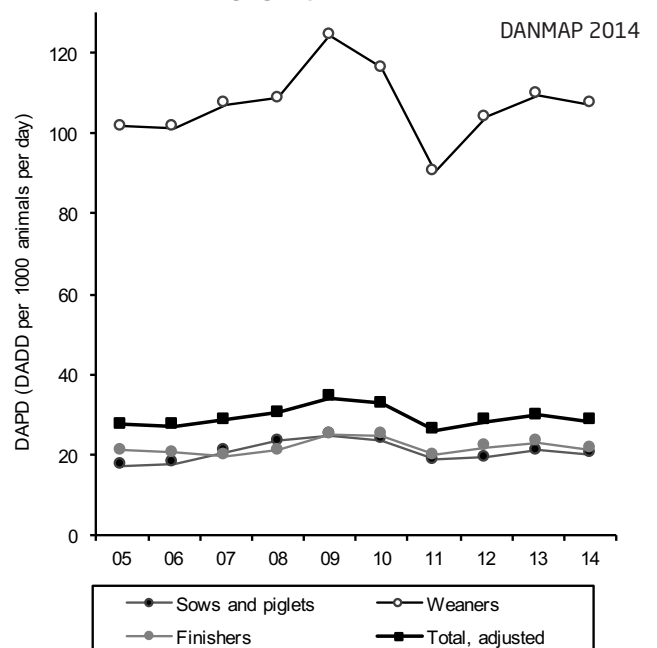
In 2014, the total antimicrobial consumption in pigs was 86.0 tonnes active compound (Table 4.1), a decrease of 4.6 tonnes (5%) compared with 2013. The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.3 and in the web annex (Table A4.2).

The treatment proportion (DAPD) is much higher in the weaning pigs, compared with finishers and sows (Figure 4.3). However, the biomass of the weaning pigs is very small (7.5-30 kg, 4 weeks), compared with the finishers (31-107 kg, 12 weeks) and the sows.

The DAPD of the total population should reflect the trends in selection pressure in the population. Due to the differences in treatment proportion between age groups, the DAPD of the total population is affected by changes in population structure. For example, an increased export of live pigs at 30 kg would, in itself, cause an increase in DAPD for the remaining population, because the DAPD for finishers is relatively low. Any changes in export and productivity must therefore be taken into account to get a true impression of the antimicrobial consumption pattern and selection pressure in the pig production.

Historically, the treatment proportion (DAPD) increased from 2004 to 2009, followed by a decrease in 2010 and 2011, which most likely was a result of DFVA's implementation of the "yellow card initiative" - a special provision for reduction of an-

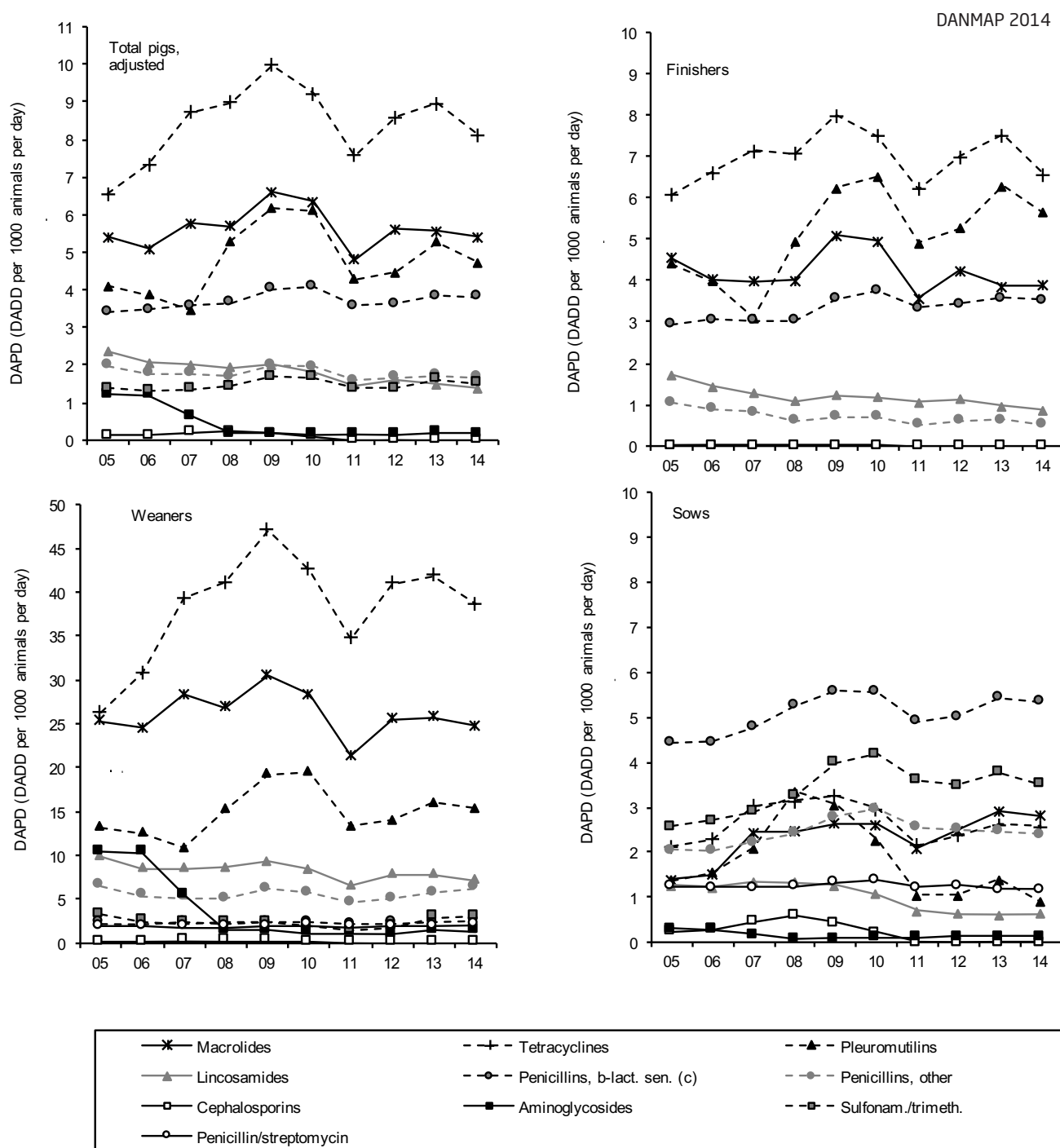
Figure 4.3. Antimicrobial consumption^(a) in the pig production, and the distribution on age groups, Denmark DANMAP 2014



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

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

Figure 4.4. Antimicrobial consumption^(a) in the total pig production^(b), and in finishers, weaners, sows and piglets, Denmark



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

a) The DAPP is calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes).

b) Total pigs produced includes pigs exported at 30 kg, which has increased in numbers from 2.7 mill. in 2005 to 10.5 mill. in 2014. Consumption in these pigs is included only from birth to 30 kg body weight. See discussion in the DANMAP 2011.

c) Beta-lactamase sensitive penicillins.

antimicrobial consumption in pig production (See DANMAP 2010). The reductions in antimicrobial use were associated with increasing use of vaccines (Textbox 4.2) and a slight temporary decrease in productivity in some herds, but disease outbreaks did not increase [Danish Veterinary Bulletin no. 6, 2012]. The antimicrobial consumption in pigs in 2014 was 14% lower than in 2009 (Figure 4.3).

In 2014, the antimicrobial consumption in pigs decreased by 5% to approximately 28 DAPD (Figure 4.3) when adjusted for changes in export. Overall the number of pigs produced in 2014 increased by 3%, and the number of pigs exported increased by 13% (Table 3.1).

Within the different age groups, the DAPD decreased in all age groups; 5% in sow herds, and 2% in weaners and 7% in finishers. The decrease was associated primarily with the use of tetracyclines, and to a lesser extent, to the use of pleuromutilins and macrolides (Figure 4.4). Tetracyclines and pleuromutilins have been the most commonly used antimicrobial agents in the Danish pig production for a decade (Figure 4.4). They

are almost entirely administered orally, and particularly used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall treatment proportion (DAPD) of pleuromutilins decreased by 11%, while the use of tetracyclines decreased by 9%.

For the critically important antimicrobial agents, the use of flouroquinolones decreased markedly from 11 kg to 4 kg, because a clinical trial for which the flouroquinolones have largely been used for over the past years did not continue in 2014. The use of cephalosporins continued to increase (from 3 to 4 kg), but still constitutes less than 1 per mille of the total consumption in pigs (Figure 4.4).

In 2014 the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% by the end of 2015.

DANMAP 2014 includes an overview of the use of vaccines and consumption of zinc in the Danish pig production, see Textbox 4.2. and 4.3.

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

DANMAP 2014

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Doses per antimicrobial class	DADDs (1000's)									
Penicillins ^(a)	201	211	211	236	282	314	318	324	311	317
Aminoglycoside-benzylpenicillin combinations ^(b)	130	104	101	101	110	93	48	47	58	90
Cephalosporins, 1st generation	103	98	89	85	89	89	99	105	111	113
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34	30	24	21
Others ^(c)	21	20	16	15	14	12	9	8	0	0
Total	566	558	544	549	570	559	508	514	504	541
Total DADD per cow per year	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9	1.0

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

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

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

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

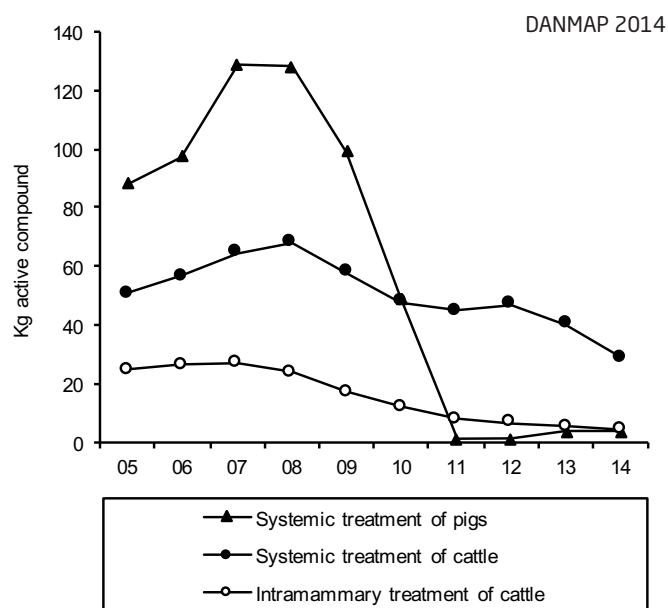
Table 4.3. Number of treatments with antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2014

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Total doses per indication ^(a)	DADDs (1000's)									
Drying off treatment (4 teats)	73	75	71	76	82	99	97	117	125	140
Therapeutic treatment (2 teats)	420	408	388	377	378	350	307	279	253	259

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

Figure 4.5. Consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark



4.3.2 Antimicrobial consumption in poultry

In Denmark, poultry production comprises mainly the broiler production (*Gallus gallus*), followed by egg layers (*Gallus gallus*) and turkey production. In addition, there is a very small production of ducks, geese, and game birds.

In Denmark, the antimicrobial consumption in the broiler production is generally low compared with other species and, a few disease outbreaks in some farms can seriously affect the national consumption, causing considerable fluctuations in annual consumption data.

In 2014, the total antimicrobial consumption in poultry (all poultry) was 1,548 kg active compound, an increase of 22% compared with 2013, and was the highest amount recorded for more than a decade. The increase was marked in both turkeys (12%) and in other poultry (incl. *Gallus gallus*) (38%). The increase was mainly driven by a remarkable increase in the use of tetracyclines, and to a lesser extent broad spectrum penicillins and macrolides (not in turkeys) (Table 4.1). There appear to be several reasons for these increases. In the broiler production there has been widespread problems particularly with bacterial arthritis and gastrointestinal infections, in 2013 and 2014. The turkey production has been challenged with prevalent respiratory disease during the same period.

For broilers, amoxicillin has been the most commonly used antimicrobial agent for at least a decade. Fluoroquinolones were the second most commonly used antimicrobial agent until 2007 (when new medicines were approved for poultry), but has not been used in broilers in 2010-2014.

4.3.3 Antimicrobial consumption in cattle

In 2014, the consumption continued to increase to approximately 13 tonnes, which represents an 8% increase compared with 2013. The production of veal and beef remained more or less at the same level from year to year, while the milk production increased (Table 3.1). The increase in antimicrobial consumption was mainly driven by a 13% increase in the use of beta-lactamase sensitive penicillins for adult cattle, from 6,636 kg in 2013 to 7,532 kg in 2014.

The majority of the parenteral use in cattle is for cows (Table 4.1), and is mainly prescribed for mastitis. The use of fluoroquinolones in cattle has been low for the last decade and no use of fluoroquinolones was reported for cattle in 2014. Approximately 29 kg of cephalosporins were used systemically, which represents a 27% decrease compared with 2013 and a 38% decrease compared with 2012 (Figure 4.5).

From 2005 to 2013 there was a slight reduction in the overall level of intramammary treatment; however in 2014 the use of intramammary treatment increased (Table 4.2). The main increase was seen for drying-off treatment, which increased by 12%. Therapeutic treatment, however, increased by only 2% (Table 4.3). A "milk quality campaign" conducted by the Danish Cattle Association (Agriculture and Food Council) since 2010, most likely has contributed to this trend. 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 increased monitoring of cell counts to determine the need for treatment.

Order (DK) 785/2010 provides legal regulations of use of antimicrobial agents for mastitis in cattle (recommending using simple penicillins). Furthermore the industry has emphasized that farmers should use narrow spectrum penicillins to treat mastitis caused by Gram-positive bacteria, unless sensitivity testing reveals resistance towards these antimicrobials.

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The overall antimicrobial consumption in aquaculture increased by 43% to 5,116 kg in 2014 compared with 2013 (Table 4.1). Measured in kg active compound sulfonamide/trimethoprim comprised 61%, quinolones 33% and amphenicols 6%. The antimicrobial consumption in both 2012 and 2011 was very low compared to previous years, probably due to cold summers in 2011-2012. The increases observed in both 2013 and 2014 are explained by the extraordinary warm summers, leading to high water temperatures and increased occurrence of bacterial infections [personal communication: N. H. Henriksen, Danish Aquaculture]. However, the aquaculture industry continues to focus on developing new and better vaccines, as well as improving vaccination strategies to reduce the risk of diseases that may require antibiotic treatment.

In 2014, the production of mink increased slightly to 17.9 million mink from 17.2 million in 2013 (Source: Copenhagen

Fur). Antimicrobial consumption in fur animals decreased by approximately 14% to 4,202 kg active compound compared with 2013 (Table 4.1). This is the second year in a row with a decrease in consumption for fur animals, which is in contrast to the increase in consumption observed from 2008-2012. In recent years, the industry, farmers and veterinarians have had increased focus on the consumption of antimicrobial agents, quality of feed and animal welfare in the mink production, and this may explain the reduced consumption. Use of fluoroquinolones and cephalosporins in fur animal production has been close to zero for several years.

The information available on antimicrobial consumption in companion animals is not as detailed as for production animals. In 2014 the overall antimicrobial consumption in pets amounted to 2,017 kg active compound, which was almost at the same level as in 2013 (1,989 kg in 2013).

A large proportion of antimicrobials used for pet animals are prescribed for treatment of chronic or recurrent disease, mainly

dermatitis. Particularly the consumption of critically important antimicrobial agents in pet animals could pose an important risk to owners of diseased dogs that are frequently treated.

In 2014, the use of fluoroquinolones for use in pets was 9 kg. Thus, the amount of fluoroquinolones used in pets constituted nearly 47% the total veterinary use (kg) of fluoroquinolones. Similarly, the pets accounted for a significant proportion (211 kg or 66%) of the use of cephalosporins used in animals. However, over the past two years the consumption of fluoroquinolones and cephalosporins has been reduced by 8% and 22%, respectively, while the consumption of other antimicrobial classes such as penicillins and sulphonamides and trimethoprim increased. The small shift in use of the different antimicrobial classes may be an effect of the treatment guidelines put out by Danish Veterinary Association in November 2012 that call for critically important antimicrobials to be avoided as much as possible,

Birgitte Borck Høg, Leonardo de Knegt

Textbox 4.1

National actions for prudent antimicrobial use in animals

Implementation of differentiated taxes on antimicrobial agents for veterinary use

In September 2013 the Danish Veterinary and Food Administration, in cooperation with the Danish Health and Medicines Authority, implemented differentiated taxes on the sales of antimicrobials and other medicines for veterinary use (Table 1). The initiative was part of the Second Veterinary Action Plan running from 2013 to 2016 [FVST 2012, Forlig om veterinærområdet, in Danish]. The action plan is supported politically by the entire Danish Parliament.

The initiative strengthens the incentive to choose alternatives to antimicrobial treatment (e.g. vaccines) or when antimicrobial treatment has to be used, to choose the most responsible antimicrobial treatment in a One Health perspective.

The tax is fixed by law [BEK 534 of 27/05/2014, in Danish] and gives a yearly income of approximately 8.3 mill. DKK (1.1 mill. Euro), which contributes to the financing of the Danish effort of responsible and prudent use of veterinary antimicrobial agents. In practice, the tax is collected when pharmacies and other distributors sell veterinary medicines.

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Table 1. Differentiated taxes on sales of antimicrobials and other medicines for veterinary use.

	DANMAP 2014
Medicine group	Tax level ^(b)
Vaccines	No tax
Penicillins, simple and narrow spectrum	0.8%
Other veterinary antimicrobials	5.5%
Critically important antimicrobials ^(a)	10.8%
Other veterinary medicines (not antimicrobials)	0.8%

a) 3rd and 4th generation cephalosporins and flouroquinolones.

b) Previously, sales of antimicrobials and other medicines for veterinary use was taxed by 0.84%. The differentiated taxes was implemented in September 2013.

New legislation on antibiotic treatment of groups of pig

In June 2014, as part of the Second Veterinary Action Plan, the Danish Veterinary and Food Administration introduced legislation on treatment of flocks of pigs. The purpose of the legislation is to make veterinarians choose the most effective treatment and thereby reduce the use of antibiotics in pigs in order to maintain the low level of antimicrobial resistance in Denmark.

The new legislative requirements apply for veterinarians and pig farmers with a Veterinary Advisory Service Contract (VASC). When veterinarians prescribe antibiotic treatment for respiratory or gastrointestinal infections for administration through feed and water the veterinarian must - in order to verify her/his clinical diagnosis - take samples for laboratory testing. The samples must be submitted to a laboratory approved by the Danish Veterinary and Food Administration or a foreign laboratory that has been approved [BEK 534 of 27/05/2014, § 46.1].

Veterinary Advisory Service Contracts

The first statutory order on Veterinary Advisory Service Contracts (VASC) in herds of cattle and pigs came into force in 1995. Since then, the concept has been improved and extended several times. Until 2010, the signing of a VASC was optional. Today the VASC is mandatory for large herds of cattle, pigs and mink farms and voluntary for smaller herds. On one hand, the VASC results in frequent veterinary advisory visits to the farm and consequently a close contact between the veterinarian and the farmer. On the other hand it provides the farmer with extended treatment possibilities.

The aims of the VASC are to:

- Focus on advice and prevention of illness rather than treatment
- Improve standard of animal health
- Minimise risk of infectious diseases
- Optimise use of antimicrobials in order to prevent antimicrobial resistance
- Improve animal welfare
- Incorporate an incentive scheme where documented prudent use of antibiotics may lead to a reduced frequency of veterinary advisory visits.

The farmer is free to choose between a basic or an advanced VASC. With an advanced VASC, the farmer is given extended rights in return for which he must have more advisory visits. Only an advanced VASC allows the farmer to initiate treatment and gives him access to veterinary medicine products for a prolonged period of time compared with the five days, which apply for farmers with a basic or no VASC.

The number of required advisory visits differs between species, age groups and category.

VASCs are categorised as 'non-compliance', if convicted for violating legislation regarding animal health, animal welfare or use of veterinary medicine or extended use of antimicrobials within the past two years. The Herds in the non-compliance category have more advisory visits and shorter periods for prescription of veterinary medicine products. The VASCs in the compliance category have the lowest number of advisory visits, and the veterinarian can prescribe veterinary medicine products for longer than a five day period.

All VASCs have to be electronically registered in a database at www.vetreg.dk. The database calculates and displays type of VASC, number of advisory visits, prescription periods and current category for the farmer, the veterinarians and the veterinary control officer.

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If the laboratory test shows that the initiated treatment is not optimal, the veterinarian must evaluate and correct treatment if necessary. If the laboratory test result is non-conclusive, the veterinarian must take samples for additional testing according to laboratory guidelines.

Following the initial laboratory test, the veterinarian must submit samples to the laboratory for verification. The intervals for re-sampling depend on the compliance category of the VASC.

The prescription period for antibiotics for treatment of groups of pigs is shortened compared to the general prescription rules relating to VASC. Since veterinarians may only prescribe veterinary medicinal products (VMP) in connection with a visit to the farm, the veterinarian must consequently visit the farm repeatedly if there is need for continued use of medication for group treatment.

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Textbox 4.2

Use of vaccines in the Danish pig production 2004-2014

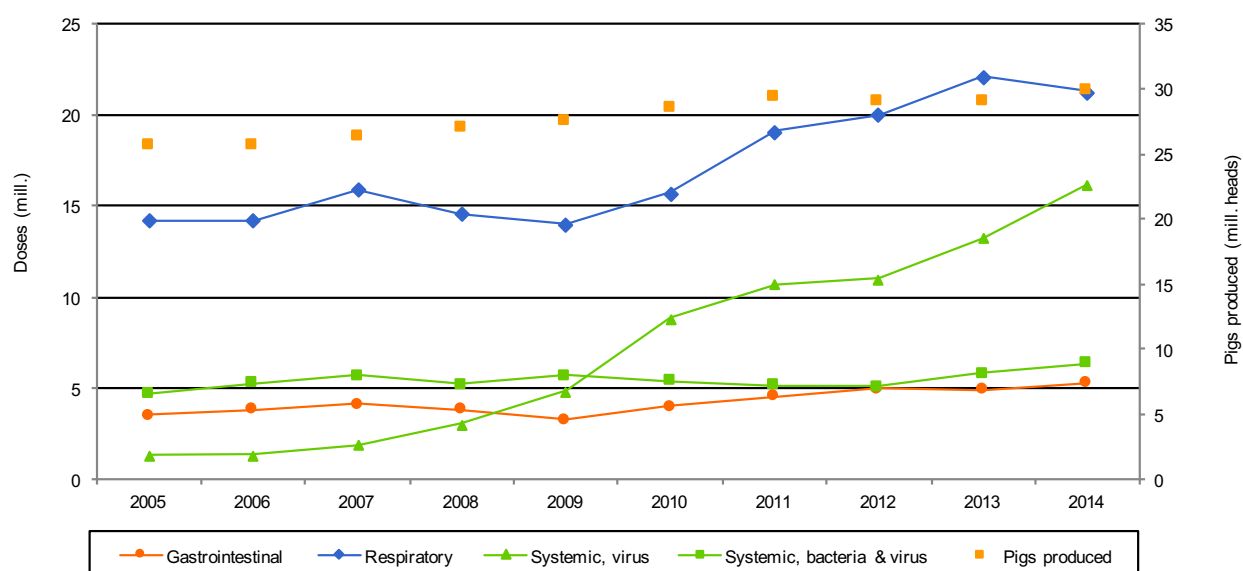
Background: During the last 10 years there has been a remarkable increase in the use of vaccines in the Danish pig production. The development over this period is described below by dividing the vaccines in four groups, i.e. vaccines for: 1) gastrointestinal infections (GI), 2) respiratory infections, 3) systemic bacterial infections or bacterial and viral infections in combination, and 4) viral infections (monovalent vaccines).

Vaccines for gastrointestinal infections: This group includes vaccines used for prevention of enteric diseases caused by *Escherichia coli*, *Clostridium perfringens* and *Lawsonia intracellularis* and reached 5.3 mill. doses in 2014. Vaccines against different types of *E. coli* and *C. perfringens* are used for prevention of neonatal diarrhea while the vaccine against *L. intracellularis* is used for prevention of enteritis in weaners and finishers. From 2004 to 2014 the usages increased by 56% (Figure 1); caused by introduction of a vaccine against *L. intracellularis* in 2005 reaching 1.9 mill. doses in 2014. In contrast, the use of vaccines for *E. coli* and *C. perfringens* remained almost constant throughout this period.

Vaccines for respiratory infections: This group includes vaccines used for prevention of diseases caused by swine influenza, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and toxin producing *Pasteurella multocida*. There has been an increase of 55% from 2004 till 2014 (13.7 to 21.3 mill. doses) which was mainly due to a 40% increase in vaccines for *M. hyopneumoniae* reaching 15 mill. doses in 2014 (Figure 1). Vaccines for *A. pleuropneumoniae* increased 115% reaching 4.8 mill. doses in 2014, and influenza vaccines increased 14 times from 0.1 mill doses in 2004 to 1.4 mill. doses in 2014.

Figure 1. Use of different groups of vaccines for pigs, Denmark 2004-2014

DANMAP 2014



Vaccines for systemic bacterial infections: There has been a 27% increase reaching 6.3 mill. doses in 2014 of this group of vaccines which include vaccines against erysipelas, leptospirosis, *Haemophilus parasuis* and a few combi-vaccines also including porcine parvovirus. The increase is related to increased vaccination against reproductive problems caused by swine specific leptospira.

Vaccines for systemic virus infections: This group comprises vaccines against PRRS (porcine respiratory and reproductive syndrome), PCV2 (porcine circovirus type 2) and PPV (porcine parvovirus). There has been a dramatic increase in the use of virus vaccines from 1.4 mill. doses in 2004 to 16.2 mill. doses in 2014.

The major part of the increase is due to the introduction of vaccines against PCV2 in 2006, reaching 14.6 mill. doses in 2014. PCV2 is causing PMWS (post weaning multisystemic wasting syndrome) and PCVAD (porcine circo virus associated diseases). From 1999 onwards a spread of PMWS was recognized globally that resulted in reduced production and increased use of antibiotics in many pig herds. The introduction of PCV2 vaccines was quite effective in reducing the prevalence of PMWS and PCVAD. Hence, these vaccines have become very popular worldwide.

The use of vaccines against PRRS increased from 0.3 mill. doses to 1,4 mill. doses. However, the use of PRRS vaccines is relatively limited in Denmark, partly due to a relatively high health status of Danish pig herds. Many herds are free from PRRS as well as other important swine specific diseases.

Remarks concerning relations between use of vaccination and antibiotics: The effect of vaccination on the antimicrobial use cannot be estimated by simply comparing the total consumption of antimicrobial agents and application of different groups of vaccines. Correlations between the two strategies for prevention and treatment of infectious diseases must be analysed applying herd level data. However, some general comments may be given.

The use of vaccination against *L. intracellularis* usually replaces a significant amount of antimicrobial treatments for diarrhea in the pig herd. *Lawsonia* infections are usually treated with tetracyclines, macrolides and pleuromutilines. These antimicrobial agents are also used for treatment of respiratory diseases, but most of the consumption in pigs is related to diarrhea in weaners and finishers. Most vaccines for pigs are used for prevention of systemic viral and respiratory diseases; infections that often cannot be treated by antibiotics. However, it is without doubt that the vaccination strategies against bacterial as well as viral infections tend to diminish antimicrobial consumption in Denmark.

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Textbox 4.3

Use of zinc oxide in the Danish pig production 2005-2014

Zinc is a critical trace element for animals (and humans). In humans, zinc deficiency may result in growth retardation, skin diseases, impaired wound healing and last but not least diarrhoea. Furthermore, undernourished children suffering from diarrhoea recover when given extra zinc. Generally, extra zinc is added to pig diets as the inherent amount of zinc in feeding-stuffs and mixed diets is not sufficient to fulfil the dietary requirement of the piglets. The most commonly used source of zinc is zinc oxide (ZnO) which contains 80% zinc.

The use of zinc oxide prescribed by veterinarians has increased markedly over the last 10 years (Figure 1). From 2005 to 2011 a three-fold increase in use of zinc and zinc oxide was reported to VetStat, whereas the number of pigs produced in Denmark increased by 14%. Since 2011, the consumption has been relative stable at approximately 500 tonnes of zinc oxide (ZnO) equivalent to 400 tonnes of zinc (Zn).

Since the late 1980s, the practice of adding high amounts of zinc oxide to the feed has become widespread in commercial pig production to prevent or alleviate post-weaning diarrhoea in early weaned piglets. Many hypotheses about the mode of action behind the beneficial effects of high inclusion levels of zinc oxide have been developed and tested. However, there is growing evidence that the hypothesis stated in 1989 that piglets suffer from transient zinc deficiency after weaning is credible. The original results showed that 2,500 ppm zinc oxide/kg feed resulted in a 60% reduction of in the diarrhoea incidence in piglets, compared with lower dietary zinc levels (addition of 0, 100, 200, or 1,000 ppm zinc as zinc oxide)[Poulsen, 1989]. The author found that the alleviating effect of high dietary levels of zinc oxide was accompanied by an increase in plasma zinc concentration (0.2 mg/100 mL) in piglets fed 2,500 ppm zinc (as zinc oxide), whereas the plasma zinc concentration remained constant at 0.1 mg/100 mL in pigs fed 0, 100, 200, or 1,000 ppm zinc as zinc oxide. The 1989-study also demonstrated that two weeks of high dietary zinc levels were needed to obtain the alleviating effect of 2,500 ppm zinc.

Piglets must be fed a sufficient amount of zinc every day, since the immediately available plasma and body zinc pool is very limited. Since the feed intake is very low right after weaning, the concentration of zinc in the diet must be increased to counterbalance this. Two weeks after weaning, the feed intake has increased to a normal level and lower dietary zinc content of about 100 ppm is sufficient.

There is an apparent need to develop a strategy, balancing the nutritional requirements and the animal welfare of weaned piglets, against the ability of zinc to select for antimicrobial resistant bacteria, as well as considering the environmental impact of zinc.

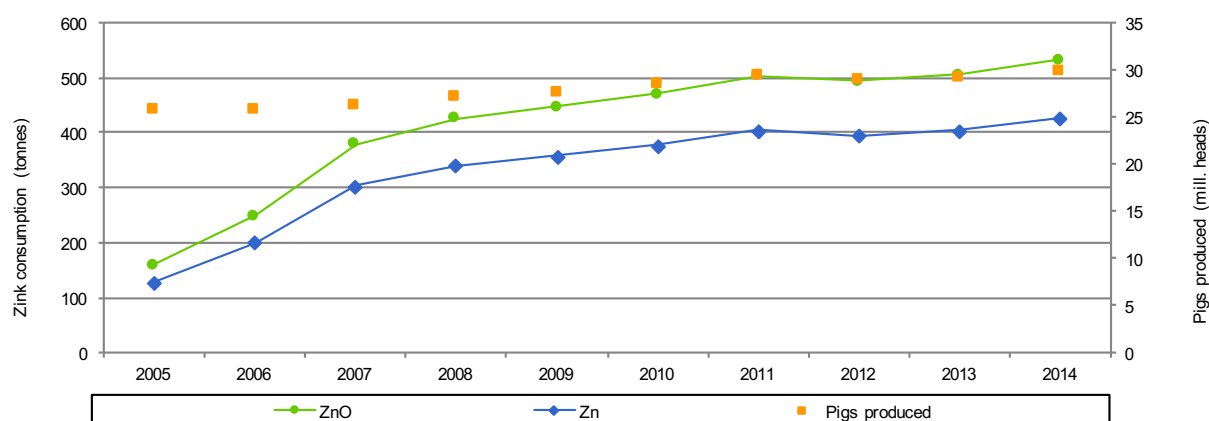
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Figure 1. Consumption (tonnes) of zinc oxide (ZnO) and zinc (Zn) in the pig production, Denmark 2005-2014

DANMAP 2014



Note: In addition to medicinal ZnO, Zn is added to all compound feeding stuffs for pigs, up to a level of 150 ppm.

5

ANTIMICROBIAL CONSUMPTION IN HUMANS



5. Antimicrobial consumption in humans

5.1 Introduction

In Denmark, the Register of Medicinal Product Statistics at Statens Serum Institut records all use of antimicrobial agents for humans. Monitoring has been performed since 1994 and all data on consumption in both primary health care and hospital care are registered. Primary Health care is in this setting defined as all non-hospital activity and corresponds to antimicrobials sold upon prescriptions from general practitioners, medical specialists as well as clinicians at hospitals when discharging a patient.

In this part, the term 'antimicrobial agents' covers only antibacterial agents for systemic use in humans; topical agents as well as agents used for treatment of viral and fungal infections are not included. The report covers antibacterials listed in the Anatomical Therapeutic Chemical (ATC) Classification System under the code J01. Currently available antimicrobial agents for systemic treatment in humans (and in animals) are listed in Table 3.2.

Data on oral (and rectal) metronidazole (registered under the code for the antiparasitic products P01AB01) and oral vancomycin (registered under intestinal antiinfectives A07AA09) are included for the first time in this report. The oral and rectal preparations of these drugs are first line choice in the treatment of *Clostridium difficile*, which has been an emerging problem since 2006. Figures and tables are updated 10 years back.

Previously in DANMAP, antimicrobial agents have been grouped into narrow- and broad-spectrum activity and their consumption reported as such. In 2014, this classification has been abandoned due to a lack of a national or international consensus on the definition. The focus in this report is placed on evaluation of the total consumption as well as consumption of the most important single agents.

5.2 Total consumption (primary health care and hospital care)

In 2014, the total consumption of antimicrobial agents for systemic use (primary health care and hospital care) was 18.58 DID, which is 3% lower than the previous year corresponding to a decrease of 0.5 DID (19.10 DID in 2013). Notably the majority of this reduction was due to decreased use of tetracyclines in primary health care (see text 5.3.1). Consumption in the primary sector accounted for 16.40 DID and at hospitals for 2.18 DID (Figure 5.1). This corresponds to 52,819 kg active substance (Table A5.1 in web annex).

Since the first DANMAP report in 1997 the total consumption increased with 4.99 DID (approximately 40%) and from 2005 with 1.96 DID (11%), reaching a peak in 2011 with a total DID of 19.31. Since 2011, the consumption, when measured in DID, has stagnated.

Primary health care accounts for approximately 90% of the total antimicrobial consumption and thus significant changes in prescription here will markedly affect the total consumption. The distribution between the sectors for the individual antimicrobials is shown in Figure 5.2.

Figure 5.1. Total consumption of antimicrobial agents in humans in primary health care vs hospital care, Denmark. DANMAP 2014

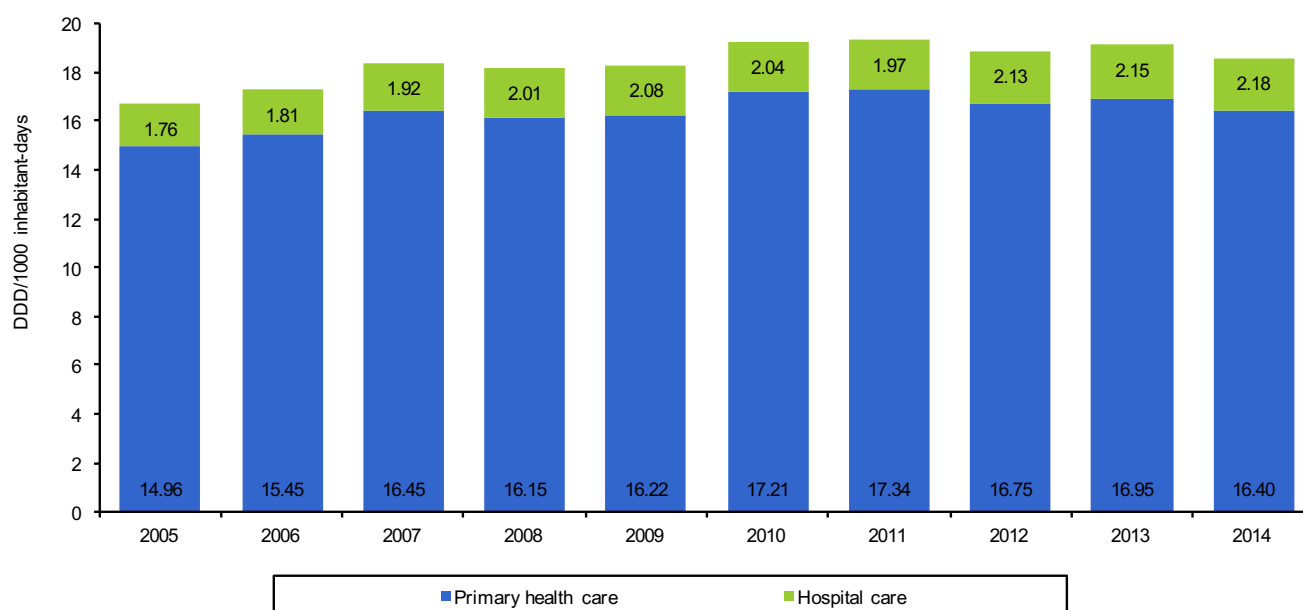


Figure 5.2. Distribution of DIDs between primary health care and hospital care, Denmark

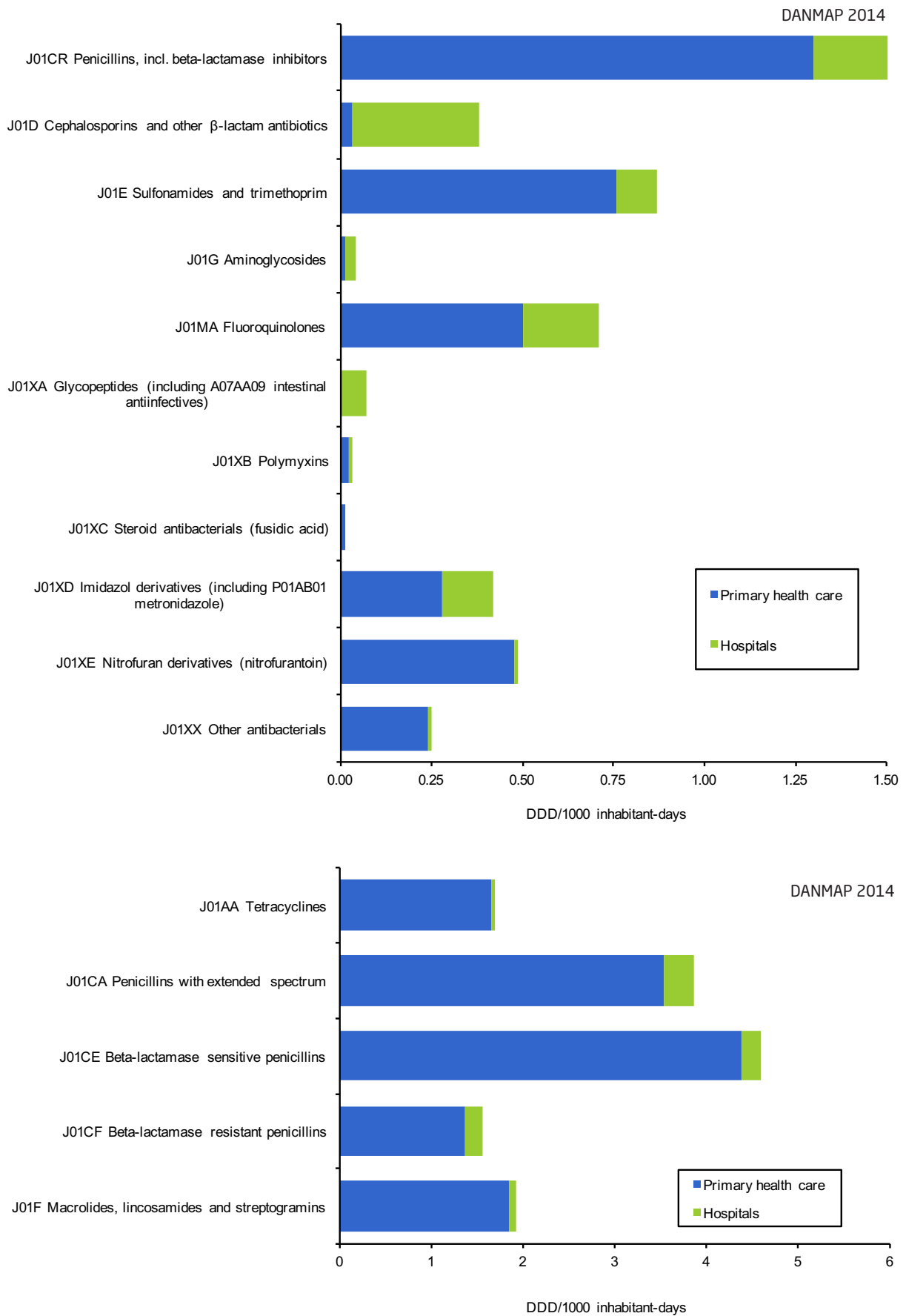


Table 5.1. Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

ATC group ^(a)	Therapeutic group	Year									
		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	1.28	1.38	1.48	1.54	1.61	1.69	1.64	1.76	1.96	1.66
J01CA	Penicillins with extended spectrum	2.79	2.95	3.25	3.26	3.29	3.47	3.41	3.40	3.48	3.53
J01CE	Beta-lactamase sensitive penicillins	5.28	5.40	5.67	5.30	5.12	5.25	5.31	4.68	4.65	4.38
J01CF	Beta-lactamase resistant penicillins	0.97	1.05	1.09	1.12	1.13	1.17	1.14	1.21	1.30	1.36
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.08	0.12	0.19	0.27	0.45	0.68	0.89	1.05	1.22	1.30
J01D	Cephalosporins and other β -lactam antibiotics	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.44	0.47	0.49	0.49	0.48	0.51	0.50	0.52	0.53	0.55
J01EB	Short-acting sulfonamides	0.35	0.35	0.31	0.28	0.27	0.26	0.24	0.22	0.22	0.21
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00
J01FA	Macrolides	2.41	2.31	2.42	2.28	2.21	2.44	2.47	2.19	1.93	1.79
J01FF	Lincosamides	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.04	0.05	0.05
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
J01MA	Fluoroquinolones	0.33	0.37	0.44	0.51	0.52	0.57	0.57	0.55	0.52	0.50
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	0.03	0.02	0.02	0.02
J01XC	Steroid antibacterials (kombination fusidic acid)	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.45	0.46	0.47	0.47	0.49	0.51	0.50	0.49	0.49	0.48
J01XX	Other antibacterials (methenamine >99%)	0.28	0.27	0.26	0.27	0.26	0.27	0.26	0.25	0.24	0.24
J01XD and P01AB	Nitroimidazole derivatives (metronidazole)	0.21	0.22	0.23	0.24	0.27	0.28	0.28	0.28	0.28	0.28
J01	Antibacterial agents for systemic use (total)	14.96	15.45	16.45	16.15	16.22	17.21	17.34	16.75	16.95	16.40

a) From the 2014 edition of the Anatomical Therapeutic Chemical (ATC) classification system.

Figure 5.4. Consumption of leading antimicrobial groups for systemic use in primary health care, Denmark

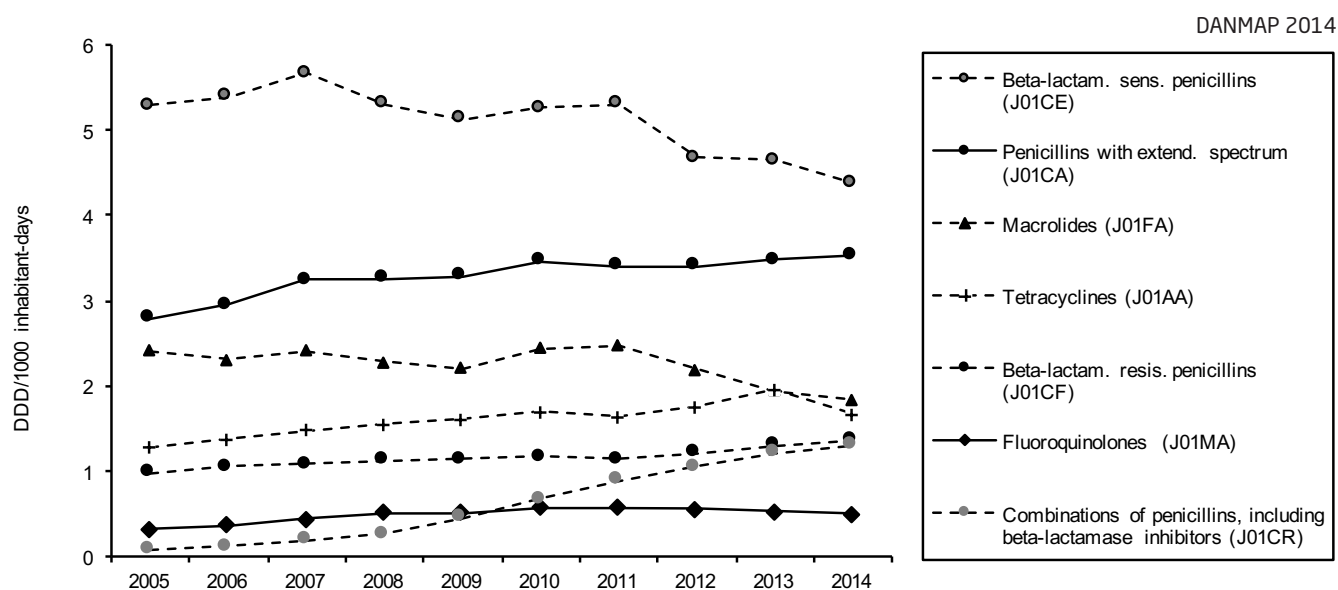
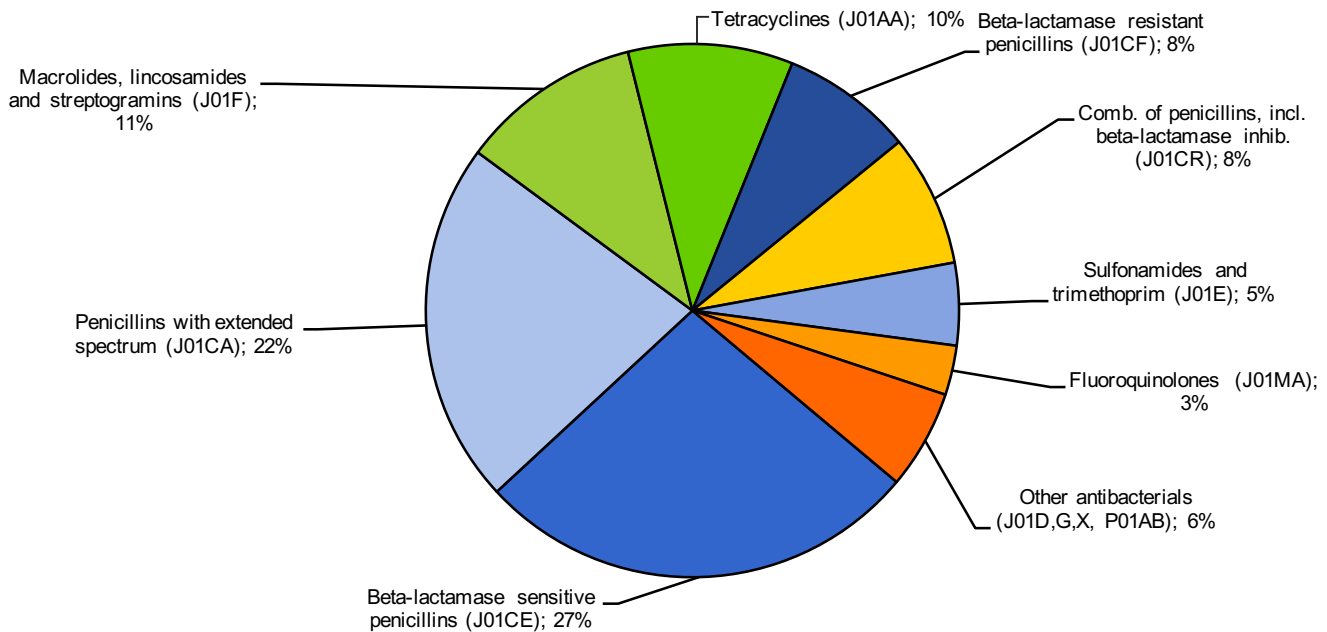


Figure 5.3. Distribution of the total consumption of antimicrobial agents in primary health care, Denmark



5.3 Primary health care

5.3.1 Total consumption in primary health care

In primary health care, the consumption of antimicrobial agents was 16.40 DID in 2014, which is approximately 3% lower than observed in 2013 (Table 5.1). However, this is largely due to a decrease in consumption of tetracyclines, especially doxycycline, which had an unusual peak in 2013. The reason for these fluctuations is presently unknown.

During the last decade the consumption increased from 14.96 DID in 2005 to 17.34 DID in 2011 (16%), but has since stagnated.

Beta-lactamase sensitive penicillins represented the largest therapeutic group of antimicrobial agents consumed (27%) - and penicillins in general (including combinations with beta-lactamase inhibitors) accounted for 65% of the total consumption in 2014. Macrolides accounted for 11%, tetracyclines for 10%, sulfonamides and trimethoprim for 5%, nitrofurantoin and fluoroquinolones for each 3% (Figure 5.3).

Metronidazole (J01XD and P01AB01) accounted in 2014 for 0.28 DID (approximately 2%). The consumption increased from 0.21 DID in 2005. The oral and rectal preparations of metronidazole are registered as an antiparasitic drug but consumption also covers treatment of anaerobic and mixed anaerobic infections, such as gynaecological or odontological infections. Since abdominal infections with *Clostridium difficile* became an emerging problem in hospitals in 2006, treatment of these

have also become quite common in outpatient care. Patients might be infected at hospital, but treatment most often continues for weeks to months after discharge.

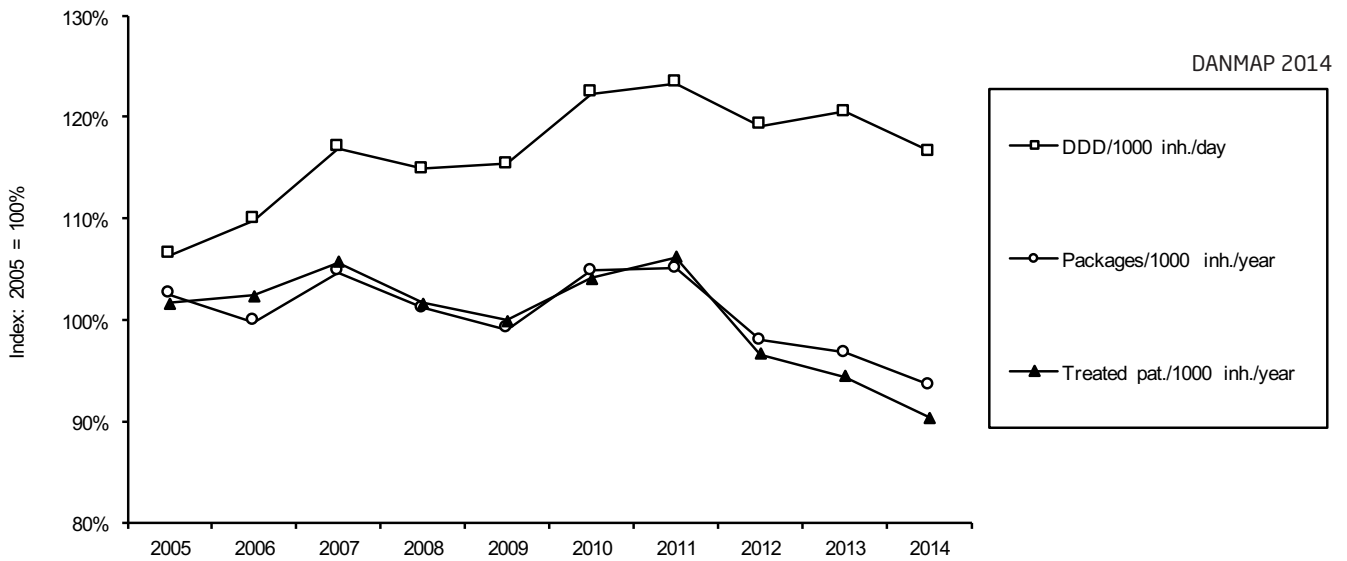
Changes over time for the consumption of the leading antimicrobials are presented in Figure 5.4. Fluoroquinolones (primarily ciprofloxacin) increased from 2005 to 2011 (0.33 DID to 0.57 DID), but have since 2012 shown a decreasing trend. In 2014 the consumption of ciprofloxacin was 0.50 DID.

5.3.2 Measures at treated patient level

In 2014, each treated patient received 21.5 DDDs per year (Table 5.2). The number of patients treated was slightly lower compared to 2013 while the DDD per package remained at the same level (Table A5.2 and A5.3 in web annex). During the past decade, the total number of DDDs per package has increased by 22%, reaching the highest level in 2013. Similarly, the number of DDDs per patient has also increased notably since 2005 while the number of packages per patient has remained relatively constant. The biggest changes in DDD/patient since 2005 have been observed for tetracyclines (from 39.0 to 49.9 DDD/patient), for combination penicillins (from 16.8 to 23.2 DDD/patient) and for beta-lactamase resistant penicillins (from 12.7 to 17.1 DDD/patient) (Table 5.2 and Table A5.3 in web annex).

For tetracyclines, both the number of DDDs per patient and DDDs per package are significantly higher than for any other antimicrobial group, primarily due to the fact that tetracyclines are commonly used for acne treatment with higher

Figure 5.5. Indicators of antimicrobial consumption (J01) in primary health care, Denmark



dosages given in treatments of typically three, but up to six months.

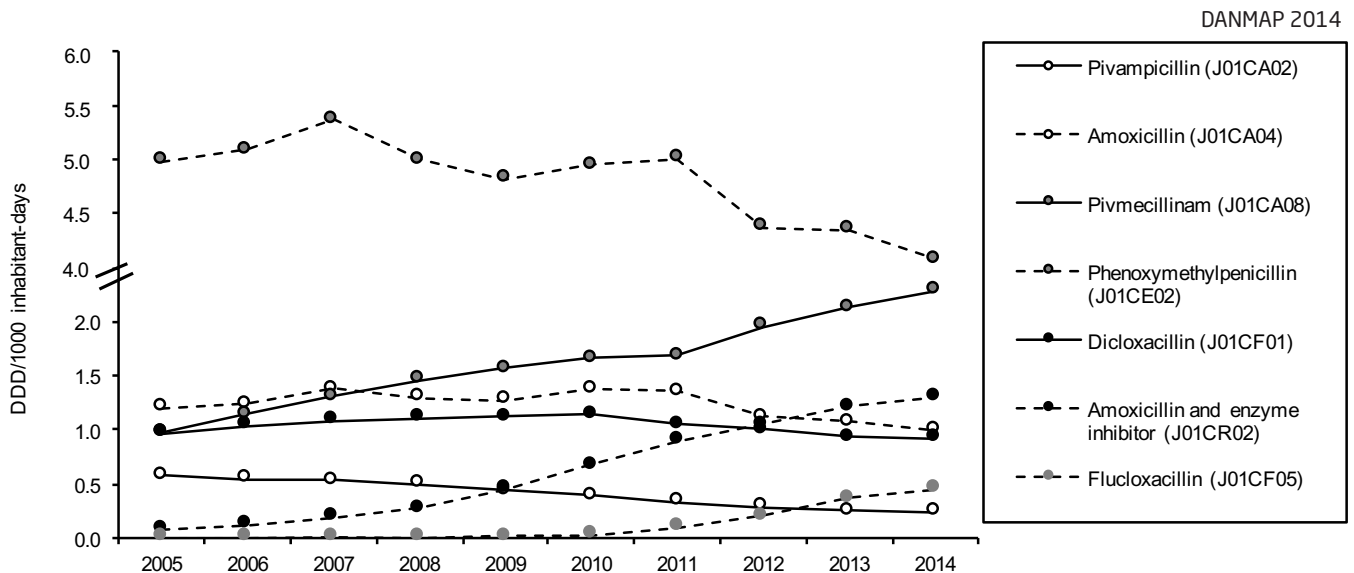
When examining the three different indicators at patient level in 2014, a slight decrease was observed for DIDs (Figure 5.5), thus receding to the same level as in 2009, while the number of packages and treated patients has remained relatively constant for the past decade. In other words, while the dosage prescribed for each patient and in each package has increased significantly since 2005 the data for 2014 might point towards a possible change in prescription and thus a drop in consumption.

5.3.3 Penicillins

Penicillins account for 2/3 of all antimicrobial consumption in primary health care, but while this has remained unchanged since 2005 individual changes in the consumption of the different groups of penicillin have been observed.

The overall consumption of penicillins remained largely the same in 2014 (10.58 DID) compared to 2013 (10.65 DID), the first time in a decade where no apparent increase has been observed. From 2005 to 2014 the consumption of penicillins increased by 16% (9.12 DID in 2005).

Figure 5.6. Consumption of leading penicillins in primary health care, Denmark



The consumption of beta-lactamase sensitive penicillins continued to decrease from 2013 to 2014 (6%), whereas increases in consumption were observed for ‘combination penicillins’ (7%), beta-lactamase resistant penicillins (5%) and penicillins with extended spectrum (1.5%).

The consumption of these three groups has also increased considerably over the past decade with particular increases for beta-lactamase resistant penicillins from 0.97 DID to 1.36 DID (40%), penicillins with extended spectrum from 2.79 DID to 3.53 DID (27%) and the combination penicillins with beta-lactamase inhibitors from 0.08 DID to 1.30 DID (1600%) (Table 5.1).

At the substance-level, phenoxymethylpenicillin continues to be the most commonly consumed penicillin despite a continuous decrease in consumption from 2005 to 2014 (from 5.28 DID to 4.38 DID, a decrease of 17%). Similarly pivampicillin and amoxicillin show a decrease over the last decade from 0.58 DID to 0.24 DID and from 1.21 DID to 0.99 DID, respectively. Meanwhile the consumption of three other substances continued to increase; amoxicillin and enzyme inhibitor from 0.08 DID to 1.3 DID, pivmecillinam from 0.97 DID to 2.28 DID and flucloxacillin from 0.01 DID to 0.45 DID (Figure 5.6).

A likely explanation for the increase in ‘combination penicillins’ might be changes in national recommendations for treatment of patients with exacerbation of chronic obstructive pulmonary disease (COPD) where amoxicillin with clavulanic acid has become a first line drug. Still increases in consumption have also been observed for other patient and age groups e.g. small children, which points towards an altogether change in prescription habits.

The increased consumption for flucloxacillin was accompanied by a minor decrease in dicloxacillin but as for the other broader penicillin groups the total increase in consumption remains yet to be explained.

It is assumed that patients’ demands on receiving antibiotic treatment upon illness might play an important role in the increasing total consumption, as well as for changes in the consumption of individual antimicrobial agents. National campaigns targeting the problem have been launched. Focus in campaigns and in local guidelines, is placed on reducing the amount of antibiotics consumed altogether, as well as choosing the agent with the most narrow spectrum when treatment seems necessary.

Further investigations on prescription habits and trends and the increase in consumption of different penicillin groups will be undertaken over the next years.

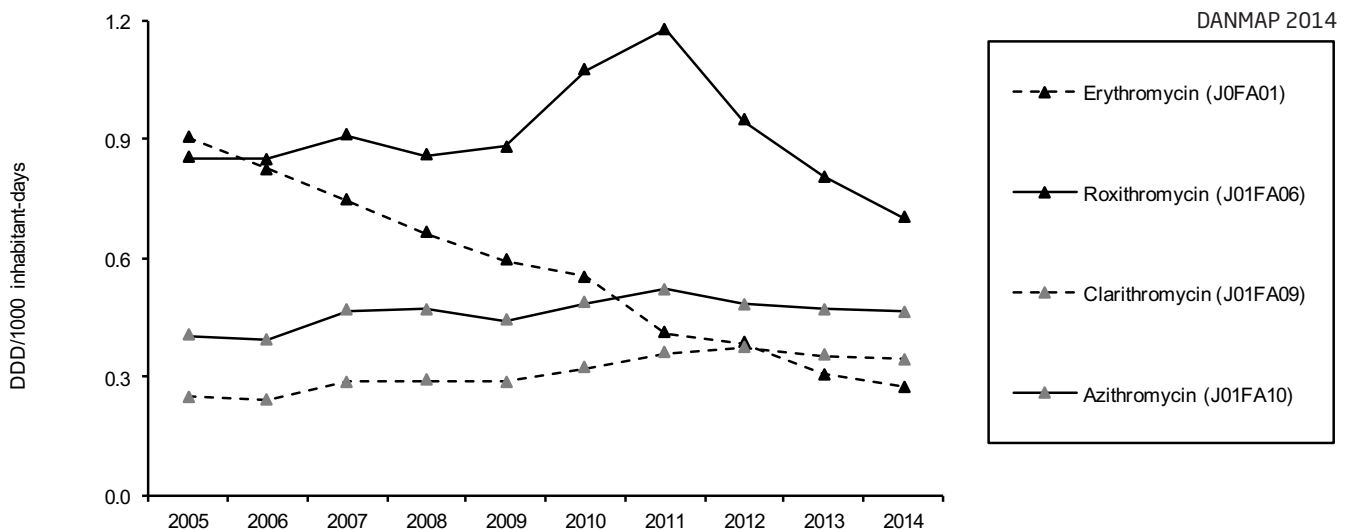
5.3.4 Macrolides

The consumption of macrolides in Denmark often fluctuates from year to year depending primarily on the presence or absence of *Mycoplasma pneumoniae* outbreaks among the population.

The consumption in 2014 (1.79 DID) was the lowest observed this decade. This follows the two ‘epidemic’ years of 2010 and 2011 (DANMAP 2010- 2012), after which no significant *M. pneumoniae* outbreaks have been observed and the consumption of macrolides has been accordingly low (Table 5.2).

At the substance level, the consumption of clarithromycin and azithromycin increased, while the consumption of erythromycin decreased substantially for the whole period. These

Figure 5.7. Consumption of macrolides in primary health care, Denmark



changes in consumption were observed for all age groups (not shown). For roxithromycin, the consumption decreased since 2011. (Figure 5.7).

5.3.5. Tetracyclines

For the first time since 2005, the consumption of tetracyclines has decreased by 15% from 2013 to 2014 (1.96 DID to 1.66 DID). The decrease was observed for all tetracyclines, however the reduction is primarily due to a marked decrease in the use of doxycycline, showing an unexplained increase from 2012 to 2013 but returning to the 2012 level in 2014 (Figure 5.8).

For tetracycline and lymecycline trends in consumption paralleled, increasing steadily from 2005 (0.61 and 0.18 DID respectively) until 2013 (0.77 and 0.39 DID) and showing similar decrease from 2013 to 2014 (0.69 and 0.36 DID).

5.4 Hospital care

5.4.1 Introduction

The antimicrobial consumption for hospital care reported was related only to bed-days and admissions in public somatic hospitals. Specialized hospitals (psychiatric hospitals, hospices and rehabilitation centers) were omitted as they contribute a large proportion of bed-days and admissions but only a small proportion of antimicrobial consumption (approximately 3%),

The consumption of antimicrobial agents in hospital care is presented as DDD per 100 occupied bed-days (DBD) and as DDD per 100 admissions (DAD) to account for hospital activity. Data are also presented as DID to enable comparison with primary health care.

During the past decade, the hospitalization pattern in Denmark has changed notably: more people are admitted to somatic hospitals while the average length of stay is considerably shorter (Figure 5.9, Table A5.4 and A5.5 in web annex). Selec-

tion pressure for the emergence of antimicrobial resistance increases with increasing hospital activity and thus the selection pressure has increased considerably from 2005 to 2014.

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

In 2014, the consumption of antimicrobial agents in somatic hospitals increased from 99.88 DBD in 2013 to 104.30 DBD in 2014 (4%) (Table 5.3). In the past decade, the consumption increased from 66.32 DBD in 2005, an increase of 57%. This reflects a combination of the described increased hospital activity and decreased number of hospital bed-days with an increase in DDDs.

In 2014 extended spectrum penicillins represented the largest group of antimicrobials consumed (16%), closely followed by combination penicillins (15%). Penicillins in general accounted for 50% of the total consumption. Cephalosporins (11%) and fluoroquinolones (9%) were also still among the most commonly consumed antimicrobial agents in hospitals (Figure 5.10).

The total consumption of antimicrobials showed only minor changes compared to previous years. Nevertheless, from 2013 to 2014 marked changes were observed for the imidazole derivatives from 4.08 DBD to 4.48 DBD (9%) and within the group of beta-lactams. Increases were thus observed in the consumption of combination penicillins from 13.64 DBD to 16.04 DBD (18%) and in penicillins with extended spectrum from 15.06 to 16.40 DBD (9%), while a decrease was observed for 2nd generation cephalosporins from 12.32 DBD to 11.68 DBD (5%) (Table 5.3).

These shifting trends in prescription of betalactams in somatic hospitals described for the period 2013 to 2014 were a continuation of changes observed for the last decade (Figure 5.11 and table 5.3). Combination penicillins increased from 1.16 DBD in 2005 to the mentioned 16.04 DBD in 2014 (380%),

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

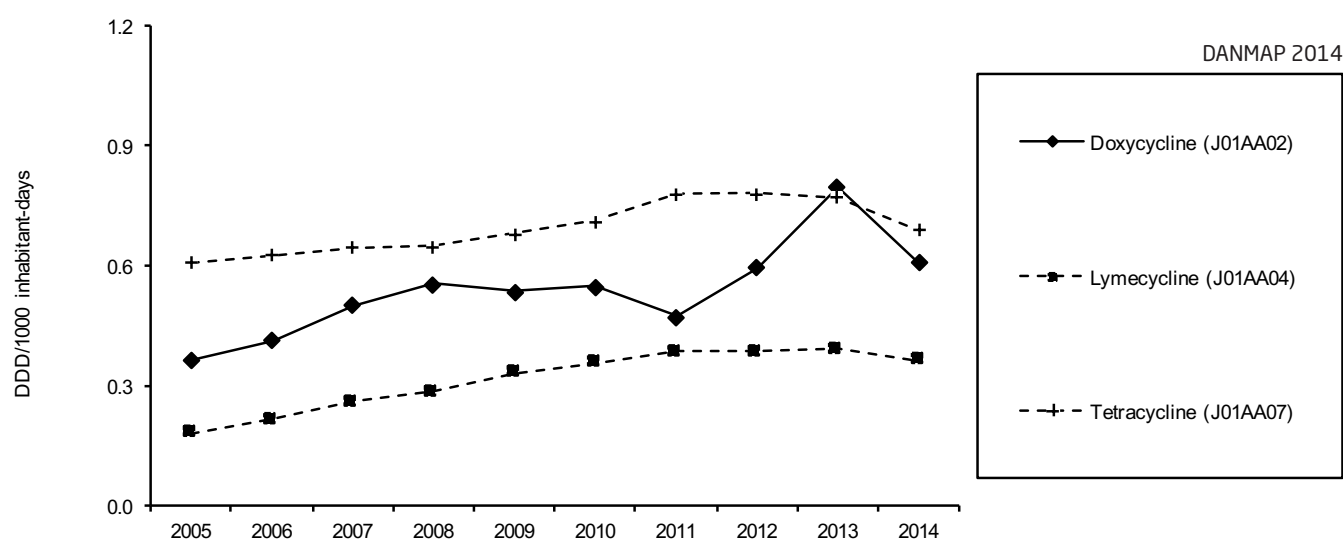
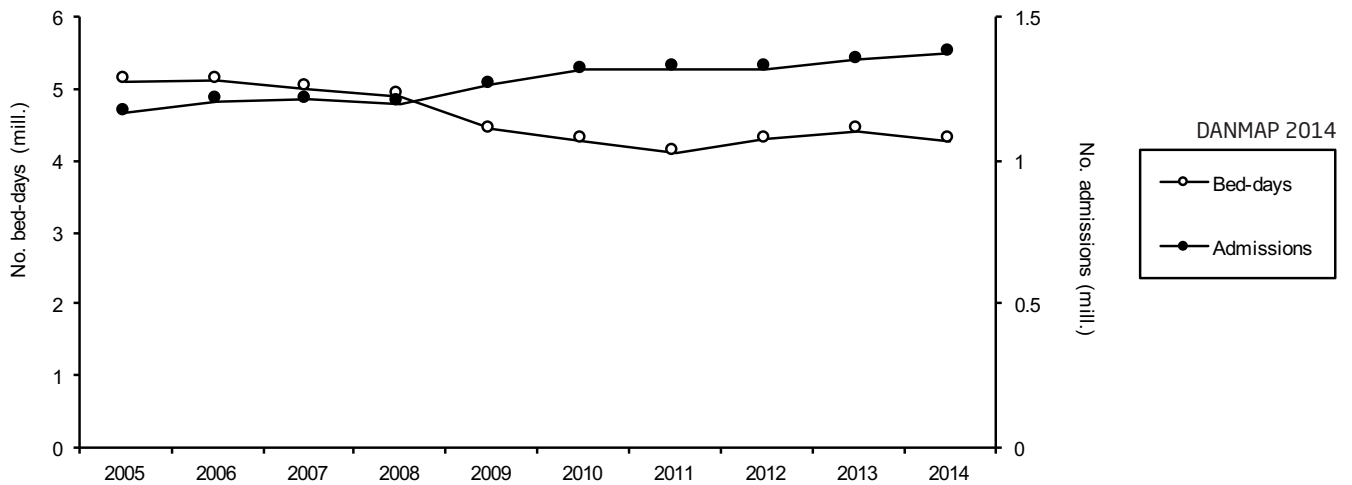


Figure 5.9. Number of bed-days and admissions in somatic hospitals, Denmark



cephalosporins increased from 9.37 DBD in 2005 to 17.66 DBD in 2011, but returned in 2014 with 12.76 DBD to a level slightly lower than in 2007 (13.47 DBD). Beta-lactamase sensitive penicillins decreased from 12.17 DBD in 2005 to 9.32 DBD in 2011, but increased again to 10.31 DBD in 2014.

The observed increase in consumption of 'combination penicillins' was most likely attributable to a change in empiric treatment from cefuroxime to piperacillin with tazobactam for severe infections. These changes were implemented during 2011 to 2014 as recommended by the National Board of Health to lower the selection pressure for ESBL producing *Enterobacteriaceae* (DANMAP 2012). In the same period piperacillin with tazobactam became in many hospitals the first line drug in recommendations for the treatment of complicated pneumonia. These changes were paralleled by an increase in the recommended dosages of amoxicillin-clavulanic acid for treatment of acute exacerbation of chronic obstructive pulmonary diseases (COPD).

Although the recent changes in treatment of critically ill patients have had a significant effect on reduction in the consumption of cephalosporins, cefuroxime still accounts for 11% of the total consumption, mainly because it is still used as a first line drug in antibiotic prophylaxis for different surgical interventions. From 2005 to 2014 2nd generation cephalosporins increased with 39% (from 8.39 to 11.68 DBD), peaking in 2010 with 16.21 DBD.

Fluoroquinolones (primarily ciprofloxacin) showed an increase in consumption from 6.14 DBD in 2005 to 10.70 DBD in 2011 (74%) but since then the consumption has stagnated and shown a decreasing trend for the last three years. In 2014, the consumption of fluoroquinolones was 9.88 DBD.

The consumption of carbapenems was 4.09 DBD in 2014, which is slightly higher than 2013 (4.02 DBD). From 2005 to

2011, an increase of 370% was observed (1.16 to 4.16 DBD). Since then the consumption stagnated. Thus in spite of recommendations from the National Board of Health on the reduced use of carbapenems and delivery problems for meropenem during the summer of 2014 no apparent decrease in meropenem consumption was observed.

5.4.3 Other measures of somatic hospital consumption DDD per 100 admissions (DAD)

Because of the observed changes in the number of hospital bed-days over time, the consumption of antimicrobials in Danish hospitals may also be measured in relation to admissions (i.e. DDD per 100 admissions, DAD).

When expressed as DAD, the total consumption of antimicrobial agents in somatic hospitals showed only a slight decrease from 2013 to 2014 (from 325.2 DAD to 324.1 DAD) (Table 5.4). During the past decade, DAD increased by 12%; an increase primarily driven by a higher number of DDDs but counterbalanced by an increase in the number of hospital admissions.

With respect to individual antimicrobial groups, increases were observed for 'combination penicillins', beta-lactamase resistant penicillins and combinations of sulfonamide and trimethoprim. As observed since 2012, the consumption of 2nd generation cephalosporins and fluoroquinolones also decreased in 2014 by 8.9% and 4.3%, respectively. The same trends were seen when consumption was expressed as DBD (Table 5.3)

DDD per 1,000 inhabitants per day (DID)

The consumption of antimicrobial agents in somatic hospitals increased from 2.15 DID in 2013 to 2.18 DID in 2014. In the past decade, the DID consumed in hospitals have increased by 22% (1.76 DID in 2005), (Table A5.4 in web annex).

Katrin Gaardbo Kuhn, Maja Laursen
and Ute Wolff Sönksen

Figure 5.10. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark

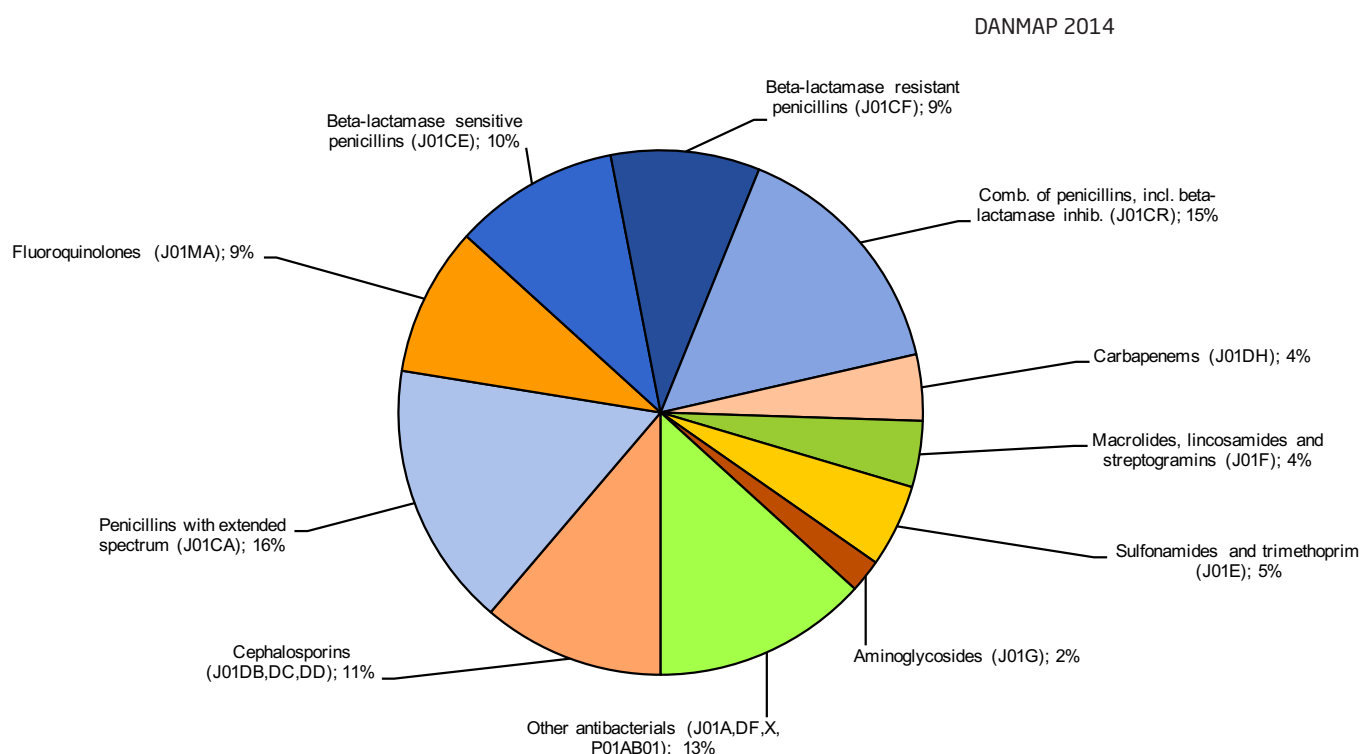


Table 5.2. Number of DDDs and packages per treated patient among leading groups of antimicrobial agents in primary health care, Denmark

ATC group ^(a)	Therapeutic group	Indicator	Year									
			2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	DDD / patient	39.0	40.9	43.0	44.4	45.2	45.9	44.0	47.6	51.6	49.9
		Packages / patient	2.0	1.9	2.0	2.0	2.0	2.0	1.9	2.1	2.1	2.1
		DDD / package	19.6	21.0	22.0	22.7	22.7	22.7	22.6	23.1	25.5	23.8
J01CA	Penicillins with extended spectrum	DDD / patient	13.9	14.2	14.4	14.7	14.8	14.9	14.8	16.1	16.7	17.2
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.7
		DDD / package	8.5	8.9	9.0	9.2	9.2	9.0	9.2	9.7	10.0	10.0
J01CE	Beta-lactamase sensitive penicillins	DDD / patient	11.3	11.5	11.7	11.8	11.8	11.8	11.8	11.8	12.0	11.9
		Packages / patient	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
		DDD / package	7.7	8.0	8.2	8.2	8.4	8.4	8.4	8.4	8.5	8.5
J01CF	Beta-lactamase resistant penicillins	DDD / patient	12.7	13.0	13.4	13.7	13.9	14.2	13.8	15.5	16.4	17.1
		Packages / patient	1.6	1.5	1.5	1.5	1.5	1.5	1.4	1.6	1.7	1.8
		DDD / package	8.0	8.6	8.7	9.0	9.1	9.3	9.6	9.7	9.4	9.7
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDD / patient	16.8	19.3	19.1	19.9	20.4	21.1	21.9	22.3	22.6	23.2
		Packages / patient	2.0	1.8	1.6	1.6	1.5	1.5	1.6	1.6	1.6	1.6
		DDD / package	9.3	10.7	11.7	12.4	13.3	13.7	14.1	14.3	14.3	14.3
J01FA	Macrolides	DDD / patient	12.4	12.6	12.4	12.5	12.5	12.2	11.5	12.4	12.6	12.8
		Packages / patient	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.6	1.6
		DDD / package	8.0	8.3	8.1	8.1	8.1	8.1	7.9	8.0	8.0	7.9
J01MA	Fluoroquinolones	DDD / patient	9.6	10.3	10.6	11.0	11.2	11.2	11.5	11.7	11.8	11.9
		Packages / patient	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDD / package	6.5	6.9	7.0	7.5	7.6	7.6	7.7	7.8	7.8	7.9
J01	Antibacterial agents for systemic use (total)	DDD / patient	17.5	17.9	17.3	18.9	19.2	19.6	19.4	20.6	21.3	21.5
		Packages / patient	2.1	2.0	1.9	2.1	2.1	2.1	2.1	2.1	2.1	2.2
		DDD / package	8.3	8.7	8.9	9.1	9.3	9.3	9.3	9.7	9.9	9.9

a) From the 2014 edition of the Anatomical Therapeutic Chemical (ATC) classification system.

DANMAP 2014

Figure 5.11. Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), Denmark

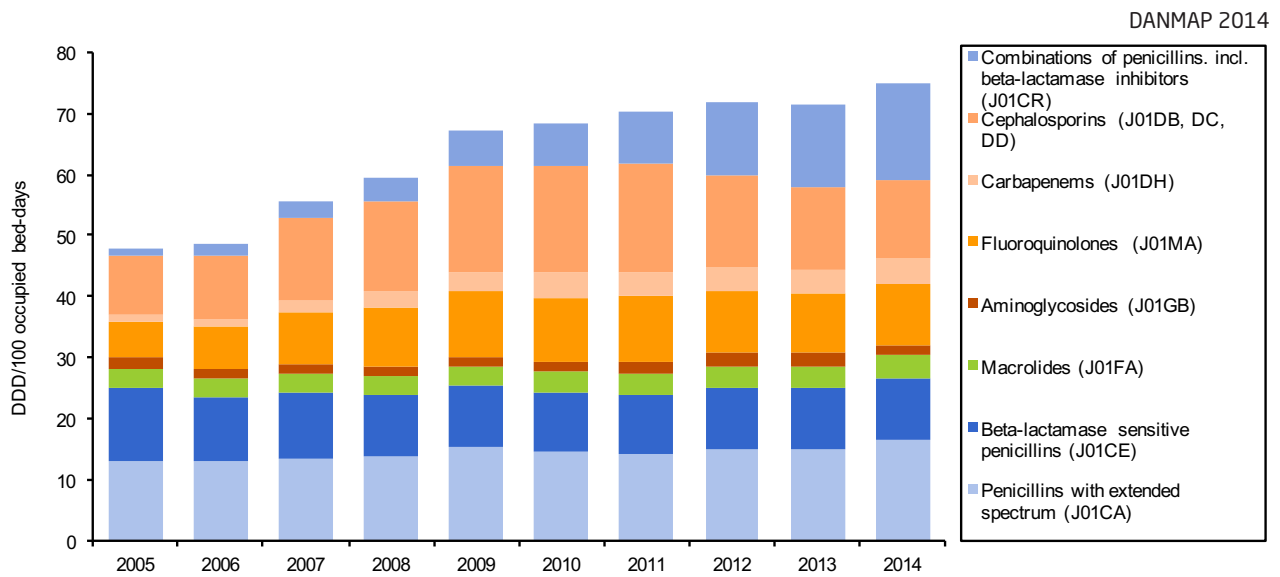


Table 5.3. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark

ATC group ^{a)}	Therapeutic group	Year									
		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	0.33	0.39	0.63	0.78	1.04	1.09	1.18	1.58	1.52	1.73
J01CA	Penicillins with extended spectrum	12.90	13.00	13.42	13.96	15.37	14.61	14.41	14.90	15.06	16.40
J01CE	Beta-lactamase sensitive penicillins	12.17	10.67	10.79	9.98	9.90	9.49	9.32	10.13	10.13	10.31
J01CF	Beta-lactamase resistant penicillins	6.71	6.51	6.70	6.81	7.40	7.71	7.30	8.37	9.07	9.57
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	1.16	1.83	2.95	4.00	5.65	7.13	8.51	12.00	13.64	16.04
J01DB	First-generation cephalosporins	0.15	0.14	0.13	0.18	0.13	0.13	0.13	0.12	0.11	0.06
J01DC	Second-generation cephalosporins	8.39	9.38	12.31	13.32	15.76	16.21	16.14	14.15	12.32	11.68
J01DD	Third-generation cephalosporins	0.83	0.83	1.03	1.25	1.42	1.26	1.39	1.07	1.08	1.02
J01DF	Monobactams	0.00	0.00	0.04	0.07	0.06	0.09	0.19	0.15	0.14	0.06
J01DH	Carbapenems	1.16	1.38	2.13	2.70	3.15	4.02	4.16	3.86	4.02	4.09
J01EA	Trimethoprim and derivatives	0.41	0.42	0.44	0.44	0.44	0.36	0.36	0.38	0.41	0.52
J01EB	Short-acting sulfonamides	0.99	0.75	0.34	0.35	0.35	0.33	0.25	0.20	0.20	0.18
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	2.11	2.12	1.52	1.95	2.28	3.04	4.11	3.33	4.21	4.70
J01FA	Macrolides	2.89	2.83	3.08	3.06	3.42	3.52	3.69	3.56	3.39	3.88
J01FF	Lincosamides	0.24	0.31	0.35	0.41	0.50	0.47	0.53	0.62	0.64	0.65
J01GB	Aminoglycosides	1.95	1.81	1.79	1.64	1.56	1.71	1.91	2.14	2.13	1.61
J01MA	Fluoroquinolones	6.14	6.74	8.16	9.53	10.71	10.44	10.70	10.02	9.77	9.88
J01XA	Glycopeptides	0.52	0.56	0.63	0.68	0.99	1.07	1.24	1.29	1.29	1.15
J01XB	Polymyxins	0.12	0.12	0.05	0.05	0.07	0.10	0.09	0.09	0.16	0.27
J01XC	Steroid antibacterials (fusidic acid)	0.25	0.28	0.28	0.26	0.31	0.34	0.27	0.23	0.22	0.23
J01XD	Imidazole derivatives	2.62	2.78	2.62	3.27	3.84	3.93	4.19	4.16	4.08	4.48
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.29	0.29	0.28	0.29	0.36	0.31	0.33	0.34	0.38	0.36
J01XX05	Methenamine	0.08	0.11	0.09	0.10	0.09	0.08	0.10	0.09	0.09	0.07
J01XX08	Linezolid	0.15	0.20	0.16	0.21	0.22	0.22	0.32	0.32	0.35	0.34
J01XX09	Daptomycin	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03
P01AB01	Nitroimidazole derivatives (metronidazole)	1.87	2.13	2.20	2.43	2.91	2.99	3.12	2.89	2.70	2.48
A07AA09	Intestinal anti-infectives (vancomycin)	1.87	2.11	2.19	2.43	2.93	2.96	3.12	2.98	2.75	2.55
J01	Antibacterial agents for systemic use (total)	66.32	67.71	74.33	80.14	90.87	93.67	97.08	98.94	99.88	104.30

a) From the 2014 edition of the Anatomical Therapeutic Chemical (ATC) classification system.

Table 5.4. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

		DANMAP 2014									
ATC group ^{a)}	Therapeutic group	Year									
		2005	2006	2007	2008 ^{b)}	2009	2010	2011	2012	2013	2014
J01AA	Tetracyclines	1.45	1.67	2.59	3.19	3.63	3.55	3.66	5.15	4.97	5.37
J01CA	Penicillins with extended spectrum	56.43	55.13	55.39	57.18	53.76	47.46	44.77	48.60	47.90	50.95
J01CE	Beta-lactamase sensitive penicillins	53.20	45.26	44.55	40.90	34.61	30.83	28.98	33.04	33.13	32.03
J01CF	Beta-lactamase resistant penicillins	29.33	27.60	27.64	27.89	25.86	25.04	22.71	27.30	29.64	29.73
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	5.09	7.77	12.17	16.37	19.74	23.15	26.47	39.14	44.60	49.81
J01DB	First-generation cephalosporins	0.67	0.60	0.55	0.72	0.46	0.43	0.41	0.40	0.37	0.20
J01DC	Second-generation cephalosporins	36.70	39.76	50.81	54.55	55.12	52.65	50.19	46.17	40.27	36.29
J01DD	Third-generation cephalosporins	3.62	3.53	4.24	5.10	4.98	4.10	4.33	3.50	3.53	3.18
J01DF	Monobactams	0.02	0.00	0.18	0.27	0.21	0.29	0.60	0.48	0.45	0.19
J01DH	Carbapenems	5.05	5.86	8.78	11.08	11.01	13.07	12.55	12.60	13.14	12.70
J01EA	Trimethoprim and derivatives	1.78	1.78	1.81	1.80	1.56	1.17	1.11	1.23	1.33	1.62
J01EB	Short-acting sulfonamides	4.32	3.18	1.41	1.43	1.21	1.09	0.78	0.63	0.62	0.55
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	9.21	8.98	6.28	7.98	7.96	9.88	12.79	10.87	13.76	14.53
J01FA	Macrolides	12.64	12.01	12.70	12.53	11.97	11.45	11.47	11.61	11.08	12.04
J01FF	Lincosamides	1.05	1.31	1.46	1.69	1.74	1.52	1.63	2.01	2.09	2.03
J01GB	Aminoglycosides	8.55	7.68	7.39	6.71	5.45	5.56	5.95	6.99	6.97	5.01
J01MA	Fluoroquinolones	26.87	28.58	33.66	39.04	37.45	33.92	33.30	32.67	31.96	30.69
J01XA	Glycopeptides	2.28	2.38	2.61	2.77	3.48	3.47	3.87	4.20	4.22	3.58
J01XB	Polymyxins	0.54	0.53	0.22	0.21	0.24	0.32	0.28	0.30	0.54	0.85
J01XC	Steroid antibacterials (fusidic acid)	1.11	1.19	1.17	1.05	1.09	1.12	0.85	0.76	0.71	0.71
J01XD	Imidazole derivatives	11.47	11.81	10.83	13.39	13.43	12.76	13.03	13.55	13.33	13.93
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	1.28	1.24	1.17	1.19	1.27	1.01	1.02	1.12	1.25	1.13
J01XX05	Methenamine	0.36	0.46	0.38	0.43	0.31	0.27	0.32	0.28	0.30	0.22
J01XX08	Linezolid	0.64	0.86	0.68	0.84	0.76	0.72	0.99	1.04	1.14	1.05
J01XX09	Daptomycin	0.00	0.00	0.03	0.06	0.06	0.07	0.05	0.06	0.07	0.10
P01AB01	Nitroimidazole derivatives (metronidazole)	8.18	9.02	9.10	9.99	10.20	9.72	9.70	9.44	8.83	7.72
A07AA09	Intestinal anti-infectives (vancomycin)	8.16	8.95	9.10	9.96	10.20	9.73	9.71	9.54	9.00	7.92
J01	Antibacterial agents for systemic use (total)	290.0	287.1	306.9	328.3	317.8	304.3	301.9	322.7	325.2	324.1

a) From the 2014 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.

Textbox 5.1

Antibiotic coverage as guidance for empirical antibiotic treatment of bacteraemia

Optimization of an antibiotic stewardship program

Objectives: When using a guideline for the empirical treatment of bacteraemia, there is a need to assess its quality to ensure its applicability and the safety of the patients. During 2011 Herlev Hospital introduced a new antibiotic guideline for empirical treatment. The primary recommendation for bacteraemia in the old guideline was cefuroxime, optionally in combination with gentamicin. We changed this recommendation to a combination therapy of ampicillin and gentamicin. There were two reasons for introducing the new guideline, firstly to increase the antibiotic coverage of the empirical treatment and secondly to reduce the consumption of cephalosporins and fluoroquinolones due to increasing rates of multiresistant bacteria and *Clostridium difficile* infections. This study aimed to develop an easily feasible method to compute the antibiotic coverage and to assess the use of coverage as a quality indicator for guidelines for empirical antibiotic treatment.

Methods: We conducted our study at Herlev Hospital, Denmark, which is a 736-bed university hospital covering a population of 425,000 inhabitants and we included all episodes of bacteraemia from January 2008 to December 2014. Episodes from the Department of Haematology and the Intensive Care Unit were also included, although the empirical guideline did not cover these departments. 5,381 episodes of bacteraemia were identified and out of these 138 fungemias were excluded. We combined results of antibiotic resistance testing with EUCAST Expert Rules by using an algorithm we developed in SAS 9.4. We defined antibiotic coverage for a given antibiotic as the number of episodes with susceptible bacteria compared with the total number of episodes tested for the antibiotic, and we computed the coverage quarterly.

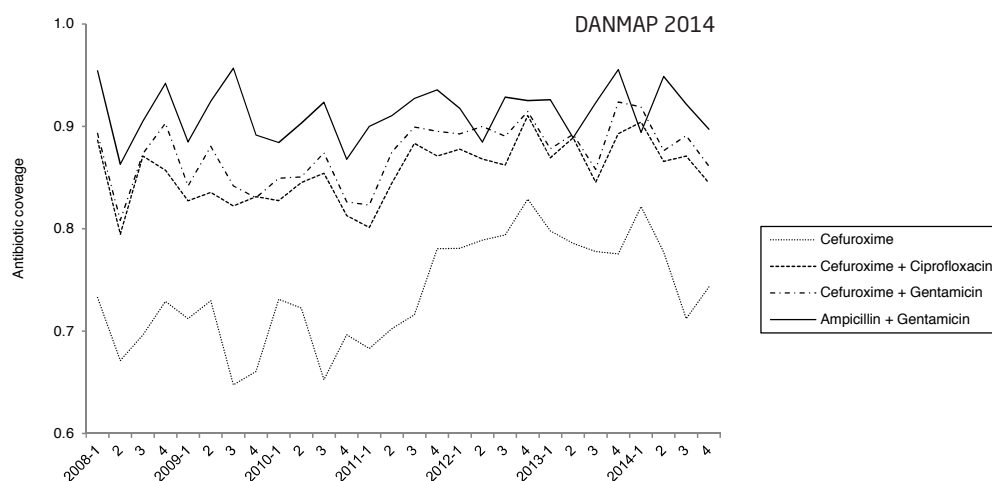
Results: During the study period, 5,243 episodes of bacteraemia, with an average of 749 episodes per year were identified. The average antibiotic coverage of cefuroxime before 2011 was 69.9% and in combination with gentamicin 85.6%. This was below our target of 90% coverage for empirical treatment. To increase the coverage we changed our recommendation to the combination of ampicillin and gentamicin, which had an average coverage of 90.7% before 2011. After the implementation of the new guideline in 2011, the coverage of ampicillin + gentamicin remained above the target with a small increase to 91.8% while the coverage of cefuroxime increased to 78.1% and in combination with gentamicin to 89.1%. In the same period the consumption of cefuroxime decreased with 77.3% to 35.2 DDD/occupied bed-days.

Conclusion: This study demonstrates that it is possible to use an algorithm to compute antibiotic coverage of bacteraemia and to use this coverage to evaluate the quality of guidelines for empirical antibiotic treatment. By decreasing the consumption of cefuroxime, we have diminished its impact on the development of resistance to cephalosporins and thus increased its usefulness, giving us a larger armamentarium for serious infections.

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Figure textbox 5.1, Antibiotic coverage as guidance for empirical antibiotic treatment of bacteraemia



Textbox 5.2

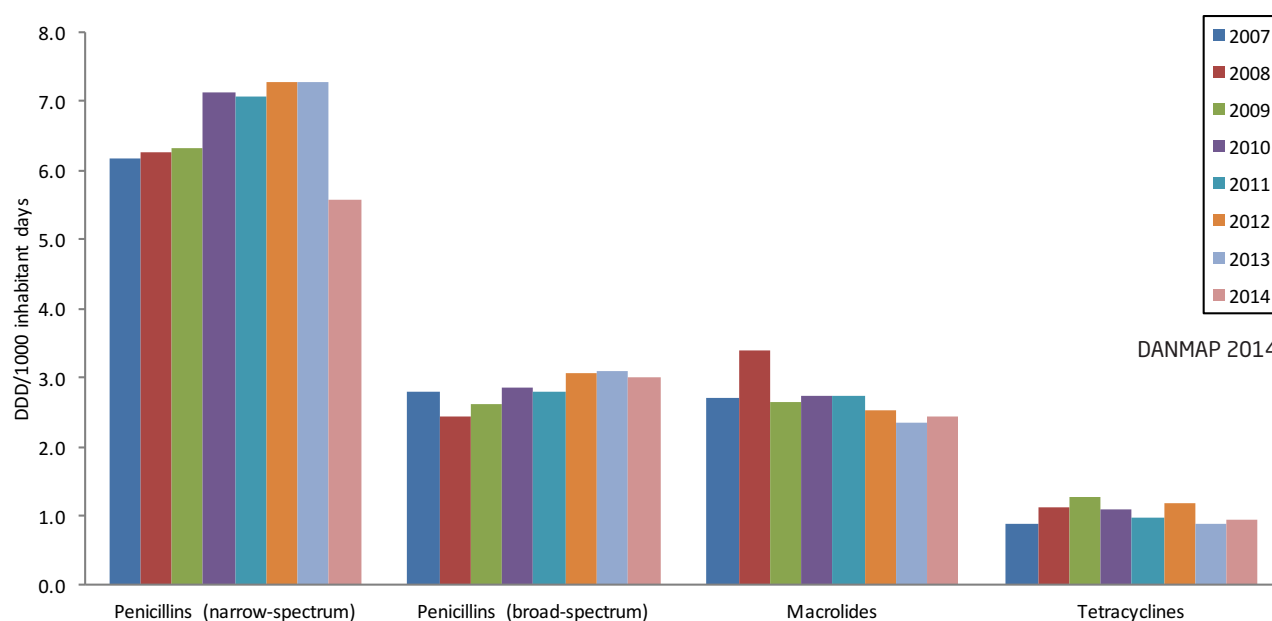
Consumption of antimicrobial agents and incidence of multi-resistant bacteria in Greenland

Background: Greenland has a population of 56,282 inhabitants (January 2014) and Nuuk is the capital with around 16,000 inhabitants. Greenland has its own Ministry of Health and the country is divided into five health regions: Qeqqa (the former health districts Sisimiut and Maniitsoq), Disko (the former health districts Aasiaat, Kangaatsiaq, Qeqertarsuaq and Qasigi-annguit), Avannaq (the former health districts Ilulissat, Uummannaq, Upernavik and Qaanaaq), Sermersooq (the former health districts Nuuk, Paamiut/Ivittuut, Tasiilaq and Illoqqortoormiut), and Kujataa (the former health districts Qaqortoq, Nanortalik and Narsaq).

The largest hospital, Dronning Ingrid's Hospital, is situated in Nuuk (185 beds). There are several smaller hospitals and health care centres in the five health regions. Around 15-16,000 persons are admitted to hospital once or several times a year. The primary health care is organized different from that in Denmark: In Nuuk, a large health care center has combined function as medical clinic, emergency room and primary health care with doctors and nurses. The settlements have nursing stations (supervised by doctors via phone or telemedicine and doctors visiting three to four times a year). Medication on prescription is free of charge. Patients with specific/serious diseases are transferred to Denmark or Iceland for further treatment (e.g. hemodialysis, cancer treatment, brain surgery etc.).

Resistant bacteria: Surveillance of resistant bacteria was begun in 2000. From then until 2014, 16 patients have been diagnosed with MRSA, 49 patients with ESBL-producing *Enterobacteriaceae*, and 45 out of 104 patients with *Clostridium difficile* infection with serotype O27. Most of these resistant bacteria were imported from Denmark or abroad, but in some cases, especially in patients with an ESBL-producing *Enterobacteriaceae*, treatment with broad-spectrum antimicrobial agents in Greenland has probably selected for these bacteria. Since 2011 there has been an increasing problem with *C. difficile* infec-

Figure 1. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1,000 inhabitants/day) 2007-2014: (a) consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines; and (b) consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin. (Note: Narrow-spectrum penicillins include benzympenicillin, phenoxymethylpenicillin and dicloxacillin, and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor).



DANMAP 2014

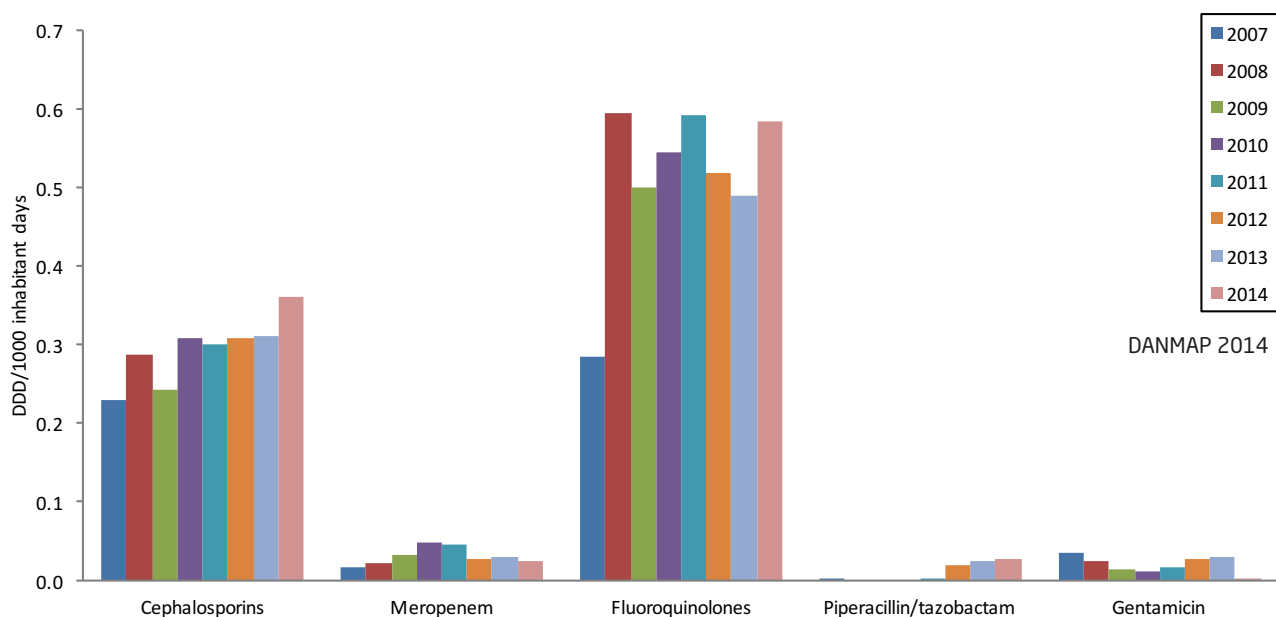
tions (mainly type O27) in the hospitals and transmission within the country has occurred. A project focusing on identification of risk factors for acquisition of *C. difficile* infections, mapping of *C. difficile* types and prevention strategies will be performed the next two years.

Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and disseminated from the National Pharmacy, who reports to Statens Serum Institut. Because of the small population, purchase and delivery to the clinics varies over time; thus fluctuations in consumption are seen. Consumption has been monitored since 2007.

The figures show the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2007 to 2014. Penicillins are grouped in narrow spectrum (including benzylpenicillin, phenoxymethylpenicillin and dicloxacillin) and broad spectrum (including ampicillin, pivampicillin, amoxicillin and amoxicillin/clavulanic acid). From 2007–2013, an increase of narrow-spectrum (18%) and broad-spectrum penicillins (12%) has been seen, but from 2013 to 2014 decreases have occurred (23% and 4% respectively). Not shown are variations within the group: Phenoxymethylpenicillin is the most consumed penicillin, accounting for 46% of penicillin consumption and 31% of the total antimicrobial consumption in 2014, but changes in the consumption over the last decade have been observed. Thus the consumption of benzylpenicillin/phenoxymethylpenicillin, amoxicillin and pivampicillin has been steadily declining, while the consumption of dicloxacillin and the combination penicillin amoxicillin/clavulanic acid has been rising simultaneously. From 2013 to 2014 an increase in broad-spectrum antimicrobial agents such as macrolides (4%), tetracyclines (5%), fluoroquinolones (19%), and cephalosporins (16%) was observed. Meropenem decreased with 19% from 2013 to 2014. From 2012 to 2014 piperacillin-tazobactam increased with 42%.

Conclusion: The consumption data of antimicrobial agents are based on purchases, and fluctuations are therefore seen from year to year. However, as a result of the increased focus on prescription of antibiotics (especially at Dronning Ingrid's Hospital where antibiotic audits are conducted and teaching carried out every year) a decrease in purchase of meropenem and an increase in purchase of piperacillin-tazobactam have been seen. Continued focus on the use of broad-spectrum antimicrobial agents - both in hospitals and in primary health care - and the incidence of multi-resistant bacteria in Greenland is very important in the future.

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Textbox 5.3

Hospital-Acquired Infections Database (HAIBA): monitoring hospital-acquired infections using existing data sources

Hospital-acquired infections (HAI) constitute a large burden for both patients and the healthcare system. An estimated 60.000 Danish patients acquire such infections each year. With increased antimicrobial resistance, treatment options become limited and prevention becomes more important. Surveillance of these infections can play a crucial role in the prevention of disease.

The Hospital-Acquired Infections Database (HAIBA) was launched on the 4th of March 2015 with the aim to provide timely and continuous data for the most frequent HAI using existing data sources. HAIBA initially showed numbers and incidences of hospital-acquired bacteremia and *Clostridium difficile* infections (CDI). Data are publicly available through eSundhed, the internet portal hosted by Statens Serum Institut which presents healthcare data at regional, hospital and municipal level. Later in 2015, surveillance data for specific surgical site infections and for urinary tract infections will be added.

Since HAIBA is based on existing data sources, there is no additional workload on healthcare personnel. Linking data from the National Patient Registry (NPR), the Danish Microbiology Database (MiBa) and regional medication modules allows computer algorithms to be created which help to identify the different types of infections.

The case definition for bacteremia is divided into two parts: (1) bacteremia based on at least one positive blood culture with a pathogenic bacterium and (2) a probable bacteremia, where three negative blood cultures were taken within 48 hours and relevant antibiotic treatment was given and/or a diagnosis code (ICD-10) for bacteremia was recorded. A bacteremia was considered hospital-acquired if the sample was taken more than 48 hours after admission and up to 48 hours after discharge.

In accordance with the European surveillance for CDI, the case definition for CDI was divided into Hospital Onset Hospital Acquired (HOHA) and Community Onset Hospital Acquired (COHA). This case definition was based on detection of *Clostridium difficile* through culture or PCR. Samples for which it was indicated that they contained a non-toxigenic strain were excluded. A CDI was considered a HOHA if the sample was taken more than 48 hours after admission and up to 48 hours after discharge. A CDI was registered as a COHA if the sample was taken between 48 hours after discharge and up to 4 weeks after discharge, or in the same period after an outpatient visit.

These data are publicly available in aggregate form, and can be accessed at different levels: national, regional, hospital and hospital department. The database contains data from both public and private hospitals, and includes cases which are associated with transfer from one hospital to another.

In the future, it will be possible for Departments of Clinical Microbiology (DCMs) to access data on individual patients in order to carry out infection control measures on a more detailed level. This will, for example, allow DCMs to look up resistance data in their own systems and closely investigate the patterns and trends in their hospitals on-site.

HAIBA is envisioned to be a practical infection control tool for microbiologists, infection control nurses and clinicians, and to provide transparency for regional and national politicians as well as citizens. Unlike prevalence studies, HAIBA allows following trends over time and therefore is valuable for more timely infection control measures. In the coming period, it will be interesting to learn from the first experiences in using HAIBA and to evaluate how it contributes to the quality of infection control.

Sophie Gubbels, Jens Nielsen, Brian Kristensen, Marianne Voldstedlund, Kåre Mølbak

More information

HAIBA surveillance data: <http://www.esundhed.dk/sundhedskvalitet/HAIBA/Sider/HAIBA.aspx>

HAIBA background: <http://www.ssi.dk/English/News/EPI-NEWS/2015/No%209%20-%202015.aspx>

CDI in HAIBA: <http://www.ssi.dk/English/News/EPI-NEWS/2015/No%2010%20-%202015.aspx>

Bacteremia in HAIBA: <http://www.ssi.dk/English/News/EPI-NEWS/2015/No%2011%20-%202015.aspx>

Contact: haiba@ssi.dk


 The logo for HAIBA (Hospital-Acquired Infections Database) features the word "HAIBA" in a bold, blue, sans-serif font. The text is centered within a light blue, horizontally-oriented oval shape that has a slight gradient and a soft shadow effect.



6

RESISTANCE IN ZOOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food. Zoonotic bacteria, such as *Salmonella* and *Campylobacter*, can develop resistance towards antimicrobial agents as a result of treatment of both animals and humans, which subsequently may lead to limited treatment possibilities or even treatment failure of human infectious diseases. Especially successful multi-resistant *Salmonella* clones have spread extensively during the last years, resulting in a very complex relationship between antimicrobial use and levels of resistance.

A more detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2014 [www.food.dtu.dk].

6.1 *Salmonella*

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

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

For *Salmonella*, DANMAP 2014 includes isolates from healthy pigs (caecum samples) and pork (carcass swabs) collected at the slaughterhouses as part of national surveillance and control programmes and from human cases. *Salmonella* isolates from broiler, layer and cattle farms as well as isolates from other types of meat (Danish and imported) are not presented as relatively few isolates were obtained. Only one isolate per farm, meat sample or human case was included in the analysis.

The report primarily presents resistance among *S. Typhimurium*. During the last ten years, the numbers of poultry flocks and meat samples infected or contaminated with *S. Enteritidis* has decreased, and therefore resistance in *S. Enteritidis* will not be presented in this report. Resistance in all *Salmonella* serotypes from pigs and Danish pork is presented for 2011 and onwards; the year when Denmark started to susceptibility test all serotypes according to EU legislation. For details on methodology see Chapter 9, Materials and Methods.

Isolates from all reported human *S. Typhimurium* cases are included. In contrast, *S. Enteritidis* isolates from human

infections are not susceptibility tested, so resistance levels including all *Salmonella* serotype cannot be calculated correctly. Thus, please note that the overall resistance levels of *Salmonella* spp. from human cases in Denmark presented in the European Summary Report on Antimicrobial Resistance [EU Summary Report on AMR 2013. ECDC/EFSA] does not include *S. Enteritidis*; a serotype which commonly has a relatively low level of resistance.

In DANMAP, *S. Typhimurium* includes the monophasic variants with antigenic formulas *S.* 4, [5],12:i:, as recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826]. In the text, generic *S. Typhimurium* indicates results only covering isolates of the non-monophasic variants.

MIC distributions for *S. Typhimurium* from pigs, pork and humans, as well as for all *Salmonella* serotypes from pigs and Danish pork in 2014 are presented in the web annex (Tables A6.1 - A6.5).

In 2014 the panel for susceptibility testing was changed. For *Salmonella*; apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin were excluded, whereas the new panel requested by EFSA includes ceftazidime, meropenem and tigecycline. A *Salmonella* isolate is considered fully sensitive if susceptible to all 13 antimicrobial agents included in the test new panel, and multi-resistant if resistant to 3 or more of the 11 antimicrobial classes (Table 9.2). Thus, 2014 calculations of fully sensitive and multi-resistance isolates cannot be directly compared with results from previous years. Please note that occurrences of fully sensitive and multi-resistant isolates were not estimated for *Salmonella* from Danish pork, as these isolates were tested using the old DANMAP panel.

6.1.1 *Salmonella* in pigs and domestic-produced pork - all serotypes

As in the previous years, *S. Typhimurium* (including the monophasic variants) and *S. Derby* were the most common serotypes isolated from Danish pigs (40% and 56%, respectively) and pork (43% and 47%, respectively) in 2014. In general, the serotype distribution in the Danish pork is similar to the distribution among isolates from pigs (Table 6.1).

Among the *Salmonella* isolates from healthy Danish pigs ($n = 173$), we observed high levels (range: 34% - 49%) of resistance to ampicillin, sulfonamide, and tetracycline (Table 6.2). Resistance to sulfonamide increased from 2013 to 2014, whereas resistance to ampicillin and tetracycline was not significantly higher than in 2013. The other tested antimicrobial agents had similar resistance levels as in 2013 (Figure 6.1).

Multi-resistance was observed in 32% of the isolates from healthy pigs; the isolates were primarily co-resistant to ampicillin, sulfonamide and tetracycline (ASuT co-resistance). As 22% of the healthy pigs tested *Salmonella* positive in 2014 [Annual Report on Zoonoses in Denmark 2014], it is estimated that multi-resistant *Salmonella* was present in approximately 7% of the Danish pigs at slaughter in 2014.

The occurrence of resistant *Salmonella* (all serotypes) from Danish pork resembles the occurrences in the isolates from pigs (Figure 6.1), however only 1% of the tested pig carcasses was estimated to be *Salmonella* positive [Annual Report on Zoonoses in Denmark 2014].

The majority of the *S. Derby* isolates from pigs were fully sensitive (50%) or resistant to tetracycline only (23%, data not presented), and the overall occurrences of resistance among all *Salmonella* serotypes in pigs and Danish pork were lower than observed among the *S. Typhimurium* isolates only. Even though *S. Derby* is very common among pigs, only few human *S. Derby* cases (N = 21) were reported in Denmark in 2014 [www.ssi.dk].

None of the *Salmonella* isolates from pigs or pork were resistant to cephalosporins (cefotaxime, ceftiofur and ceftazidime), quinolones (ciprofloxacin, nalidixic acid) or carbapenems (meropenem).

6.1.2 *S. Typhimurium* in pigs, domestic-produced and imported pork

Levels of resistance were comparable among *S. Typhimurium* isolates from Danish pigs and pork (Table 6.3), and resistance to the tested antimicrobial agents were similar to levels reported in 2013.

S. Typhimurium isolated from pigs (n = 70) had very high levels of resistance to ampicillin (66%), sulfonamide (74%), and tetracycline (66%, Table 6.3). The majority of isolates (64%) were multi-resistant; primarily with the ASuT co-resistance. The monophasic *S. Typhimurium* variants constituted 56% of the total number of *S. Typhimurium* isolates from pigs, representing 71% of the multi-resistant isolates.

The occurrence of ASuT co-resistance has been increasing among *S. Typhimurium* from pigs since 2010, partly due to an

Table 6.1. Serovar distribution (%) among *Salmonella* from pigs and pork, Denmark

Antimicrobial agent	DANMAP 2014	
	Pigs %	Pork Danish %
Derby	56	47
4,[5],12:i:-	23	28
Typhimurium	18	15
Infantis	1	8
Goettingen	1	-
Rissen	1	-
Livingstone	1	-
Not typeable	-	2
Number of isolates	173	60

Table 6.2. Resistance (%) among all *Salmonella* serotypes from pigs and pork, Denmark

Antimicrobial agent	DANMAP 2014	
	Pigs %	Pork Danish %
Tetracycline	49	42
Tigecycline	2	-
Chloramphenicol	5	7
Florfenicol	-	3
Ampicillin	34	30
Cefotaxime	0	0
Ceftazidime	0	0
Meropenem	0	-
Trimethoprim	13	10
Sulfonamide	41	38
Streptomycin	-	37
Gentamicin	1	2
Neomycin	-	7
Apramycin	-	2
Ciprofloxacin	0	0
Nalidixic acid	0	0
Colistin	2	2
Spectinomycin	-	8
Fully sensitive	39	-
Multi-resistant	32	-
Number of isolates	173	60

Note: Isolates from Danish pork has been analysed using a different test panel than isolates from pigs. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to 3 or more of the 11 antimicrobial classes (see Table 9.2).

increase in the occurrence of the monophasic variants of *S. Typhimurium* often carrying the ASuT co-resistance (Figure 6.2).

In Danish pork (n = 26), 64% of the isolates were of the monophasic variants and high levels (58%) of ASuT co-resistance were observed. As in *S. Typhimurium* isolates from pigs, the occurrence of monophasic variants has generally increased since 2010.

The increased occurrence of monophasic *S. 4,[5],12:i:-* is not an isolated Danish phenomenon, but related to new pandemic strains of *Salmonella* in Europe [Hopkins et al. 2010. Eurosurveillance 3:1]. As the changes in occurrences of antimicrobial resistance among *Salmonella* are highly influenced by the spread of such successful multi-resistant clones, the relationship between antimicrobial use and levels of resistance becomes very complex.

None of the *S. Typhimurium* isolates from Danish pigs and pork were resistant to 3rd generation cephalosporins or quinolones

in 2014. Among the eight EU Member States reporting resistance in *S. Typhimurium* (incl. monophasic variants) from pigs in 2013, most Member States reported no resistance to 3rd generation cephalosporins (cefotaxime), whereas quinolone resistance was more common ranging up to 21% of the isolates [EU Summary Report on AMR 2013. ECDC/EFSA].

6.1.3 *Salmonella* in humans

In 2014, *Salmonella* continued to be the second most frequent cause of bacterial intestinal infections in Denmark. A total of 1,122 human laboratory-confirmed cases of salmonellosis was reported (19.9 cases per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2014]. The most common serotypes were *S. Typhimurium* (including the monophasic variants) and *S. Enteritidis* with 7.6 and 4.8 cases per 100,000 inhabitants, respectively.

As in previous years, SSI collected information on travel history through phone interviews. Patients were asked about the date of disease onset and whether they had travelled abroad

Table 6.3. Resistance (%) among *Salmonella Typhimurium*^(a) from pigs, Danish pork and human cases^(b), Denmark DANMAP 2014

Antimicrobial agent	Pigs	Pork	Human cases			Total %
	%	Danish %	Domestic sporadic %	Domestic outbreak %	Travel abroad %	
Tetracycline	66	69	56	63	53	57
Tigecycline	3	-	6	0	8	4
Chloramphenicol	7	8	7	0	11	6
Florfenicol	-	8	-	-	-	-
Ampicillin	66	65	51	61	55	54
Cefotaxime	0	0	2	0	3	1
Ceftazidime	0	-	1	0	5	1
Ceftiofur	-	0	-	-	-	-
Meropenem	0	-	0	0	0	0
Trimethoprim	11	8	8	1	11	7
Sulfonamide	74	73	54	61	53	55
Streptomycin	-	73	-	-	-	-
Gentamicin	1	4	2	0	5	2
Neomycin	-	4	-	-	-	-
Apramycin	-	4	-	-	-	-
Ciprofloxacin	0	0	6	0	17	6
Nalidixic acid	0	0	4	0	11	4
Colistin	0	0	4	0	6	3
Spectinomycin	-	12	-	-	-	-
Fully sensitive	21	-	29	35	31	32
Multi-resistant	64	-	45	58	47	48
Number of isolates	70	26	163	89	64	423

^(a)Note: Isolates from Danish pork have been analysed using a different test panel than isolates from pigs and humans.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to 3 or more of the 11 antimicrobial classes (see Table 9.2).

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

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

within a seven-day period prior to the onset of the disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as “domestically acquired” if the patients had not travelled within the last week prior to the onset of the disease. Patients were categorised as of ‘unknown origin’ if no telephone interview was conducted and travel information had not been reported to the general practitioners. In 2014, travel information was obtained for 71% of all reported *Salmonella* cases [Annual report on Zoonoses in Denmark 2014].

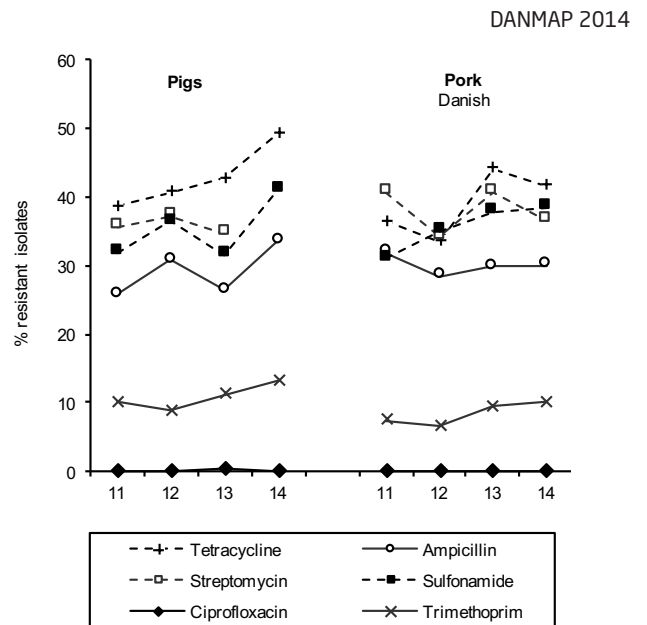
Human cases associated with a detected outbreak reported in the Annual Report on Zoonoses in Denmark in 2014 were considered ‘outbreak-related’ and all other domestic cases were considered ‘sporadic domestic’ in this report.

6.1.4 S. Typhimurium in humans

S. Typhimurium, including the monophasic variants (antigenic formula: 1,4,[5],12:i:-), was the most common serotype among the human cases (427 cases). For the first time, the monophasic variants represented more than half of these cases (230 monophasic versus 197 generic *Typhimurium*). Available isolates were susceptibility tested (n = 423).

Among the reported human *S. Typhimurium* isolates included in DANMAP, 15% of the cases were categorised as travel-associated, whereas 39% and 21% most likely had acquired their infection in Denmark as sporadic incidences or as part of detected outbreaks, respectively (Table 6.3).

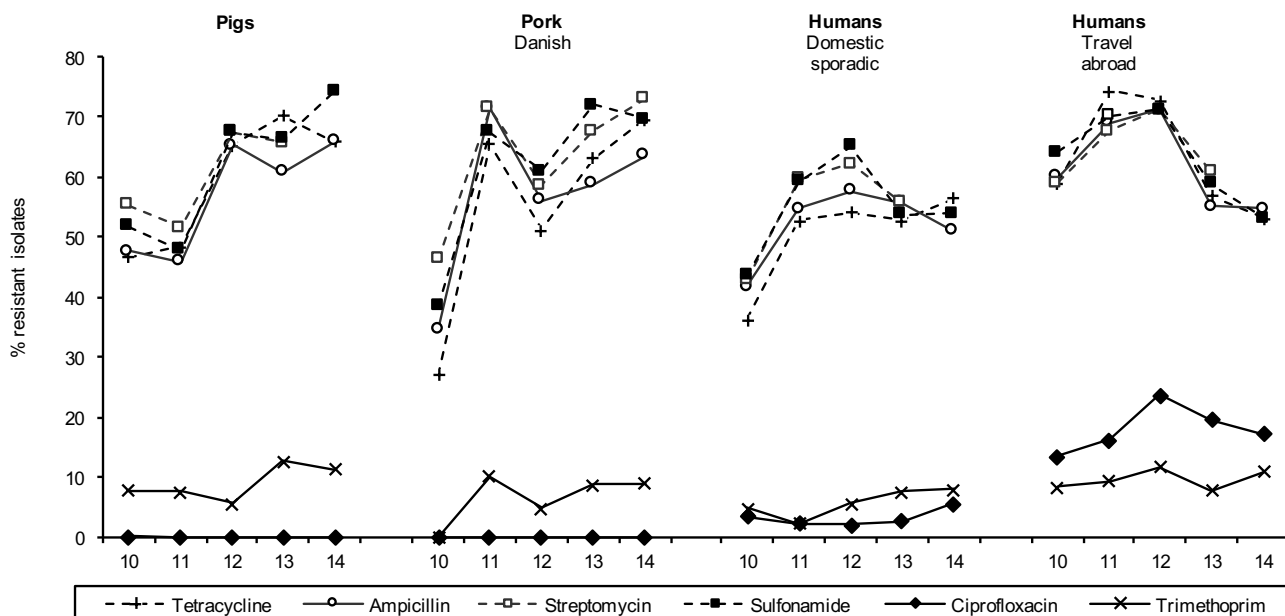
Figure 6.1. Resistance (%) in all *Salmonella* serotypes from pigs and Danish pork, Denmark



Note: The number of isolates varies between years (pigs: n = 173-404, Danish pork: n = 60-148). Before 2011, not all serotypes were susceptibility tested.

Five domestic foodborne outbreaks caused by *S. Typhimurium* were reported in 2014, representing 89 cases. Two of these outbreaks were caused by fully sensitive strains and three outbreaks were caused by monophasic strains with the typical

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



Note: The number of isolates varies between years (pigs: n = 144-434, Danish pork: n = 26-70, domestic sporadic human cases: n = 106-227 and travel related human cases: n = 51-95).

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

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

ASuT resistance profile. The proportion of outbreak-related cases in 2014 (21%) was similar to 2013 (22%).

High levels of resistance to ampicillin, sulfonamide, and tetracycline were observed. Resistance to these three antimicrobial agents occurred at similar levels (51 - 63%) among isolates from domestic and travel-associated cases (Table 6.3). Overall, 45% of all *S. Typhimurium* isolates carried the ASuT resistance profile (n = 190). Comparison with previous years showed that the proportion of isolates resistant to these antimicrobial agents have stabilised at this fairly high level in the past four years. In general, levels of resistance were similar to previous years (Figure 6.2).

The tendency seen in previous years of higher levels of resistance among travel-associated isolates compared with domestic infections is not distinct in 2014; only fluoroquinolone (ciprofloxacin) resistance was higher in isolates from travel-related cases (17%) compared with isolates from domestic cases (6%). This may reflect a higher prescription of fluoroquinolones in production animals in the countries of destination. In general, the levels of resistance in *S. Typhimurium* from domestic cases were similar to those of Danish pork and pigs, except for the occurrence of resistance to ciprofloxacin and nalidixic acid. In Denmark, fluoroquinolones are rarely used in animal husbandry (except for pet animals) since the implementation of legal restrictions in 2002-2003 [DANMAP 2010]. Ciprofloxacin or other fluoroquinolones are often used for empirical treatment of adults with severe bacterial gastroenteritis.

Regarding resistance to antimicrobial agents critical for treatment of human infections, resistance to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) was found in seven *S. Typhimurium* isolates (2% of cases); from three travel-related cases, three sporadic domestic cases and one case of unknown origin. These seven isolates were also resistant to ciprofloxacin. Co-resistance to ciprofloxacin and 3rd generation cephalosporins are of major concern as these two antimicrobial agents are considered the most important for treatment of severe salmonellosis [Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections 2009. ECDC].

Helle Korsgaard and Eva Møller Nielsen

6.2 *Campylobacter*

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

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

For *Campylobacter*, DANMAP 2014 includes randomly collected isolates from broilers and cattle at slaughter and from fresh broiler meat ready for retail. Isolates from human cases originates from three out of five geographical regions in Denmark. One isolate per farm, batch of meat or human case is included, and data are only presented if a sufficient number of isolates were obtained (>15) from a given source. For details see Chapter 9, Materials and Methods.

MIC distributions for *C. jejuni* from broilers and cattle, broiler meat and humans are presented in the web annex (Tables A6.6 - A6.8).

In 2014, the panel of tested antimicrobials was changed. For *Campylobacter*, chloramphenicol was excluded in the new panel requested by EFSA. A *Campylobacter* isolate was considered fully sensitive if susceptible to all six antimicrobial agents included in the new test panel, and multi-resistant if resistant to three or more of the five antimicrobial classes (Table 9.2). Thus, 2014 calculations of fully sensitive and multi-resistance isolates cannot be directly compared with results from previous years.

6.2.1 *C. jejuni* in broilers and domestic-produced broiler meat

In 2014, we observed moderate levels of resistance to ciprofloxacin (18%) and tetracycline (12%) among *C. jejuni* isolates from broilers (n = 165, Table 6.4) compared with 2013 (26% and 20%, respectively). The majority (74%) of isolates were fully sensitive to the six antimicrobial agents included in the test panel. From broilers, all *C. jejuni* isolates resistant to ciprofloxacin were also resistant to nalidixic acid (n = 29) and often also to tetracycline (n = 13). Only one isolate was multi-resistant (resistant to tetracycline, erythromycin, streptomycin, ciprofloxacin and nalidixic acid).

In *C. jejuni* isolates from Danish broiler meat (n = 26), resistance to ciprofloxacin (15%) and tetracycline (12%) were observed at similar levels as in 2013. Also, levels of antimicrobial resistance were comparable between *C. jejuni* isolated

from Danish broilers and Danish broiler meat (Figure 6.3), and macrolide (erythromycin) resistance has remained at a very low level for a decade.

From 2002 to 2011, a steady increase in resistance to ciprofloxacin was observed in *C. jejuni* isolates from broilers and Danish broiler meat; even after 2009 where the poultry industry stopped using fluoroquinolones in the broiler production. However, during the last five years, resistance to ciprofloxacin has fluctuated between 15% and 29% (Figure 6.3).

The consumption of antimicrobial agents in broilers is generally low, but tetracycline has been one of the most commonly used antimicrobial agents in Danish broilers over the last five years. Over the past two years their consumption of tetracycline, as well as broad spectrum penicillins and macrolide has increased considerably. However, this is not reflected in the resistance patterns in isolates from broilers or broiler meat (Figure 6.3).

6.2.2 *C. jejuni* from imported meat

In *C. jejuni* isolates from imported broiler meat (n = 45), the levels of resistance to tetracycline (64%) and nalidixic acid (76%) remained high (Table 6.4), and resistance to ciprofloxacin (82%) increased markedly compared with 2013. Compared to the Danish broiler meat, the occurrence of fully sensitive isolates (11%) was much lower in the imported meat. Almost all isolates resistant to ciprofloxacin (n = 37) was also resistant to nalidixic acid (n = 34), and often also to tetracycline (n = 25). Nine percent of the *C. jejuni* isolates were multi-resistant in 2014.

Over the past five years, the level of resistance to tetracycline and ciprofloxacin has generally been higher in isolates from imported broiler meat compared with Danish broiler meat. This corresponds with the data reported by EFSA, where Denmark has reported the lowest proportions of resistance among *C. jejuni* isolates from broiler meat [EU Summary Report 2013, ECDC/EFSA 2014].

6.2.3 *C. jejuni* in cattle

In *C. jejuni* isolates from cattle (n = 110), we observed moderate levels of resistance to ciprofloxacin (22%) and nalidixic acid (24%), whereas resistance to tetracycline (4%), erythromycin (1%) and streptomycin (1%) was low (Table 6.4). The majority (75%) of isolates was fully sensitive to the six compounds included in the test panel, none were multi-resistant. Among the resistant isolates (n = 28) almost all (n = 24) were co-resistant to ciprofloxacin and nalidixic acid.

Despite continued low consumption of fluoroquinolones in cattle, resistance to fluoroquinolones (ciprofloxacin) among *C. jejuni* from cattle has remained at a moderate level (16 - 21%) since 2006. 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 has now spread to other parts of Denmark.

Resistance to tetracycline generally increased from 2005 to 2010; however, this trend was discontinued in 2011 and in 2014 the level of tetracycline resistance is comparable to 2006-2009 (Figure 6.4).

6.2.4 *C. jejuni* in humans

In 2014, *Campylobacter* continued to be the most frequent cause of bacterial intestinal infections in Denmark. A total of 3,782 human laboratory confirmed cases of campylobacteriosis were reported (67.1 per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2014].

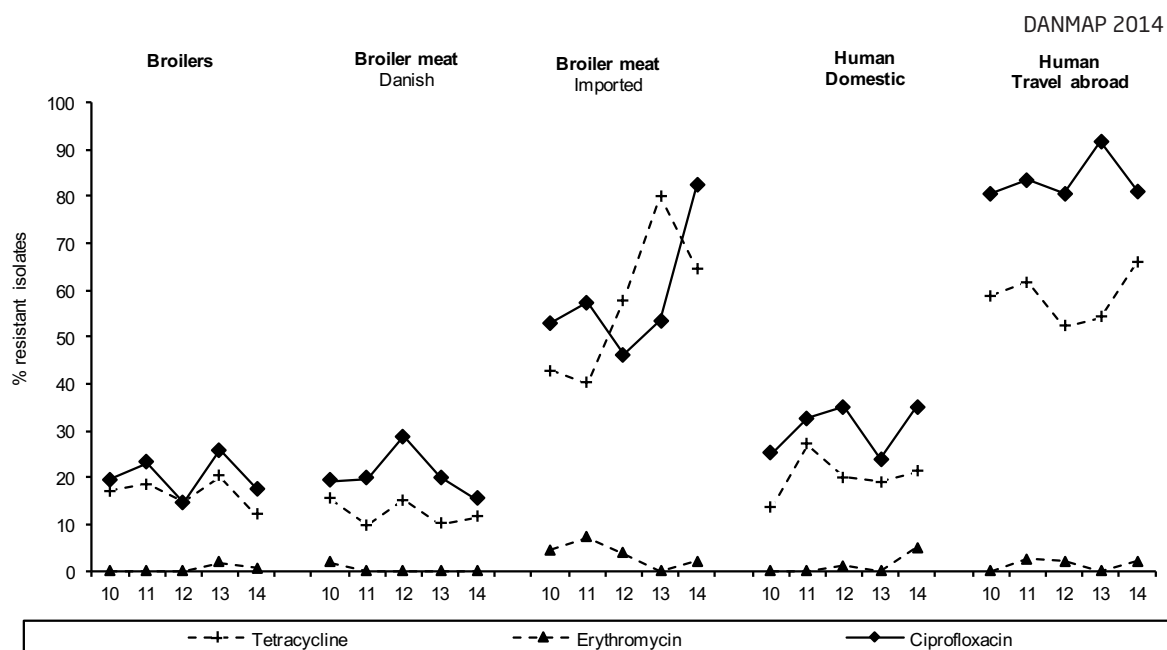
A random selection of the *Campylobacter* isolated from stool samples in three geographical regions were submitted to SSI for species identification and susceptibility testing. In 2014, 237 *C. jejuni* isolates were susceptibility tested. Among the tested isolates, 20% were from travel-associated cases and 34% were domestically acquired and 46% were of unknown

Table 6.4. Resistance (%) in *Campylobacter jejuni* from animals, meat of Danish and imported origin and human cases^{a)}, Denmark

Antimicrobial agent	DANMAP 2014						
	Cattle	Broilers	Broiler meat		Humans		Total %
	Danish %	Danish %	Danish %	Import %	Domestically acquired %	Travel abroad %	
Tetracycline	4	12	12	64	21	66	28
Erythromycin	1	1	0	2	5	2	3
Streptomycin	1	5	0	7	8	9	6
Gentamicin	0	0	0	0	1	6	3
Ciprofloxacin	22	18	15	82	35	81	46
Nalidixic acid	24	18	15	76	35	81	46
Fully sensitive	75	74	81	11	60	17	51
Multi-resistant	0	1	0	9	8	11	6
Number of isolates	110	165	26	45	80	47	237

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the five antimicrobial classes (see Table 9.2).

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

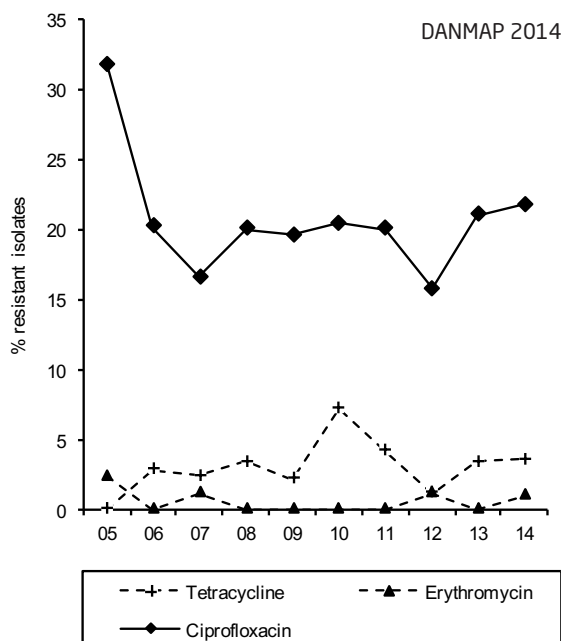
Figure 6.3. Resistance (%) in *Campylobacter jejuni* from broilers, broiler meat and human cases^a, Denmark

Note: The number of isolates varies between years (broilers: n = 41-165, Danish broiler meat: n = 26-70, imported broiler meat: n = 26-70, domestic human cases: n = 42-104 and travel-associated human cases: n = 24-78).

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

origin. As in previous years, SSI collected information on travel history through phone interviews. Patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to the onset of disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as "domestically

acquired" if the patients had not travelled within the week prior to the onset of disease.

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

Note: The number of isolates varies between years (n = 41-110).

Among the domestically acquired infections, 60% were fully sensitive to all the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower among isolates from travel associated cases (17%, Table 6.4). All multi-resistant isolates (6% of all cases) were co-resistant to ciprofloxacin and nalidixic acid as well as tetracycline (except one isolate). All isolates resistant to gentamicin or erythromycin were multi-resistant, except for one isolate resistant to erythromycin only.

The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel-associated *C. jejuni* isolates (81% and 66%, respectively) compared to isolates from domestically acquired infections (35% and 21%, respectively, Table 6.4 and Figure 6.3).

Ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with severe bacterial gastroenteritis, and the level of resistance to these antimicrobial agents is therefore of major importance. In Denmark, fluoroquinolones are rarely used in animal husbandry 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*.

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7

RESISTANCE IN
INDICATOR BACTERIA

7. Resistance in indicator bacteria

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

7.1 Enterococci

For Enterococci, DANMAP 2014 includes randomly collected *Enterococcus* isolates from healthy pigs and broilers at slaughter (*E. faecalis* only) and from domestic fresh broiler meat, pork and beef sold at wholesale and retail outlets (both *E. faecalis* and *E. faecium*). In addition, enterococci from imported broiler meat, beef and pork (*E. faecalis*) were included. We included only one isolate per farm or meat sample, and data are presented in the report where a sufficient number of isolates from a given source were obtained (>15). For details on methodology, see Chapter 9, Materials and Methods.

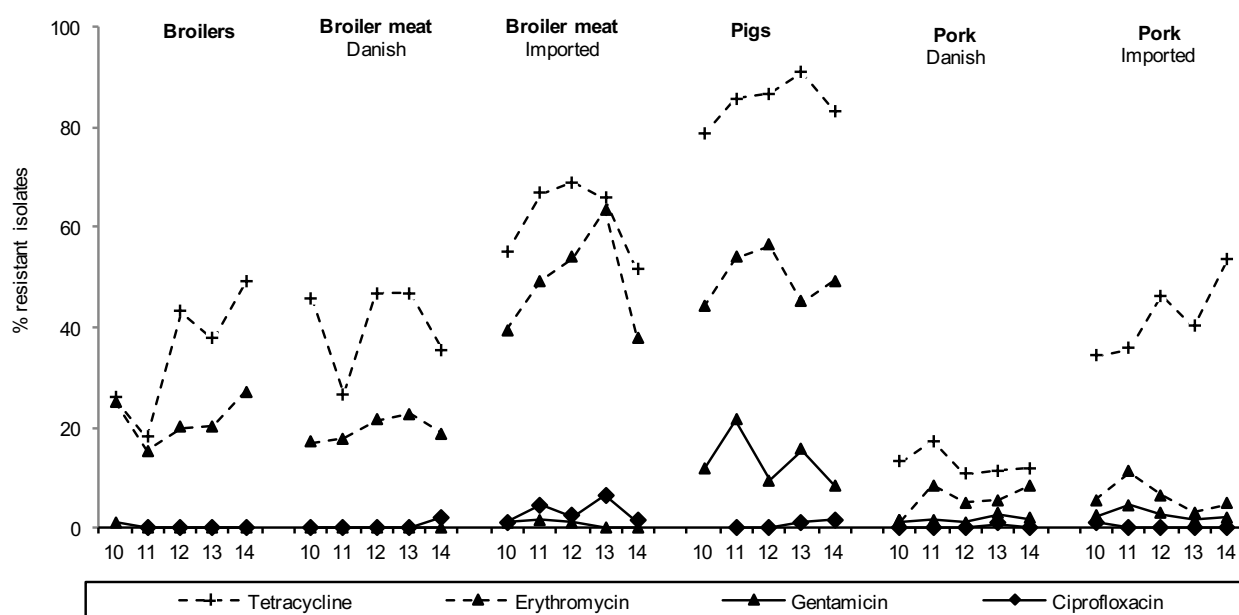
An isolate is considered multi-resistant if resistant to three or more of the ten included antimicrobial classes and fully sensitive if susceptible to all ten classes included in this survey, (Table 9.2). Please note that resistance to streptogramins (Quinupristin/dalfopristin) is not included in the definition of multi-resistance or fully sensitive isolates. In 2014, the panels used for resistance testing were changed. For *Enterococcus*; kanamycin, penicillin, salinomycin and streptomycin were excluded, whereas the new panel requested by EFSA includes daptomycin. Thus, 2014 calculations of fully sensitive and multi-resistant isolates cannot be directly compared with results from previous years.

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

7.1.1 *E. faecalis* from broilers and domestic-produced broiler meat

In *E. faecalis* isolates from broilers (n = 100), 49% of the isolates were tetracycline resistant followed by erythromycin (27%) and chloramphenicol (2%). The occurrence of resistance to tetracycline has increased over the last five years, and the level in 2014 is comparable to or higher than what was observed in 2001-2007 (27 - 46%). Only one isolate (1%)

Figure 7.1. Resistance (%) in *Enterococcus faecalis* from animals and meat of Danish and imported origin, Denmark DANMAP 2014



Note: The number of isolates varies between years (broilers: n = 86-114, Danish broiler meat: n = 34-75, imported broiler meat: n = 69-104, Pigs: n = 109-157, Danish pork: n = 84-150 and imported pork: n = 45-140).

was multi-resistant, while 42% were susceptible to all ten antimicrobial classes included in the multi-resistance definition (Table 7.1). The multi-resistant isolate was resistant to erythromycin, tetracycline and tigecycline.

The levels of resistance in *E. faecalis* isolates from domestic broiler meat were comparable to *E. faecalis* isolates from broilers (Figure 7.1). Only four isolates (8%) were multi-resistant and all of these were resistant to tetracycline and erythromycin.

7.1.2 *E. faecalis* from pigs and domestic-produced pork

A very high occurrence of resistance to tetracycline (83%) and moderate to high occurrence of resistance to erythromycin (49%) and chloramphenicol (24%) was found in *E. faecalis* isolates from pigs (n = 142). Resistance to fluoroquinolones (ciprofloxacin) was observed in one isolate (1%) from pigs. The levels of resistance were comparable with those from 2013 (Figure 7.1). All the chloramphenicol- (n = 34) and gentamicin- (n = 12) resistant isolates from pigs were resistant to tetracycline, and all but one chloramphenicol- and tetracycline-resistant isolate were resistant to erythromycin. Almost all (97%) isolates that were resistant to erythromycin (n = 70) were also resistant to tetracycline. In 2014, 26% of the isolates from pigs were multi-resistant (Table 7.1) and 15% were fully sensitive to the ten antimicrobial classes included.

A total of 88% of the *E. faecalis* isolates from Danish pork (n = 109) were fully sensitive to all ten antimicrobial classes tested (Table 7.1). The highest occurrence of resistance was seen for tetracycline (12%) followed by erythromycin (8%), and chloramphenicol (4%). Resistance to fluoroquinolones (ciprofloxacin) was not observed in isolates from Danish pork. Resistance to tetracycline remained at the same level as in 2013, at a significantly lower level than in isolates from pigs where tetracycline resistance has been 80 - 90% over the last ten years. Resistance to chloramphenicol and erythromycin are also much lower in isolates from Danish pork compared with isolates from pigs.

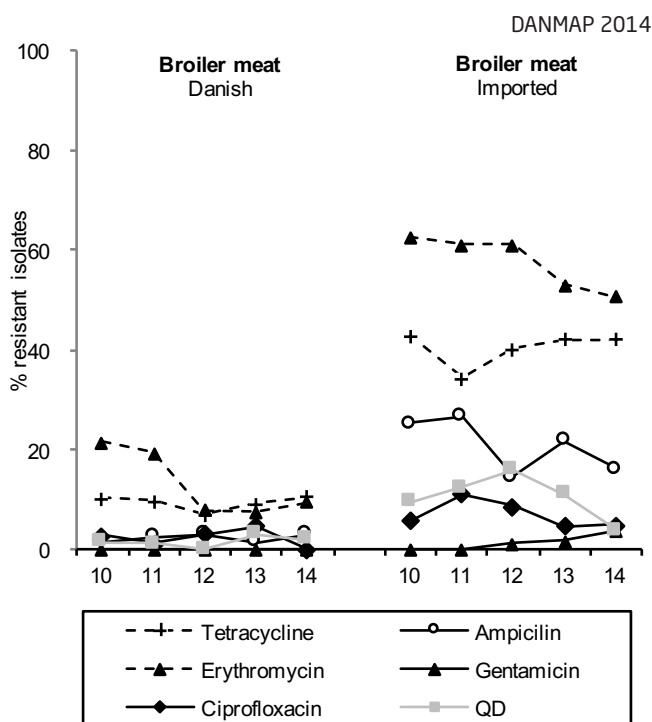
7.1.3 *E. faecalis* from domestic-produced beef

In *E. faecalis* from domestic beef (n = 57), 14% of the isolates were resistant to tetracycline and 2% resistant to erythromycin and gentamicin. Most of the isolates (86%) were fully sensitive to the ten antimicrobial classes, and only one isolate was multi-resistant (Table 7.1). Levels of antimicrobial resistances were comparable with those from 2013.

7.1.4 *E. faecalis* from imported meat

Resistance to erythromycin was lower in isolates from imported broiler meat in 2014 (n = 74) compared with 2013 (Figure 7.1). As in previous years, erythromycin resistance remained higher in *E. faecalis* from imported meat than in isolates from Danish broiler meat. None of the isolates from imported broiler meat were multi-resistant, whereas 8% of the isolates from Danish broiler meat were multi-resistant (Table 7.1).

Figure 7.2. Resistance (%) in *Enterococcus faecium* from Danish and imported broiler meat, Denmark



Note: The number of isolates varies between years (Danish broiler meat: n = 66-145, imported broiler meat: n = 64-107). QD = Quinupristin/dalfopristin.

Compared with Danish pork, *E. faecalis* isolates from imported pork (n = 105) were more frequently resistant to tetracycline, whereas resistance levels to all other antimicrobial agents were comparable. A total of 47% of the *E. faecalis* isolates from imported pork were fully sensitive to the ten antimicrobial classes compared with 88% in isolates from Danish pork. All resistant isolates from imported pork were resistant to tetracycline, and the occurrence of tetracycline-resistant isolates has increased over the last five years. In isolates from both domestic and imported pork, 5% were multi-resistant (all were resistant to tetracycline and erythromycin, Table 7.1).

The levels of resistance in *E. faecalis* isolates in Danish and imported beef (n = 42) were comparable, except for tetracycline and chloramphenicol where isolates from imported beef was more resistant (Table 7.1). Resistance to tetracycline (31%), erythromycin (12%) and chloramphenicol (10%) was detected in isolates from imported beef. Ten percent (10%) of the tested isolates from imported beef were multi-resistant compared with 2% among isolates from Danish beef. Overall, 69% of the tested isolates from imported beef were susceptible to the ten antimicrobial classes compared with 86% in Danish-produced beef.

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

Antimicrobial agent	DANMAP 2014							
	Broilers	Broiler meat		Beef		Pigs	Pork	
	Danish %	Danish %	Imported %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	49	35	51	14	31	83	12	53
Tigecycline	1	0	0	0	0	0	0	0
Chloramphenicol	2	6	1	0	10	24	4	3
Ampicillin	0	0	0	0	0	0	0	0
Erythromycin	27	19	38	2	12	49	8	5
Gentamicin	0	0	0	2	5	8	2	2
Ciprofloxacin	0	2	1	0	0	1	0	0
Vancomycin	0	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Daptomycin	0	0	0	0	0	2	0	0
Fully sensitive	42	65	41	86	69	15	88	47
Multi-resistant	1	8	0	2	10	26	5	5
Number of isolates	100	48	74	57	42	142	109	105

Note: An isolate is considered multi-resistant if resistant to three or more of the ten antimicrobial classes, and fully sensitive if susceptible to eleven antimicrobial agents included in the test panels (see table 9.2).

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

Antimicrobial agent	DANMAP 2014				
	Broiler meat		Beef		Pork
	Danish %	Imported %	Danish %	Imported %	Danish %
Tetracycline	10	42	6	10	9
Tigecycline	0	0	0	0	0
Chloramphenicol	0	0	0	0	0
Ampicillin	3	16	0	0	4
Erythromycin	9	51	0	5	0
Quinupristin/dalfopristin	2	4	0	0	0
Gentamicin	0	4	0	0	0
Ciprofloxacin	0	5	3	5	0
Vancomycin	0	0	0	0	0
Teicoplanin	0	0	0	0	0
Linezolid	0	0	0	0	0
Daptomycin	0	2	0	0	0
Fully sensitive	82	31	91	90	91
Multi-resistant	1	10	0	0	0
Number of isolates	96	81	35	21	23

Note: An isolate is considered multi-resistant if resistant to three or more of the ten antimicrobial classes, and fully sensitive if susceptible to eleven of the twelve antimicrobial agents included in the test panels (excluding quinupristin/dalfopristin, see table 9.2).

7.1.5 *E. faecium* from domestic-produced broiler meat, beef and pork

In *E. faecium* isolates from Danish broiler meat (n = 96), resistance was observed to tetracycline (10%), erythromycin (9%) and ampicillin (3%); and over the last five years, resistance to erythromycin has decreased. Only one isolate was multi-resistant (resistant to tetracycline, erythromycin and ampicillin), while 82% were susceptible to the ten antimicrobial classes included (Table 7.2). Note that resistance to quinupristin/dalfopristin is not included in the estimation of multi-resistant and fully sensitive *E. faecium* isolates.

In *E. faecium* isolates recovered from Danish pork (n = 23), resistance was observed to only tetracycline (9%) and ampicillin (4%) (Table 7.2). No multi-resistant isolates were detected, and 91% of the tested isolates were fully susceptible to the ten antimicrobial classes included.

In *E. faecium* from Danish beef (n = 35), few isolates were resistant to tetracycline (6%) and ciprofloxacin (3%). No isolates were multi-resistant, while 91% of tested isolates were susceptible to the ten antimicrobial classes.

7.1.6 *E. faecium* from imported meat

As in 2013, resistance to tetracycline, ampicillin, erythromycin and ciprofloxacin was significantly more frequent in *E. faecium* from imported broiler meat (n = 81) than in isolates from Danish broiler meat (n = 96, Table 7.2 and Figure 7.2). Overall, 10% of the *E. faecium* isolates from imported broiler meat were multi-resistant; all were resistant to both tetracycline and erythromycin.

Low levels of resistance were found in *E. faecium* from imported beef, comparable to the finding in isolates from Danish beef.

All levels of resistance in *E. faecium* from the different types of meat investigated in 2014 were comparable to levels in 2013.

7.1.7 One Health perspective

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

Apart from tetracycline, significantly higher resistance to chloramphenicol and erythromycin was found among *E. faecalis* isolated from pigs when compared with broilers, reflecting the higher usage of antimicrobials in pigs. All of these antimicrobial agents are used for human treatment (though chloramphenicol for eye infections only).

As in previous years, the occurrence of antimicrobial resistance in *E. faecalis* from Danish pork is much lower than from Danish pig. This is not observed among *E. faecalis* from broiler meat, where we find equal levels of resistance in broilers and broiler meat. This may indicate that enterococcal populations in live animals and on pork constitute different sub-populations. Pork cuts for sampling are collected from wholesale and retail outlets. It is possible that enterococci on these products may reflect the processing environment rather than direct contamination of the meat during slaughter and dressing. In contrast, the cutting of broilers is done in slaughter plants, which may explain why the enterococcal populations from live broilers and from broiler meat appear more similar.

The tendency that imported meat (both for *E. faecalis* and *E. faecium*) contains isolates with higher levels of resistance is generally seen for broiler meat, beef and pork. All multi-resistant enterococci were tetracycline and erythromycin resistant no matter what reservoir they were isolated from, indicating a close physical connection between these two antimicrobial resistance mechanisms. Furthermore, in all reservoirs, isolates that were tetracycline and erythromycin resistant was found in higher numbers than multi-resistant isolates, indicating that additional resistance mechanism(s) may be added to this structure.

Lars Bogø Jensen, Tina Birk and Helle Korsgaard

7.2 Indicator *Escherichia coli*

For indicator *E. coli*, DANMAP 2014 includes randomly collected isolates from healthy pigs, broilers and cattle at slaughter and from fresh broiler meat, beef and pork sold at wholesale and retail outlets (domestic and imported meat). We included only one isolate per farm or meat sample, and present only data where a sufficient number of isolates were obtained (>15). For details on methodology see Chapter 9, Materials and Methods.

The MIC distributions and occurrence of resistance among *E. coli* are presented in the web annex (Tables A7.4 and A7.5). Textbox 7.1 presents data for *E. coli* isolated after selective enrichment with 3rd generation cephalosporins (ceftriaxone) examined for carbapenemase and ESBL resistance genes.

In 2014, the panel of tested antimicrobials was changed. For *E. coli*; apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin have been excluded, whereas the new panel requested by EFSA includes ceftazidime, meropenem and tigecycline. An *E. coli* isolate is considered fully sensitive if susceptible to all 13 antimicrobial agents included in the new test panel, and multi-resistant if resistant to 3 or more of the 11 antimicrobial classes (Table 9.2). Thus, 2014 calculations of fully sensitive and multi-resistance isolates cannot be directly compared with results from previous years.

7.2.1 Indicator *E. coli* from broilers and domestic-produced broiler meat

Among the *E. coli* isolates from broilers (n = 191), a significant increase in resistance to gentamicin (from none to 3%) and nalidixic acid (3% to 11%) was observed compared with observations from 2013 and 2012; while reduced resistance was seen for tetracycline (15% to 6%), ampicillin (26% to 14%), trimethoprim (19% to 7%) and sulfonamide (26% to 13%) (Table 7.3). Gentamicin-resistant isolates were only found in broilers tested during 2014 (n = 6).

In contrast, the use of antimicrobial agents in the broiler production has increased since 2012. This increase applies to several therapeutic groups e.g. tetracycline, penicillins (not beta-lactamase sensitive), sulfonamides and trimethoprim (Table 4.1); a trend not reflected in the reduced resistance observed in broiler isolates in 2014.

We found 3% of the *E. coli* broiler isolates resistant to nalidixic acid and 6% resistant to ciprofloxacin. From 2003 to 2007, fluoroquinolone consumption in poultry was significantly higher than for the other production animals in Denmark, because antimicrobial agents approved for poultry were limited to amoxicillin and fluoroquinolones. However, fluoroquinolones have not been used in the broiler production for the last five years.

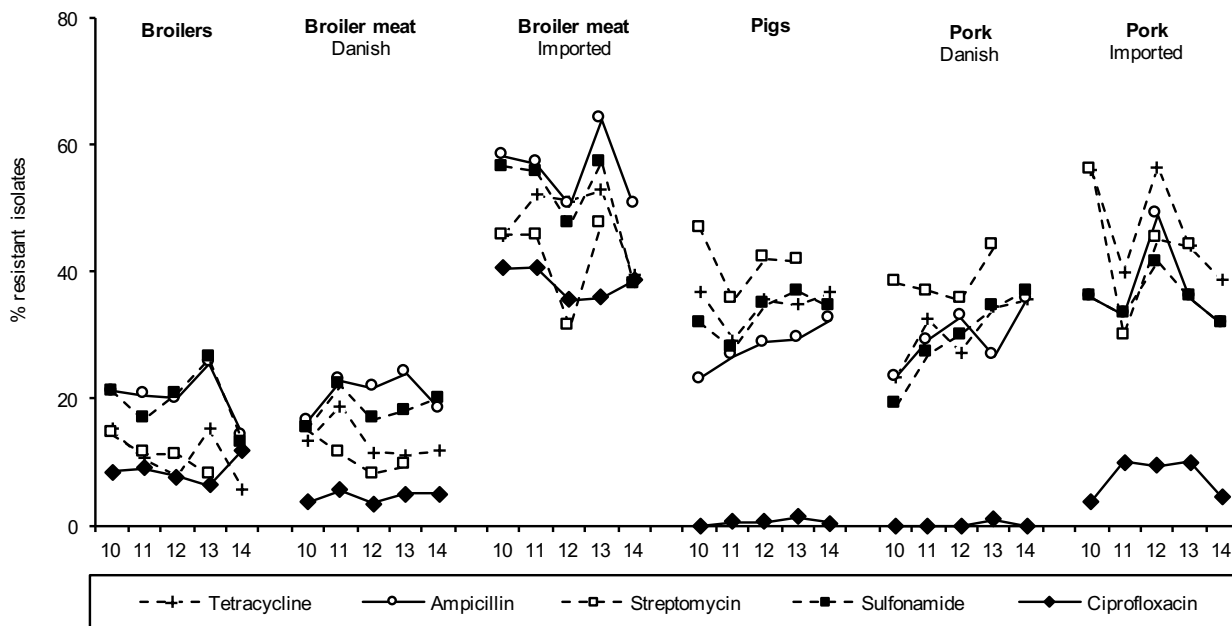
Table 7.3. Resistance (%) among *Escherichia coli* from animals and meat of Danish and imported origin, Denmark DANMAP 2014

Antimicrobial agent	Broilers		Broiler meat		Cattle		Beef		Pigs		Pork	
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %
Tetracycline	6	12	39	12	11	9	37	36	39			
Tigecycline	0	0	0	0	0	0	0	0	0			
Chloramphenicol	0	1	5	5	4	0	2	5	5			
Ampicillin	14	19	51	8	11	9	33	36	32			
Cefotaxime	0	1	3	0	4	0	0	0	0			
Ceftazidime	0	1	2	0	2	0	0	0	0			
Meropenem	0	0	0	0	0	0	0	0	0			
Trimethoprim	7	14	30	2	4	9	24	30	20			
Sulfonamide	13	20	38	12	9	9	34	37	32			
Gentamicin	3	2	2	0	0	0	1	3	5			
Ciprofloxacin	12	5	39	0	4	0	0	0	5			
Nalidixic acid	11	4	36	0	2	0	0	0	5			
Colistin	0	0	1	0	0	0	0	0	0			
Fully sensitive	70	70	24	86	83	84	43	48	48			
Multi-resistant	7	13	39	7	7	9	25	29	25			
Number of isolates	191	135	160	136	46	32	209	73	44			

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the eleven antimicrobial classes (see Table 9.2).

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

DANMAP 2014



Note: The number of isolates varies between years (broilers: n = 115-191, Danish broiler meat: n = 116-197, imported broiler meat: n = 136-177, pigs: n = 146-209, Danish pork: n = 68-93 and imported pork: n = 30-53). Isolates are not tested for resistance to streptomycin in 2014.

In *E. coli* isolates (n = 135) from Danish broiler meat, 20% of the isolates were resistant to sulfonamide followed by ampicillin (19%) and trimethoprim (14%). Occurrence of resistance to ciprofloxacin and nalidixic acid remained at a low level (5% and 4%, respectively), and generally, levels of resistance were comparable with the levels observed in 2013.

Higher levels of resistance to tetracycline (12%) and trimethoprim (14%) was found in *E. coli* from Danish broiler meat than in isolates from Danish broilers, while lower prevalence was found for the quinolones (nalidixic acid and ciprofloxacin) (Table 7.3).

Among *E. coli* isolates from Danish broilers and broiler meat, 70% of the tested isolates were fully sensitive to all tested antimicrobials, while 7% and 13%, respectively, were multi-resistant. Among the multi-resistant isolates, all of these were resistant to sulfonamide (n = 31), 93% resistant to ampicillin (n = 29) and around 50% to tetracycline (n = 16).

Resistance to 3rd generation cephalosporins (ceftiofur and cefotaxime) was not found in broiler isolates recovered using the non-selective method, but from 1 - 2% of the *E. coli* isolates from Danish broiler meat. Based on a more sensitive selective enrichment method, ESBL-producing *E. coli* was recovered from 9% of the tested samples of Danish broiler meat (n = 137, see Textbox 7.1). None of the isolates showed any indication of carbapenemase (meropenem) resistance.

7.2.2 Indicator *E. coli* from cattle and domestic-produced beef

In *E. coli* isolated from cattle (n = 136), 12% of the isolates were resistant to tetracycline and sulfonamide, followed by ampicillin (8%) (Table 7.3). Occurrences of resistance were comparable with levels in 2013.

As in isolates from cattle, resistance in *E. coli* isolates from Danish beef (n = 46) was low, and at the same level as in 2013. One isolate from Danish beef was resistant to ciprofloxacin. Among the isolates from cattle and Danish beef, 7% were multi-resistant, while 86% and 83% were fully sensitive, respectively (Table 7.3). Among the multi-resistant isolates, all were resistant to ampicillin and sulfonamide (n = 12); and 83% to tetracycline (n = 10).

None of the isolates from cattle and only two isolates from Danish beef were resistant to 3rd generation cephalosporins (ceftiofur and cefotaxime). No ESBL-producing *E. coli* was recovered from the tested samples of Danish beef (n = 121) using the selective enrichment method (see Textbox 7.1). None of the isolates showed any indication of carbapenemase (meropenem) resistance.

7.2.3. Indicator *E. coli* from pigs and domestic-produced pork

In *E. coli* from pigs (n = 209), 37% of the isolates were resistant to tetracycline followed by sulfonamides (34%) and ampicillin

(33%) (Table 7.3). The resistance levels were comparable to those observed in 2013, and the trend analysis over the period 2010-2014 shows an increasing occurrence of resistance to ampicillin from 23% in 2010 to 34% in 2014 (Figure 7.3).

In the *E. coli* isolates from Danish pork (n = 73), 37% were resistant to sulfonamide followed by tetracycline (36%) and ampicillin (36%). Generally, levels of resistance were comparable to 2013 (Figure 7.3 and Table 7.3).

Among the isolates from pigs and Danish pork, 25% and 29% of the isolates were multi-resistant while 43% and 48% were fully susceptible to all tested antimicrobials, respectively. Among the multi-resistant isolates from pigs and Danish pork (n = 74), almost all were resistant to ampicillin (n = 73) and sulfonamide (n = 72); and 70% to tetracycline (n = 52).

Quinolone resistance was not detected among isolates from pigs or Danish pork reflecting the low usage due to a voluntary ban by the industry in 2001. Furthermore, no resistance to 3rd generation cephalosporins (ceftiofur and cefotaxime) was detected in 2014 using non-selective methods. Based on a more sensitive selective enrichment method, ESBL-producing *E. coli* was recovered from one of the tested samples of Danish pork (n = 182, see Textbox 7.1). None of the isolates showed any indication of carbapenemase (meropenem) resistance.

7.2.4 Indicator *E. coli* from imported meat

In *E. coli* from imported broiler meat (n = 160), 51% of the isolates were resistant to ampicillin followed by 38 - 39% resistance to tetracycline, sulfonamide and ciprofloxacin. Five isolates (3%) were resistant to 3rd generation cephalosporins. A significant reduction in prevalence of resistance to tetracycline, ampicillin, cefotaxime and sulfonamide was detected when comparing results from 2014 with 2013 (Figure 7.3). Compared with Danish broiler meat, *E. coli* isolates from imported broiler meat had higher levels of resistance to tetracycline, ampicillin, trimethoprim, sulfonamide, nalidixic acid and ciprofloxacin. Furthermore, a higher proportion of the isolates from imported broiler meat were multi-resistant (39%), and less were fully sensitive (24%) to all tested antimicrobials, compared with the isolates from Danish broiler meat (Table 7.3). Among the multi-resistant isolates from imported broiler meat (n = 62), 87% were resistant to sulfonamide (n = 54), 85% to ampicillin (n = 53), while 63% (n = 39) were resistant to tetracycline.

In *E. coli* from imported beef (n = 32), 9% of the isolates were resistant to tetracycline, ampicillin, sulfonamide and/or trimethoprim. These levels are comparable with 2013 and the findings in Danish beef. No resistance to nalidixic acid and ciprofloxacin was observed. Three isolates were multi-resistant (9%), all of them were resistant to ampicillin, sulfonamide and trimethoprim, while one isolate was also resistant to tetracycline (Table 7.3).

In *E. coli* isolates from imported pork (n = 44), 39% of the isolates were resistant to tetracycline followed by ampicillin (32%) and sulfonamide (32%). Two isolates were resistant to nalidixic acid and ciprofloxacin. No changes in resistance levels were observed from 2014 to 2013, and resistance in imported pork is comparable to Danish pork (Table 7.3). Among isolates from imported pork, 25% were multi-resistant while 48% were fully susceptible to all tested antimicrobials. Among the multi-resistant isolates (n = 8), all isolates were resistant to sulfonamide, while seven were resistant to tetracycline and ampicillin.

Resistance to 3rd generation cephalosporins (ceftiofur and cefotaxime) was only found in isolates from imported broiler meat (3%). Based on a more sensitive selective enrichment method, ESBL-producing *E. coli* was recovered from 25% of the tested samples of imported broiler meat (n = 167), but not from the tested samples of imported beef (n = 87) and pork (n = 163) (see Textbox 7.1). None of the isolates showed any indication of carbapenemase (meropenem) resistance.

7.2.5 One Health perspective

We use *E. coli* as an indicator organism for antimicrobial resistance because it is a commensal in both mammals and birds and commonly present on raw meat. Using phenotypic resistance as a marker, our data indicate that *E. coli* in slaughter animals and in the derived meat constitute overlapping bacterial populations (Table 7.3). Therefore, meat has the potential to act as a vehicle transferring antimicrobial resistance from food animals to humans.

Transfer of genes coding for resistance to antimicrobial agents that are critically important in human medicine such as 3rd generation cephalosporins is of major concern, as is the transmission through food of *E. coli* resistant to gentamicin and fluoroquinolones. Resistance to fluoroquinolones is generally low in Danish animals (only detected in broilers), but higher in imported meats, in particular imported broiler meat (Figure 7.3). Resistance to fluoroquinolones (ciprofloxacin) was detected in *E. coli* from Danish beef, but at low levels. A significant reduction in ciprofloxacin resistance was seen when comparing isolates from broilers with isolates from Danish broiler meat. Resistance to cephalosporins is presently increasing internationally in the animal reservoir, causing great concern both nationally and internationally. Cephalosporin resistance commonly resides on mobile genetic elements, e.g. plasmids, and therefore may be transferred between bacteria, in addition to clonal spread of *E. coli* strains. No resistance to cephalosporins was detected using the DANMAP setup in isolates from broilers, pigs or cattle in 2014, but it was found in low levels in Danish broiler meat and Danish beef.

The study of ESBL /AmpC in fresh meat available in Danish retail (Textbox 7.1) and among patients with ESBL-producing *E. coli* blood stream infections (textbook 8.1) shows that in Denmark consumption of meat may currently be considered an

insignificant source for the human infections. Only for one of the 241 human isolates could an isolate with matching ESBL gene and MLST type be identified among the typed isolates from meat.

Multi-resistance is of importance because high levels of resistance decrease the number of first choice antibiotics available for treatment of infections in humans and because it increases the risk of selection of antimicrobial resistance. As an example, if resistance to fluoroquinolone (chromosomal) or ESBL (plasmid) develops, the risk of co-selection through use of "old antimicrobial agents" (such as tetracycline, sulfonamide and penicillin) in the same bacterium increases with the occurrence of multi-resistance. Resistance to these antimicrobial agents is common in *E. coli* from Danish pigs and pork, as well as from imported broiler meat, where we also observed high levels (25%-29%) of multi-resistance (Table 7.3).

With co-resistance to critically important antimicrobial agents, the risk of maintenance and spread of the critically important antimicrobial resistance through use of another class of antimicrobial agents increases markedly, in particular when resistance determinants are located on mobile genetic elements. High levels of resistance and multi-resistance contribute to a reduced number of antimicrobial agents available for use in human medicine, thus limiting the solutions for treating human illness, see section 8.1 regarding human *E. coli* infections.

Lars Bogø Jensen, Tina Birk and Helle Korsgaard

Textbox 7.1

Reduced occurrence of ESBL-producing *Escherichia coli* in meat from Danish retail and comparison to isolates from human bloodstream infections

Background: Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide in both humans and production animals. Lately, several studies have found similar ESBL genes, plasmids and clones of *E. coli* isolates from animals, meat and human infections, suggesting a zoonotic link. Furthermore, the occurrence of carbapenemase-producing Enterobacteriaceae (CPE) is an even greater threat in human medicine, since carbapenems are the last line antimicrobial agents for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Carbapenemase-producing bacteria are currently almost exclusively isolated from human cases, primarily in the non-zoonotic *Klebsiella pneumoniae*, but carbapenemase resistant *E. coli* have recently been discovered in pets, pigs and rivers [Woodford et al, 2014 J Antimicrob Chemother 69:287-291]. To date no carbapenemase resistant bacteria has been found in the Danish surveillance of food or food producing animals.

ESBL and carbapenem resistance can be driven by usage of cephalosporins and/or carbapenems as well as antimicrobial usage in general, through co-selection. 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 (Table 4.2). Cephalosporins have not been used in the Danish broiler production for at least a decade, but were before May 2012 used in the foreign production of grandparent animals of the Danish broilers [DANMAP 2012]. At present, carbapenems are not licensed for use in food-producing animals in Denmark or other parts of the world [EFSA Journal 2013;11(12):3501].

The aim of this study was to investigate the occurrence of carbapenemase- and ESBL-producing *E. coli* in fresh meat at retail and investigate if there was a clonal relationship between the ESBL-producing *E. coli* from retail meat and strains from human bloodstream infections (see section 10, for definition of ESBL).

Materials and methods: During January through December 2014, samples of broiler meat (Danish: n = 150, imported: n = 167), beef (Danish: n = 121, imported: n = 87) and pork (Danish: n = 182, imported: n = 163) were collected randomly at wholesale and retail outlets in all regions of Denmark. ESBL-producing *E. coli* were isolated from 5 g of meat after culturing in selective McConkey media enriched with ceftriaxone (1 µg/ml). Whole genome sequencing (WGS) was used to describe the genetic background for ESBL- and carbapenem-resistance. The reads were assembled de novo prior to prediction of genes, and the web-server ResFinder (www.genomicepidemiology.org) was used to identify acquired ESBL and CPE genes in the WGS data. In isolates where no genes were detected, the sequences were investigated for up-regulation of chromosomal AmpC by use of CLCbio Genomic Workbench v8. The multi locus sequence typing was determined using the web server: www.genomicepidemiology.org (version 1.7).

ESBL-producing *E. coli* isolates from meat and from human bloodstream infections obtained during 2014 (see Textbox 8.1) were compared for clonal relationship by whole-genome-based SNPs analysis, if they had the same ESBL gene and belonged to the same sequence types (STs).

Results: None of the isolates from Danish or imported meat contained any known carbapenemase genes.

Ceftriaxone resistant *E. coli* was found in 9% of Danish broiler meat and 25% of imported broiler meat samples. As in previous years, the occurrences were generally low (0-1%) in the Danish and imported beef and pork; only one sample from Danish pork contained ceftriaxone resistant *E. coli* (CTX-M-1) in 2014.

The occurrence of ESBL-producing *E. coli* in Danish broiler meat has decreased significantly, since the highest occurrence was detected in 2011 (44% in 2011, 25% in 2013 and 9% in 2014, Figure 1). The decrease was mainly due to a reduction in the occurrence of CMY-2 (Table 1). A significantly lower level of ESBL-producing *E. coli* was also observed in imported broiler meat from 2013 to 2014 (52% to 25%, Figure 1), where most of the reduction was due to less samples containing CMY-2, but also

Table 1. Distribution (%) of ESBL enzymes detected in *E. coli* isolates from broiler meat, Denmark DANMAP 2014

Enzyme	Danish						Import					
	2009	2010	2011	2012 ⁽¹⁾	2013	2014 ⁽¹⁾	2009	2010	2011	2012 ⁽¹⁾	2013 ⁽¹⁾	2014 ⁽¹⁾
CMY-2	50	81	95	88	83	23	48	37	62	36	38	33
CTX-M-1	50	19	5	11	14	77	25	44	23	40	32	45
CTX-M-14	-	-	-	-	-	-	-	-	-	2	-	-
CTX-M-2	-	-	-	-	-	-	3	3	-	-	-	-
HV-28	-	-	-	-	-	-	-	-	-	1	-	-
SHV-1	-	-	-	-	-	-	-	-	-	-	1	-
SHV-12	-	-	-	-	3	-	16	10	12	19	18	10
SHV-2a	-	-	-	-	-	-	-	1	-	-	-	-
TEM-11	-	-	-	-	-	-	-	-	-	-	1	-
TEM-116	-	-	-	1	-	-	-	-	-	-	1	-
TEM-20	-	-	-	-	-	-	1	-	-	-	-	-
TEM-30	-	-	-	-	-	-	-	-	-	1	3	-
TEM-52	-	-	-	-	-	-	1	4	1	-	3	-
TEM-52B	-	-	-	-	-	-	-	-	-	-	-	5
Unknown	-	-	-	-	-	-	4	2	1	2	3	-
AmpC upregulation	-	-	-	-	-	-	1	-	-	-	-	7
Total number of :												
Enzymes	4	16	58	76	36	13	69	113	73	116	97	42
Meat samples	121	187	132	206	146	150	193	226	152	178	172	167

Note: Proportion of total number of enzymes.

1) In some isolates more than one enzyme was detected: CTX-M-1 and CMY-2, SHV-12, -28 or TEM-11, -30; CMY-2 and SHV-12 or TEM-30, -52, -116; SHV-12 and TEM-30.

CTX-M-1 and SHV-12 were found less frequently. The ESBL occurrence was significantly higher in imported meat than in broiler meat produced in Denmark.

As in previous years, the most common ESBL enzymes in Danish and imported broiler meat were CTX-M-1 and CMY-2 (Table 1). More than one ESBL enzyme were detected in isolates from two samples of Danish broiler meat (CTX-M-1 and CMY-2; CTX-M-1 and SHV-28) and in one sample of imported broiler meat (CTX-M-1 and CMY-2).

The 27 CTX-M-1 producing *E. coli* isolates from broiler meat and the one from pork belonged to 19 sequence types (STs). The 14 CMY-2 producing isolates from broiler meat belonged to 11 STs. The two *E. coli* isolates from broiler meat co-producing CTX-M-1 and CMY-2 both belonged to two STs also detected among the broiler *E. coli* isolates producing CTX-M-1 only. The four SHV-12 producing *E. coli* isolates from broiler meat belonged to four different STs.

Similar combination of ESBL geno-types and STs were detected from human bloodstream *E. coli* isolates and *E. coli* isolates from imported broiler meat on five occasions; CTX-M-1 belonging to ST23, ST117 and ST131 (4 meat samples in total) and CMY-2 ST38 (1 meat isolate). Two highly similar isolates (ST23 containing CTM-X-1) were found from a sample of imported broiler meat and in a human bloodstream infection (11 SNPs were detected between the two isolates). Whereas the human and the meat isolates belonging to ST23, ST38 and ST131 did not have similar SNP-profiles. Thus, we only detected one case (ST23, CTX-X-1), with a possible transfer of an ESBL-producing *E. coli* between broiler meat and humans.

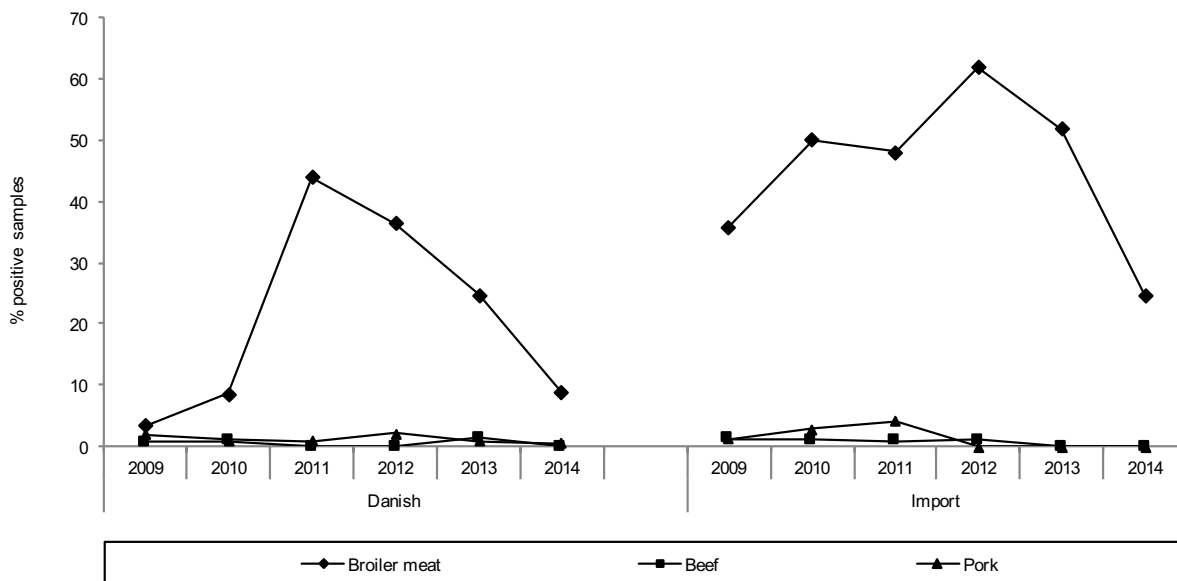
Discussion and conclusion:

The decreasing occurrence of ESBL-producing *E. coli* in Danish broiler meat continued in 2014 and for the first time a significant reduction was also observed in imported broiler meat. The reductions are, most likely a result of the discontinued use of 3rd generation cephalosporins in the top of the breeding pyramids in the country producing the grandparents. This has led to

continued ... Textbox 7.1

Figure 1. Occurrence (%) of samples with ESBL-producing *Escherichia coli* in meat, Denmark

DANMAP 2014



a reduction in imported parent flocks harbouring ESBL genes. A significant reduction in ESBL-producing *E. coli* from broilers was also observed in Sweden, where the poultry production uses the same suppliers as Denmark [SWEDRES-SWARM 2014]. The reduction in imported broiler meat may be explained by a general reduction in antimicrobial consumption in EU countries exporting broiler meat to Denmark.

CTX-M-15, CTX-M-14, CTX-M-27 are the major enzymes detected among the ESBL-producing *E. coli* isolates from human bloodstream infections, whereas CTX-M-1 (4%) and CMY-2-variants (7%) are detected to a lesser extent (Textbox 8. 1). None of the isolates from Danish or imported meat contained any known carbapenemase gene. Therefore, we conclude that presently fresh meat available at the Danish market seems to be a minor source for the human ESBL-producing bacteria causing bloodstream infections, and not a source for carbapenemase producing bacteria causing human infections in Denmark. However, two highly similar *E. coli* isolates were detected; one from human bloodstream infection and the other from imported broiler meat - indicating a possible zoonotic transmission, which is in accordance with a previous report [Leverstein-van Hall *et al.* 2011. *Clin. Microbiol.Infect.* 17:873-880].

Besides clonal spread of ESBL-producing bacteria, genes encoding ESBL-production can be transferred horizontal by plasmid transfer between the animal reservoir and human reservoir. Recently, similar plasmids with genes encoding ESBL-production have been detected from broilers and humans in the Netherlands. [de Been *et al.* 2014 *PLOS Genetics* 10:12]. However, plasmid comparison was not investigated in the present study, but will be investigated in the future.

In summary, the investigation of bloodstream isolates from human cases of septicaemia allows us, for the first time, to assess the impact of zoonotic transmission of ESBL resistance genes. Human blood stream infections with *E. coli* may be seen as a result of a filtering process, where the filters include required pathogenicity traits in the bacterium and decreased resistance to invasion by the patient. The results show that humans and animals do share resistance genes and strain types. However, based on this relatively small sample from meat it is only a minority of ESBL *E. coli* bacteremias in humans that are caused by strains of possible animal origin. Further monitoring and larger studies are needed to investigate and quantify the zoonotic link between carbapenemase and/or ESBL-producing *E. coli* from meat/animals and human infections.

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8

**RESISTANCE IN HUMAN
CLINICAL BACTERIA**

8. Resistance in human clinical bacteria

8.1 *Escherichia coli*

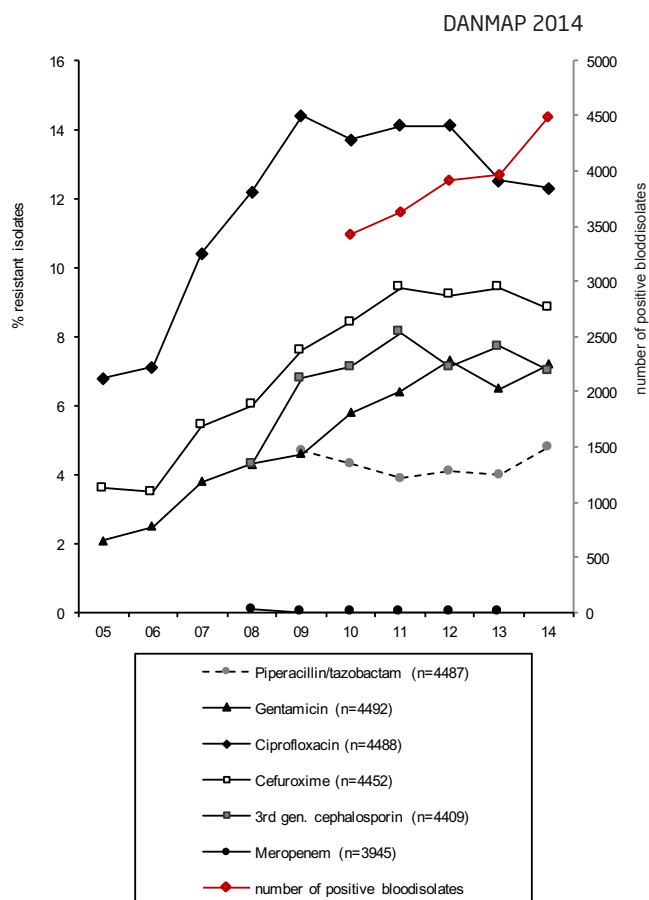
Escherichia coli is part of the normal intestinal flora in both humans and animals but also one of the most common causes of infections. In humans, *E. coli* is the most frequent cause of community- and hospital-acquired urinary tract infections and of bacteremia. *E. coli* also accounts for the majority of travel-related diarrhea. It further might cause meningitis in newborns.

For *E. coli*, DANMAP 2014 includes data on resistance referred from 10 out of 11 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. These data cover all blood and urinary isolates from hospitals and for some DCM a selected number of urinary isolates from the primary health sector. Total numbers of isolates are shown in Table 8.1.

Blood isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 4,500 *E. coli* isolates from blood (Table 8.1 and Figure 8.1).

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 2014.

The total number of *E. coli* isolates from blood has been rising in recent years, (from 3,426 in 2010 to 4,492 in 2014). (Figure 8.1). Similar increases have been observed in the number of positive bloodisolates for other bacteria. This increase might be explained by a rise in the amount of blood cultures taken at hospitals at admission but changes in blood culturing systems as well as the introduction of new blood culturing bottles might also play a role.

In 2014, ampicillin resistance was 45%, which has been unchanged since 2007. For piperacillin/tazobactam the resistance increased to 5%; resistance had been stable at 4% since surveillance was begun in 2009.

For cephalosporins the resistance to 2nd generation cephalosporins (cefuroxime) was 8.5% and resistance to 3rd generation 7%, both decreasing slightly compared to 2013. This puts Denmark back among the other Scandinavian countries and the Netherlands in the EARS-Net data, which reported resistance for 3rd generation cephalosporins in 5 - 7% of their strains. For most other European countries, the occurrence of resistance reported in 2013 was higher, ranging from 10% and up [EARS-Net 2013].

The occurrence of ciprofloxacin resistance remained stable at 12%. This is among the lowest reported resistances in Europe [EARS-Net 2013]. Historically ciprofloxacin resistance has been rising in Denmark until 2009 (15%) and has since shown a slow decrease.

Aminoglycoside (gentamicin) resistance remained with 7% at the same level as reported in 2012 and 2013. Until 2012, the resistance to gentamicin slowly increased with about 0.5 - 1% a year. Most European Countries had resistance levels of 5 to 10% [EARS-Net 2013].

No carbapenem (meropenem) resistant *E. coli* blood isolates were reported in 2014. However reporting only covers the first *E. coli* isolate per patient per year. Three carbapenemase producing blood isolates were referred to and confirmed at the reference laboratory at Statens Serum Institut.

In the 10-year period from 2005 to 2014, resistance in *E. coli* blood isolates has altogether increased steadily, the increase being most pronounced from 2006 to 2011. Since then, a slight stagnation was observed or, for a few antimicrobial agents (cephalosporins and ciprofloxacin), even a decrease. This development parallels the trends in total antimicrobial consumption, which showed pronounced increase for both drugs til 2010 and for cefuroxim a drop in consumption from 2013 to 2014 of 0,6 DID (5%) (Figure 5.1).

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

DANMAP 2014

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Ampicillin	45	42	39
Mecillinam	8	7	5 #
Piperacillin/tazobactam	5 *	4 #	
Sulfonamide		32	32
Gentamicin	7	5	
Ciprofloxacin	12	11	9 #
Cefuroxime	9	7 *	
3rd generation cephalosporins ^(a)	7	6	4
Meropenem	<1	<1	<1
Max. number of isolates tested	4,492	43,986	49,745

*) An asterisk indicates a significant increase from 2013 to 2014.

#) A number sign indicates a significant decrease from 2013 to 2014.

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

Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 44,000 *E. coli* isolates from hospital patients with a urinary tract infection (Table 8.1 and Figure 8.2). For these isolates bigger variations showed in the chosen test panel: all 10 DCM performed susceptibility testing for mecillinam and ciprofloxacin and almost all tested for ampicillin, piperacillin/tazobactam, gentamicin, cefuroxime and 3rd generation cephalosporins (most often cefpodoxim). Only five DCM tested for sulfamethizole. Routine testing for meropenem in hospital urines was performed at six DCM, the other DCM performed testing only on selected strains, thus referring fewer data to DANMAP.

Ampicillin resistance was 42%, aminoglycoside (gentamicin) resistance was slightly higher and sulfonamide resistance was 32%, which are at the same levels as in 2013.

Resistance to 2nd generation cephalosporin (cefuroxime) (7%) was slightly higher than in 2013 (6%) and 3rd generation cephalosporin resistance (6%) was at the same level as in 2013.

The occurrence of ciprofloxacin resistance was slightly lower than in 2013 (11% vs 12%), still the same development as in blood isolates was observed with a steep increase in resistance from 5% in 2005 to 13% in 2011 and since a slower decrease.

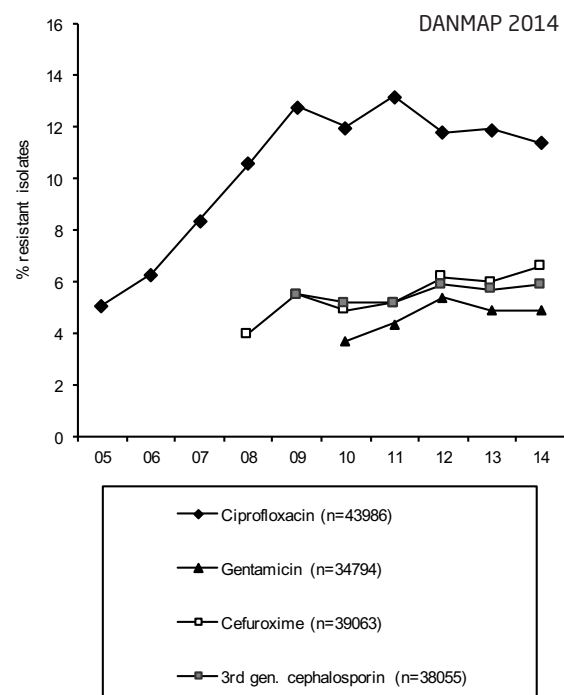
In 2014, one carbapenem (meropenem) intermediate resistant *E. coli* strain from hospital urine was reported.

Urine isolates from primary health care

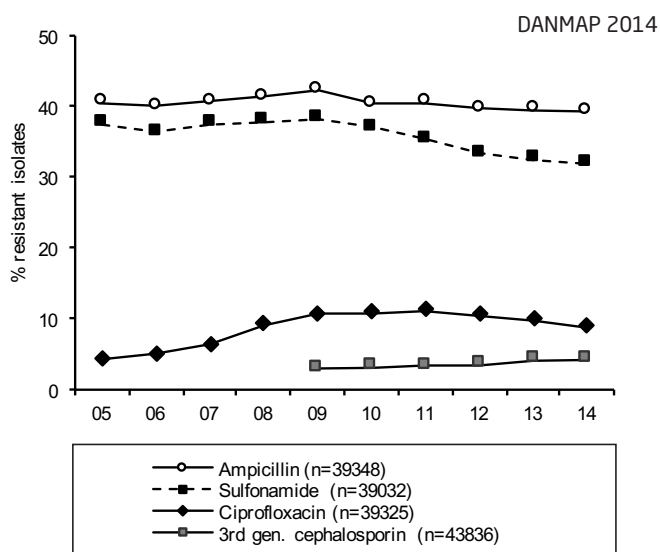
DANMAP received data on the antimicrobial susceptibility of approximately 50,000 *E. coli* isolates from urinary tract infection in patients from primary health care (Table 8.1 and Figure 8.3).

In Denmark culturing and susceptibility testing of urines from

primary health care varies substantially between the different regions. Many GP's perform local testing and only urines from patients with known resistance problems are sent to the DCM at the regional hospital. Thus the amount of culturing and susceptibility testing performed at the different DCM will vary accordingly. As for hospital urines less than half of the DCM test for sulfamethizole, the same applies also to meropenem and piperacillin/tazobactam. All DCM test for mecillinam and 3rd generation cephalosporins (cefpodoxime), most test for ampicillin and ciprofloxacin.

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 2014.

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 2014.

The occurrence of ampicillin resistance was 39% and sulfonamide resistance was 32%, both levels were similar to levels reported in 2013. Ampicillin resistance has been at the same level for the last 20 years, while sulfonamide resistance has decreased since 2009 (38%).

The occurrence of resistance to 3rd generation cephalosporin was 4%, at the same level as reported in 2013.

Resistance to ciprofloxacin was 9%, which continued the slight decrease observed for the past four years (11% in 2011). As for hospital urines and blood isolates, the resistance had been increasing from 2005 to 2011, which paralleled the increasing consumption of fluoroquinolones during the same period (Table 5.1).

In 2014, two carbapenem (meropenem) resistant and one intermediate resistant strain were reported from *E. coli* urine isolates from primary health care. Routine meropenem testing was performed at three DCM, most DCM performing only on selected strains.

Results for mecillinam resistance

Mecillinam resistance was 8% for blood isolates, which was lower than resistance reported in previous years. Mecillinam is among the most used penicillins in Denmark and the consumption of pivmecillinam in the primary sector has increased with 34% for the last decade (Figure 5.6, table 5.1). Detailed comparison of the reported resistance levels for mecillinam revealed great differences between laboratories but also for some individual laboratories over time. These differences are due to differences in reporting and change of breakpoints over time. Results for mecillinam resistance for 2014 are shown in Table 5.1, but results from the former years are not reported owing to the mentioned methodological and interpretational difficulties. This issue is now under consideration in the DANRES group.

8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the normal intestinal flora in humans but also the cause of urinary tract-, respiratory tract-, and bloodstream infections. Many of these infections are hospital acquired and can be life threatening, especially if the strains are resistant to antimicrobial agents.

For *K. pneumoniae*, DANMAP 2014 includes data on resistance referred from 10 out of 11 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. (Table 8.2).

Blood isolates from hospital patients

Statens Serum Institut has since 2006 received data on the antimicrobial susceptibility of *K. pneumoniae* isolates from blood, and following a sudden increase in resistance (due to an outbreak with the so called *epi-K.pn.in* in 2007) data were included in the DANMAP report from 2008 and onwards. In 2014 DANMAP received information on 943 *K.pneumoniae*. (Table 8.2 and Figure 8.4).

In general, the level of antimicrobial resistance has decreased, since it peaked in 2009. Piperacillin/tazobactam is the only antimicrobial with slowly increasing resistance since its first registration in the DANMAP report 2010 (Figure 8.4). This is in concordance with an increased use of this drug due to changes in the Danish antibiotic policy: increased use of beta-lactams is thus recommended to reduce the consumption of cephalosporins.

Resistance to 2nd generation cephalosporins (cefuroxime) was 12% and resistance to 3rd generation cephalosporins was 8%. The resistance to 3rd generation cephalosporins is the highest reported level among the Nordic countries (which in 2013 were reported below 5%), but remains lower than the occurrence reported by most other European countries. [EARS-Net 2013].

In 2014, three carbapenem (meropenem) resistant and one intermediate *K. pneumoniae* blood isolate were reported. These data cover the first *K. pneumoniae* isolate registered per patient per year. In addition to these a fourth carbapenemase producing blood isolate were referred to and confirmed at the reference laboratory at Statens Serum Institut.

Ciprofloxacin resistance was slightly lower than the previous year (9% in 2013, 7% in 2014), and resistance to aminoglycoside (gentamicin) remained unchanged from the year before (5%).

Compared to data reported to the EARS-Net 2013 report, the level of resistance to ciprofloxacin was higher than the levels reported from the other Nordic countries (< 5%) but levelled with resistance reported from the United Kingdom (9%) and the Netherlands (6%). Whereas the level of resistance to

aminoglycosides was the same as in the other Nordic countries and lower than most countries reporting to EARS-Net [EARS-Net 2013].

Resistance to mecillinam was 8%, at the same level as in 2013. As for *E.coli*, data on percentages of resistance to mecillinam differ in time and geographically, which is related to variations in testing as well as interpreting rules and guidelines. Data for mecillinam resistance are presented in Table 8.2, but not shown in the figures.

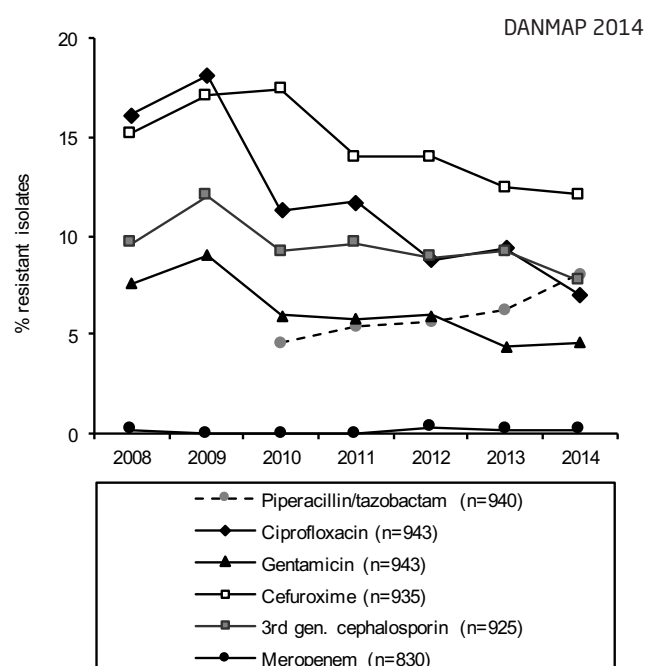
Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of 6,349 *K. pneumoniae* isolates from hospital patients with a urinary tract infection (Table 8.2 and Figure 8.5).

Resistance to mecillinam (10%), sulfonamide (19%), gentamicin (4%), 2nd generation cephalosporins (cefuroxime) (9%), 3rd generation cephalosporins (7%) and ciprofloxacin (8%) was at the same level as reported in 2013.

In 2014, carbapenem (meropenem) resistance was reported in seven and intermediate resistance in five *K. pneumoniae* urine isolates from hospitalised patients. Not all DCM performed routine susceptibility testing for meropenem, thus the number of these strains represent a selected population.

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



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2014.

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

DANMAP 2014

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary healthcare
	%	%	%
Mecillinam	8	10	9 #
Piperacillin/tazobactam	8	6 *	
Sulfonamide		19	17 #
Gentamicin	5	4	
Ciprofloxacin	7	8	7
Cefuroxime	12	9	
3rd generation cephalosporins ^(a)	8	7	5
Meropenem	<1	<1	<1
Max. number of isolates tested	943	6349	4105

*) An asterisk indicates a significant increase from 2013 to 2014.

#) A number sign indicates a significant decrease from 2013 to 2014.

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

Urine isolates from primary health care

DANMAP received data on the antimicrobial susceptibility of approximately 4,100 *K. pneumoniae* isolates from urinary tract infection in patients from primary health care. (Table 8.2 and Figure 8.6)

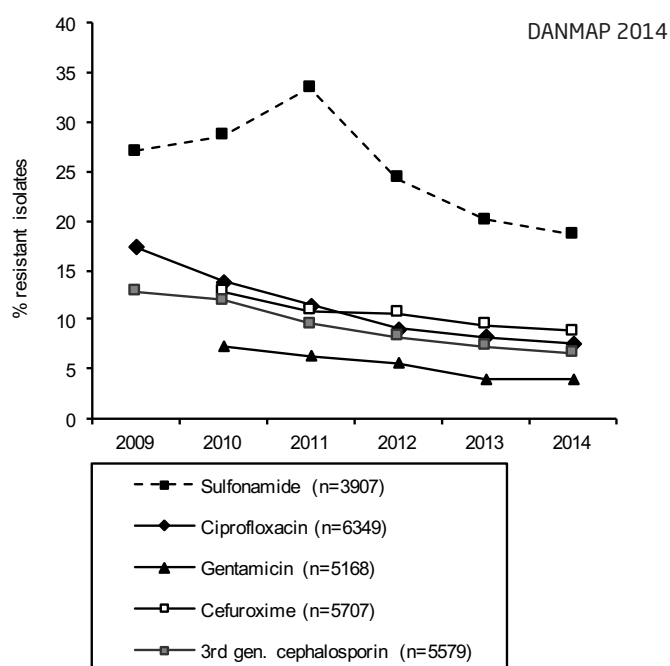
In 2014, resistance to 3rd generation cephalosporins was 5%, which is a slight decrease from the level reported in 2013 (6%).

Resistance to ciprofloxacin was 7%, which is similar to the levels reported in 2013. Resistance to mecillinam was 9% and sulfonamide resistance decreased from 22% in 2013 to 17% in 2014.

In 2014, three carbapenem (meropenem) resistant and one intermediate resistant *K. pneumoniae* urine isolates from patients in primary health care were reported. As for the hospital urines this number represents a selected population.

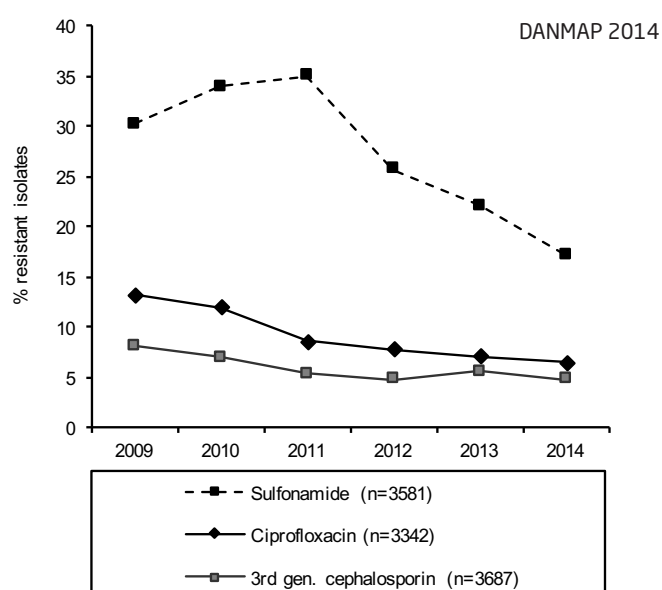
Ute Wolff Sönksen and Stefan S. Olsen

Figure 8.5. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2014.

Figure 8.6. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2014.

8.3 *Pseudomonas aeruginosa*

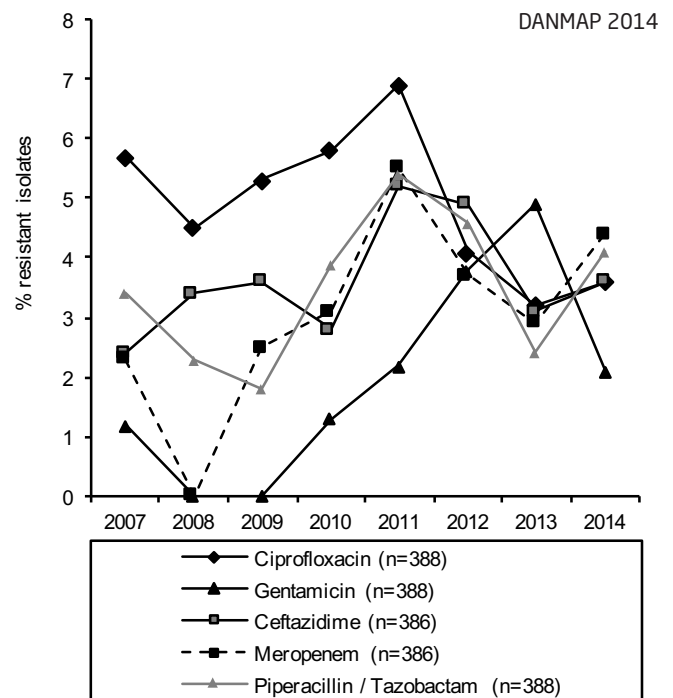
Pseudomonas aeruginosa is an opportunistic pathogen of immunocompromised individuals. The case fatality rate is high for *P. aeruginosa* infections in patients suffering from cancer, cystic fibrosis and burns. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes bloodstream infections. It is among the most frequent colonizers of medical devices (e.g. indwelling catheters). *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial agents. The antimicrobial classes which can be used for treatment include some fluoroquinolones (primarily ciprofloxacin), aminoglycosides (e.g. gentamicin, tobramycin and amikacin), some beta-lactams (piperacillin/tazobactam, ceftazidime, and carbapenems) and colistin.

P. aeruginosa blood isolates obtained from hospitalised patients

For *P. aeruginosa*, DANMAP 2014 includes data from 10 out of 11 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. DANMAP received data on the antimicrobial susceptibility of 388 *P. aeruginosa* isolates from blood. Resistance to all the tested antimicrobial agents was not significantly different from the level in 2013, except for gentamicin which decreased from 2013 (5%) to 2014 (2%), (Figure 8.7). The occurrence of resistance to fluoroquinolones, carbapenems, ceftazidime and piperacillin/tazobactam was at the same level or lower reported to EARS-Net 2013 by the Nordic countries [EARS-Net 2013].

Meropenem resistance was observed for 4% (n = 17) of the *P. aeruginosa* isolates in 2014. A Danish study of *P. aeruginosa* carbapenem non-susceptible isolates from 2011 showed that carbapenemases were present in a minority of the isolates (7%) [Hansen *et al.* 2014 *Microb. Drug Res.* 20: 22-9]. As in previous years, putative carbapenemase producing *P. aeruginosa* isolates were sent on a voluntary basis from the DCM

Figure 8.7. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2014.

to SSI for national surveillance on carbapenemase producing bacteria, including not only isolates from bloodstream infections but also from other origins. In 2014, six VIM-producing *P. aeruginosa* isolates were detected from six patients. Furthermore, two NDM-producing *P. aeruginosa* were detected from two patients. [Textbox 8.2].

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Textbox 8.1

Characterization of ESBL/AmpC-producing and carbapenemase-producing *Escherichia coli* from bloodstream infections, 2014 Denmark

Background: Third-Generation cephalosporin-resistant *Escherichia coli* (3GC-R *Ec*) is increasing in Europe [EARS-Net report, 2013]. The extended-spectrum cephalosporin-resistance in *E. coli* can be due to production of extended-spectrum beta-lactamases (ESBLs), plasmid-mediated AmpC (pAmpCs) or constitutive overexpression of the chromosomal *ampC* gene due to mutations within the promoter/attenuator region.

The worldwide spread of ESBL-producing *E. coli* is in part due to the spread of the pandemic clone O25b:-ST131. Evidently, this clone is strongly related to the presence of antimicrobial resistance genes, including CTX-M-15 and other ESBL enzymes, as well as virulence factors.

Before 2007, the occurrence of 3GC-R *Ec* was low among *E. coli* isolated from bloodstream infections in Danish patients. However, the rate of resistance among invasive *E. coli* in Denmark has increased from 2.5% in 2006 to 7.0% in 2014.

Carbapenemase-producing *E. coli* are also of concern (see Textbox 8.2 Carbapenemase-producing bacteria in Denmark, 2014). The aim of the present study was to characterize ESBL (including pAmpC) and carbapenemase-producing *E. coli* from bloodstream infections according to genes and Multilocus sequence types (MLSTs).

Material and Methods: During January 2014 through December 2014, 11 out of 12 Danish departments of clinical microbiology collected all their 3GC-R *Ec* (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime resistance) from bloodstream infections. Furthermore, meropenem non-susceptible *E. coli* from bloodstream infections were included in the study. Only one isolate per patient was included in the study. The isolates were sent to Statens Serum Institut for further characterization. Genomic DNA was extracted from the isolates (DNeasy Blood and Tissue Kit, QIAGEN, Copenhagen, Denmark). Fragment libraries were constructed using the Nextera Kit (Illumina, Little Chesterford, United Kingdom) followed by 251-bp paired end sequencing (MiSeq, Illumina) according to the manufacturer's instructions. The paired-end Illumina data was assembled using CLC bio's Genomic Workbench 7.5.

The ResFinder web server, (www.genomicepidemiology.org, version 2.1) were used to identify acquired ESBL (including plasmid AmpCs) and carbapenemase genes from the assembled WGS data.

The MLST web server (www.genomicepidemiology.org, version 1.7) was used for detection of MLST-profiles. For isolates with no ESBL genes detected, the sequences were investigated for mutations presumed to up-regulate chromosomal AmpC by the use of CLCbio Genomic Workbench.

Results: Whole genome sequence data were obtained from 261 *E. coli* isolates. Genes encoding ESBL production (including pAmpCs) and/or carbapenemase production were detected in 245 isolates. Sixteen isolates were hyper AmpC producers only; these isolates were not investigated further. The distribution of the 245 isolates with genes encoding ESBL and carbapenemase production in relation to the five Danish regions is shown in Table 1.

Demographic data was available for 240 of the 245 patients with ESBL/carbapenemase producing *E. coli*. One hundred-twenty-seven (53%) of the patients were men and the average age at diagnosis was 70 years (ranging from below one to 97 years). Twenty-seven patients (11%; 15 women and 12 men) of the 240 patients, died within 30 days of diagnosis (average age at death 75 years; ranging from 47 to 97 years).

Among the 245 isolates, 21 different ESBLs (including pAmpCs) and carbapenemases were detected (Table 2). CTX-M-15 dominated (49%) followed by CTX-M-14, CTX-M-27 and CTX-M-101. Different variants of CMY were detected in 16 (7%) isolates. Three isolates produced carbapenemases; one OXA-48 (from a patient initially hospitalized in Turkey) and two OXA-181 (from two patients with no recent history of travel abroad). In several isolates more than one gene encoding ESBLs and/or carbapenemases were detected (Table 2).

The 245 isolates belonged to 51 different MLSTs. ST131 was the most common sequence type (ST), 124 (51%) of the isolates belonged to this type. Other major types were ST38 (7%), ST69 (4%), ST405 (5%) and ST648 (3%), whereas the rest of the isolates belonged to STs, which only were detected in 1 - 5 isolates (<1 - 2% per type) (Table 3).

Among the 124 isolates belonging to ST131, CTX-M-15 (64 %) was most common, followed by CTX-M-27 (17%), CTX-M-101 (10%) and CTX-M-14 (6%). The two isolates with OXA-181 and CMY-2 belonged to ST410, whereas the isolates with OXA-48 belonged to ST443.

Conclusion: As in previous Danish studies of ESBL-producing *E. coli* from bloodstream infections, most of the isolates produced a CTX-M enzyme, most often CTX-M-15 [DANMAP 2009, DANMAP 2011, Hansen *et al.* Microb. Drug Res. 2014]. Plasmid mediated AmpC (CMY) was only present in a minor part of the isolates.

As in the study by Hansen *et al.* the worldwide disseminated CTX-M-15 ST131 was strongly represented in this study, but the Danish isolates belonged to other international STs (e.g., ST38, ST69, ST405 and 648) related to spread of ESBLs too. The finding of three *E. coli* isolates with OXA-48 group enzymes from bloodstream infection is worrying.

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Table 1. Distribution of 245 ESBL and Carbapenemase producing *E. coli* from bloodstream infections, 2014

Region	DANMAP 2014	
	Numbers	
The Capital Region of Denmark	110	
The Zealand Region	27	
Region of Southern Denmark	43	
Central Denmark Region	43	
North Denmark Region	22	
Total numbers	245	

Table 3. Distribution of MLSTs among the 245 *E. coli* from bloodstream infections, 2014

MLST	DANMAP 2014	
	Numbers of isolates	%
ST12	5	2%
ST38	18	7%
ST69	10	4%
ST95	5	2%
ST131	124	51%
ST405	13	5%
ST648	7	3%
Other STs ⁽¹⁾	63	26%

(1) less than 5 isolates per ST

Table 2. ESBL enzymes and carbapenemases detected in 245 *E. coli* from bloodstream infections, 2014

Enzyme	DANMAP 2014	
	Numbers	%
CTX-M-1	10	4%
CTX-M-14 ⁽¹⁾	38	16%
CTX-M-14a ⁽¹⁾	5	2%
CTX-M-15 ⁽¹⁾	121	49%
CTX-M-27	25	10%
CTX-M-55	8	3%
CTX-M-101	12	5%
Other ESBL enzymes	8	3%
CMY variants ⁽¹⁾	16	7%
OXA-48-group ⁽¹⁾	3	1%

(1) In some isolates more than one enzyme was detected.

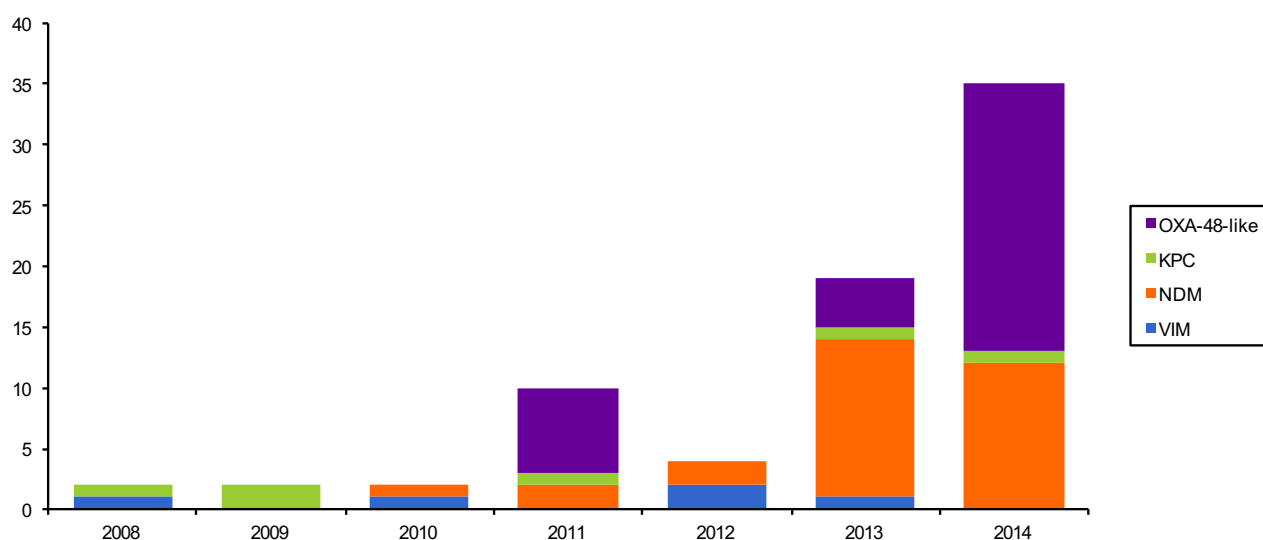
Textbox 8.2

Carbapenemase-producing bacteria in Denmark, 2014

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram-negatives like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem-resistant bacteria are often none or suboptimal. Resistance can be caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integron-encoded metallo- β -lactamase (VIM), and New Delhi metallo- β -lactamase (NDM), and Imipenemase (IMP).

In recent years, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut. The present textbox describes carbapenemase-producing *Enterobacteriaceae* (CPE), carbapenemase-producing *P. aeruginosa* and *Acinetobacter* ssp.

Figure 1. Numbers of carbapenemase-producing Enterobacteriaceae (CPE)



More than one isolate was included from the same patient, if the isolates belonged to different bacterial species and/or harboured different carbapenemases.

During 2014, 55 carbapenemase producing bacteria were detected from 48 patients. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Six of the isolates were from bloodstream infections. Thirty (65%) of the patients were men and the average age at diagnosis was 63 years (ranging from one to 97 years). Thirteen patients (28%) (nine men and four women), died within 30 days of diagnosis (average age at death 68 years; ranging from 17 to 97 years). In many cases, the sources of the carbapenemase-producing bacteria were unknown or related to spread between patients in Denmark, which was different compared to the previous years, where most of the cases were related to travel abroad.

Enterobacteriaceae: In 2014, 35 CPE (from 29 patients) were detected compared to 19 in 2013 and 20 CPE during 2008-2012 (Figure 1). Twenty-two of the 35 CPE isolates harboured OXA-48-like genes. Twelve NDM-producing isolates and one KPC-producing isolate were detected

The NDM-1 producing *C. freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2013 and 2014. Until the end of 2014, six patients were involved in this outbreak. None of these patients had a prior history of travel noted in their hospital records. The origin of the NDM-1 producing *C. freundii* was unknown.

During 2014, NDM-5 producing *K. pneumoniae* were detected from three patients at a hospital in the Capital Region of Denmark. The isolates had highly similar SNP-profiles, indicating a possible spread between the patients or a common origin. None of the patients had travelled recently, and the origin of the NDM-5 producing *K. pneumoniae* was unknown.

Acinetobacter spp: The number of carbapenemases-producing *Acinetobacter* spp was the same level as in 2013. Nine OXA-23 producing *A. baumannii* isolates were detected in 2014. Furthermore, one OXA-40-like producing *A. baumannii*, one OXA-58-like producing *A. baumannii* and one NDM-1 producing *A. Acinetobacter pittii* were detected. NDM-1 producing *A. pittii* are rare and the origin of this isolate was unknown.

P. aeruginosa: In 2014, six VIM-producing *P. aeruginosa* isolates were detected from six patients. Furthermore, two NDM-producing *P. aeruginosa* were detected from two patients.

Conclusion: The occurrence of carbapenemase-producing bacteria in Denmark is increasing, a trend worrisome to patients and clinicians. Especially the spread of CPE is of concern, since *Enterobacteriaceae* can be carried in the intestine for a long time without any symptoms of infections, which makes outbreak control difficult.

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8.4 Streptococci, including pneumococci

Streptococci include *Streptococcus pneumoniae* (pneumococci), beta-haemolytic streptococci (BHS), and non-haemolytic streptococci (NHS). The prevalence of asymptomatic carriage of pneumococci in the nasopharynx varies with age. NHS are part of the normal commensal flora of the upper respiratory tract, mouth, skin, and intestine in humans.

Pneumococci may cause common and less severe infections such as otitis media, sinusitis, pneumonia, and invasive infections such as bacteraemia, meningitis, and endocarditis.

BHS of group A cause tonsillitis, otitis media, wound infections, but also more severe infections: e.g., bacteraemia, necrotizing myo-fasciitis, and rarely meningitis. BHS of group B may be present in the vaginal flora of 20-25% of women in the child-bearing age and may therefore cause meningitis and septicaemia in the newborn. Such infections may also occur in elderly or immuno-compromised patients. BHS of groups C and G predominantly cause soft-tissue infections and sometimes bacteraemia. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group. NHS may cause invasive infections, e.g. endocarditis.

This report presents data on resistance in non-duplicate invasive isolates (i.e., from blood or cerebrospinal fluid) of pneumococci and BHS submitted to the Neisseria and Streptococcus Reference laboratory. Isolates are received from all DCMs in Denmark. Non-invasive isolates are infrequently received but are not included in this report. There is no surveillance of NHS.

Infections with pneumococci and BHS are usually treated with penicillins or macrolides. All invasive non-duplicate *Streptococcus pneumoniae* and group A, B, C and G BHS were therefore tested for susceptibility to penicillin and erythromycin. Moreover, the group A, B, C and G BHS were tested for susceptibility to clindamycin and for inducible clindamycin resistance.

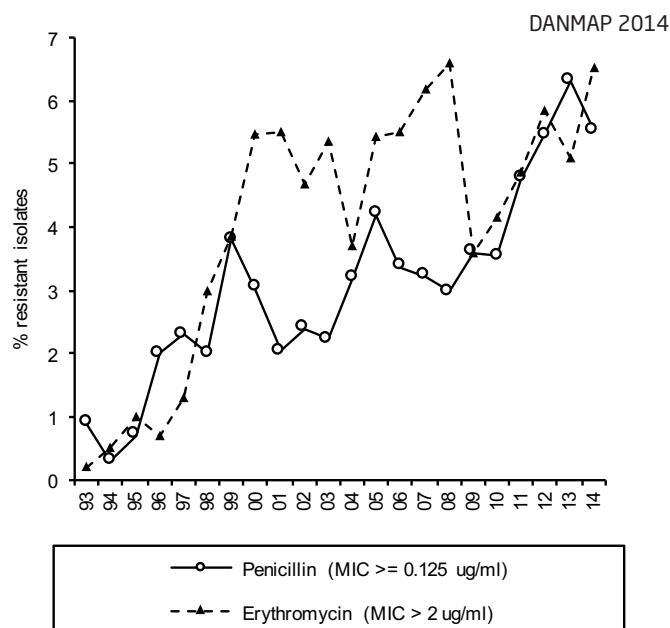
Streptococcus pneumoniae

Susceptibility testing was performed on 722 isolates of *S. pneumoniae* (Figure 8.8).

For penicillin, 40 (5.5%) isolates were non-susceptible (resistant and intermediary resistant) in 2014 compared to 6.3% in 2013 (Figure 8.8). The 40 isolates belonged to 13 different serotypes and the most commonly found penicillin non-susceptible serotypes were type 15A (n = 14), 19A (n = 5), 24F (n = 4) and 23B (n = 4). One of the 40 isolates (type 19F) was fully resistant to penicillin.

Regarding erythromycin, 47 (6.5%) isolates were non-susceptible in 2014 compared to 5.1% in 2013 (Figure 8.8). The

Figure 8.8. Nonsusceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark



47 isolates belonged to 11 different serotypes and the most commonly found erythromycin non-susceptible serotypes were type 15A (n = 15), 24F (n = 14) and 33F (n = 6).

Non-susceptibility to penicillin or erythromycin or both was more frequent for some serotypes than for others. This was the case for serotype 9V (3 of 3 received isolates), 15A (15 of 27 received isolates) and 23B (4 of 10 received isolates).

The levels of erythromycin and penicillin non-susceptibility in Denmark were similar to the levels reported in 2013 to EARS-Net by the neighboring countries Norway, Sweden, Germany, and the United Kingdom. Many other European countries reported considerably higher levels of resistance in 2013 [EARS-Net 2013].

Beta-haemolytic streptococci Group A streptococci

All 149 isolates of group A streptococci (*Streptococcus pyogenes*) were sensitive to penicillin. Erythromycin resistance was detected in one isolate (0.7%) as compared to five isolates in 2013 (3.0% of 167). This isolate was also resistant to clindamycin (0.7%). In total, 99.3% of the isolates were sensitive to both penicillin, erythromycin, and clindamycin.

Group B, C and G streptococci

All 139 isolates of group B streptococci (*Streptococcus agalactiae*) were sensitive to penicillin. Erythromycin resistance was detected in 31 isolates (22%) as compared to 22 in 2013 (17% of 129). 16 isolates (11%) were resistant to clindamycin and additionally four isolates (2.9%) showed inducible resistance to clindamycin. In total, 77% of the isolates were sensitive to both penicillin, erythromycin, and clindamycin.

All 80 isolates of group C streptococci (*Streptococcus equisimilis* and *S. zooepidemicus*) were sensitive to penicillin. Erythromycin resistance was detected in three isolates (3.8%) the same number as in 2013 (4.5% of 66). Three isolates (3.8%) were resistant to clindamycin and additionally one isolate (1.3%) showed inducible resistance to clindamycin. In total, 95% of the isolates were sensitive to both erythromycin, clindamycin and penicillin.

All 188 isolates of group G streptococci were sensitive to penicillin. Erythromycin resistance was detected in 19 isolates (10.1%) as compared to 16 in 2013 (10.4% of 154). Three isolates (1.6%) were resistant to clindamycin and additionally 13 (6.9%) showed inducible resistance to clindamycin. In total, 89.9% of the isolates were sensitive to both penicillin, erythromycin, and clindamycin.

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8.5 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but might also cause infections. Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteraemia and bacterial endocarditis. These infections can be life-threatening in humans, especially in hospitalized patients. The naturally high level of antimicrobial resistance found in enterococci 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) with an aminoglycoside (gentamicin) or a glycopeptide (vancomycin).

For *E. faecalis* and *E. faecium*, data from 10 of the 11 DCM were obtained, representing 95% of the Danish population.

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

DANMAP received data on the antimicrobial susceptibility of 738 *E. faecium* isolates and 598 *E. faecalis* isolates from blood.

As in previous years, most of the *E. faecium* isolates from bloodstream infections were ampicillin resistant. Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as a risk factor for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has been observed in hospitals in Denmark during the last decade. The antimicrobial selection pressure in a hospital environment might be a reason for the high level of ampicillin resistant *E. faecium* as a cause of bloodstream infections. In 2014, 94% of the *E. faecium* isolates were resistant to ampicillin.

Only one of the DCMs (Aalborg) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates, 30% were HLGR, whereas 68% of the tested *E. faecium* isolates were HLGR. The level of HLGR *E. faecalis* and HLGR *E. faecium* was similar to or higher than the level detected in many European countries reporting to EARS-Net in 2013 [EARS-Net 2013].

In 2014, vancomycin resistance was detected in 4.3% of the *E. faecium* isolates and 0.2% of the *E. faecalis* isolates from bloodstream infections. The *E. faecium* bloodstream isolates were part of outbreaks with vancomycin resistant (*vanA*) *E. faecium*, which is described in Textbox 8.3. The level of vancomycin resistant *E. faecium* was above or at the same level reported to EARS-Net 2013 by the other Nordic countries [EARS-Net 2013].

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Textbox 8.3

Continued increase in occurrence of clinical vancomycin resistant enterococci in Danish hospitals in 2014

Background: *Enterococcus faecalis* and *Enterococcus faecium* are commensal bacteria in the intestine of humans. *E. faecalis* and *E. faecium* can also cause urinary tract infections (UTI) and fatal infections like sepsis and endocarditis, especially among older patients. Enterococci are intrinsically resistant to a number of first-line antimicrobial agents including cephalosporins. Therefore, therapy of enterococcal infections may be difficult. Severe enterococcal infections are primarily treated with vancomycin, but recently an increase in the occurrence of vancomycin resistant enterococci (VRE) has been observed in Denmark and internationally. Many of the VRE are also resistant to ampicillin and gentamicin limiting the treatment possibilities. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents have many side effects.

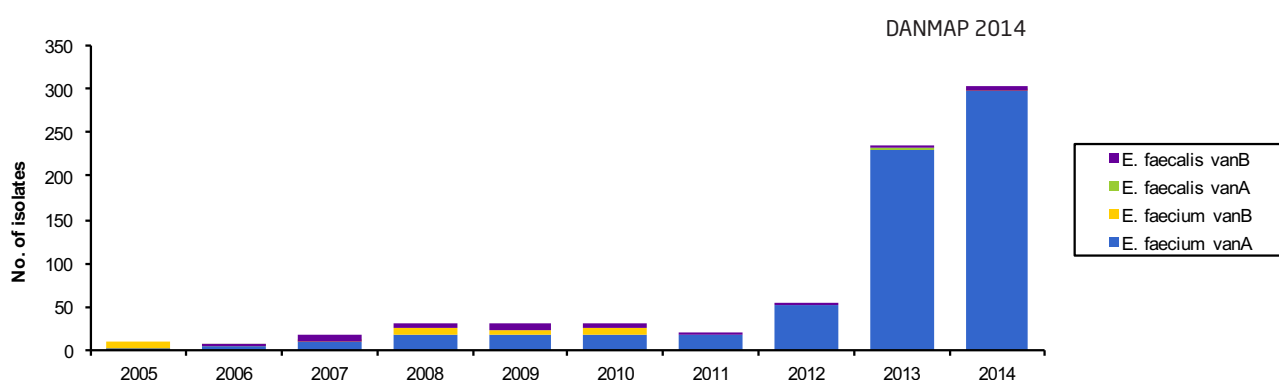
Surveillance of VRE: Since 2005, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis, submitted VRE for species identification, genotyping (*vanA*, *vanB* and *vanC*) and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

From 2005 through 2009, the occurrence of clinical VRE in Denmark has been low (<30 isolates) (Figure 1). However, in 2010 and 2011 outbreaks occurred at hospital wards in the Central Denmark Region [DANMAP 2010 and DANMAP 2011] causing an increase in the number of clinical *vanA E. faecium*. In 2013, a steep increase in *vanA E. faecium* was observed. They were primarily detected at hospitals in the Capital Region, but also from The Zealand Region and the Central Denmark Region. VRE was detected in the two other regions of Denmark too, but to a much lower extent. Typing of VRE isolates from 2012 and 2013 showed spread of several *vanA E. faecium* clones both inside hospitals and between hospitals [Pinholt *et al.* 2015, J. Antimicrobial. Chemother, 70:2474-82]. In 2014, a further increase in VRE was observed to a total of 303 isolates. Most of the VRE were *vanA E. faecium*, which increased from 231 clinical *vanA E. faecium* in 2013 to 297 in 2014 (Figure 1). Phylogenetic analysis of the whole genome sequences of the *vanA E. faecium* isolates obtained from blood and sent to the reference laboratory (n = 32) demonstrated the same clonal types on several hospitals. These isolates belonged to sequence types, which previously have been detected from clinical VRE outbreaks internationally (ST80, ST117, ST192 and ST203).

Conclusion: The continued increase in number and transmission of *vanA E. faecium* in 2014 in Denmark is worrying. VRE can be carried in the intestine for a long period without any symptoms of infection and likewise persist in the hospital environment, which makes infection control difficult but should include proper cleaning, good hand hygiene, screening for VRE and isolation of patients.

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Figure 1. Numbers of vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from clinical samples and van genes, Denmark



8.6 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the 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 causes 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 septic arthritis.

In Denmark, a voluntary surveillance programme of all *S. aureus* bacteraemia cases was established in 1957. Laboratory and clinical notification of Methicillin-resistant *S. aureus* (MRSA) has existed since November 2006. At SSI, all isolates are typed by *spa* typing, susceptibility testing is performed for 20 antimicrobials and the presence of the gene *lukF-PV* is determined. *LukF-PV* codes for a cytotoxin (Panton-Valentine leucocidin, PVL) which has been closely linked to skin abscesses and the very rare condition of severe necrotizing pneumonia. PVL is found both in MSSA and MRSA, for MRSA PVL has been closely associated with community acquired MRSA strains. In addition demographic and epidemiological information is registered. Based on the epidemiological information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO), import (IMP) and livestock-associated MRSA (CC398). For CA and HACO classification is separated into known and not known exposure.

Surveillance of bacteraemia

In 2014, 1,964 *S. aureus* bacteraemia cases corresponding to 34.9 cases per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. For the second year the number of cases was higher than in the previous years (approximately 1,500 annual cases). This may to some extent reflect underreporting in previous years. Fifty-six (2.9%) of the bacteraemia cases were caused by MRSA. This is a steep increase compared to the levels in previous years (30 cases, 1.7% in 2013), but still very low compared to most other countries participating in EARS-Net [EARS-Net 2013]. Eight of the 56 MRSA cases were caused by CC398 (4 MRSA CC398 in 2013). Four hundred and thirty seven (22%) patients died within thirty days of the onset of bacteraemia. The mortality for the MRSA bacteraemia cases was 21% (n=12). Antimicrobial resistance in *S. aureus* bacteraemia isolates from 2005–2014 is presented in Table 8.3. The highest frequency of resistance to other than penicillin, was observed for fusidic acid (15%), erythromycin (8%), clindamycin (8%) and norfloxacin (6%). Susceptibility to all tested antimicrobial agents was at the same level as in 2013, but resistance to erythromycin, clindamycin, fusidic acid and norfloxacin has increased steadily since 2005 (Table 8.3). Resistance to at least 1, 2 or 3 other antimicrobial agents in addition to penicillin was demonstrated in 29%, 16% and 9% of the cases, respectively. Typing dem-

onstrated 545 different *spa* types distributed in 28 different CC groups (the ten most prevalent *spa* types are presented in Table 8.4). PVL was demonstrated in 21 (1%) cases of which six were MRSA. The 21 PVL containing isolates were distributed over 18 different *spa* types and 10 different CC groups.

Surveillance of methicillin-resistant *S. aureus*

In 2014, 2,965 new MRSA cases were detected (52.7 per 100,000 inhabitants). This was the highest number of cases observed in Denmark (Figure 8.9). A case was defined as a person found positive for the first time with a specific MRSA strain regardless whether the patient was infected or colonised only. MRSA isolates carry either the *mecA* or more uncommonly, the *mecC* gene.

In 2014, the number of new MRSA cases increased by 42% compared to 2013 (n=2,092) and was almost five times as high as in 2007 (n=663). CC398 cases increased substantially and constituted 43% (n=1,277) of new MRSA cases in 2014 (Table 8.5). The increase was for a large part due to changes in screening procedures for patients admitted to hospitals, which from December 2012 included contact to pigs as a risk factor for acquisition of MRSA.

MRSA isolates carrying *mecC* were demonstrated in 53 cases (1.8%) in 2014 (9 in 2009, 21 in 2010, 37 in 2011, 24 in 2012 and 41 in 2013). Thirty-six of the cases (68%) had infections at the time of diagnosis. Only one possible livestock contact was registered for the 53 *mecC* cases.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.5. Most of the cases (86%) were acquired in Denmark. At the time of diagnosis, 38% (n=1,115) of cases had infection, which was lower than in 2013 (45%) due to a much lower fraction of infections among CC398 cases (n=240, 19%).

The epidemiological classification of MRSA infections 2007–2014 is shown in Figure 8.10. Despite the increasing total number of cases, the number of hospital acquired infections (n=49) and the number of healthcare-associated with community onset (HACO) infections (n=140) were at a stable very low level. The number of community-acquired (CA) infections continued the increasing trend in 2014 and was by far the largest group (n=456) while infections caused by CC398 increased in 2014 and was the second most prevalent (n=240) (Figure 8.10).

Molecular typing of the MRSA strains

In total, *spa* typing revealed 291 different strain types of which 210 types were associated with clinical infections. The 10 dominating *spa* types isolated in 2014 are shown in Table 8.4. They constituted 67% of the total number of MRSA isolates. Ten *spa* types constituted 57% of the 1,115 clinical infections with MRSA. These most prevalent *spa* types causing clinical infections at time of presentation were t034/CC398 (n=179), t002/CC5 (n=91), t008/CC8 (n=86), t019/CC30 (n=58), t127/CC1 (n=53),

Table 8.3. Resistance (%) in isolates from *S. aureus* bacteraemia cases, Denmark

DANMAP 2014

Antimicrobial agent	2005 %	2006 %	2007 %	2008 %	2009 %	2010 %	2011 %	2012 %	2013 %	2014 %
Methicillin	1.6	1.4	0.6	1.3	1.6	1.4	1.4	1.2	1.7	2.9
Penicillin	78	80	78	77	77	75	77	74	76	77
Erythromycin	5	5	4	5	7	5	7	6	7	8
Clindamycin	4	4	3	4	6	4	6	6	6	8
Tetracycline	3	3	2	3	2	3	2	2	3	5
Fusidic acid	10	10	9	9	9	13	13	14	15	15
Rifampicin	<1	<1	<1	<1	<1	<1	<1	<1	0	<1
Norflloxacin	3	2	1	2	2	3	4	4	5	6
Kanamycin	2	1	<1	1	1	1	<1	1	2	2
Linezolid	nt	nt	nt	0	0	0	0	0	0	0
Mupirocin	0	0	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	nt	nt	nt	<1	1	1	1

Note: nt = not tested. In web annex table A8.1 the distribution of MICs and resistance for all tested antimicrobial agents are shown.

Table 8.4. The ten most prevalent spa types demonstrated in SAB and in MRSA cases, Denmark 2014

DANMAP 2014

spa type	SAB		MRSA			
	CC group ^(a)	No. of cases	spa type	CC group ^(a)	No. of cases	No. causing infections (%)
t127	CC1	103	t034	CC398	990	179 (18)
t230	CC45	100	t002	CC5	181	91 (50)
t002	CC5	88	t011	CC398	180	36 (20)
t084	CC15	66	t127	CC1	136	53 (39)
t012	CC30	66	t008	CC8	119	86 (72)
t091	CC7	63	t223	CC22	98	33 (34)
t015	CC45	51	t304	CC8	79	37 (47)
t008	CC8	46	t019	CC30	71	58 (82)
t701	CC8	35	t032	CC22	70	31 (44)
t065	CC45	33	t437	CC59	54	35 (65)

a) CC = Clonal complex.

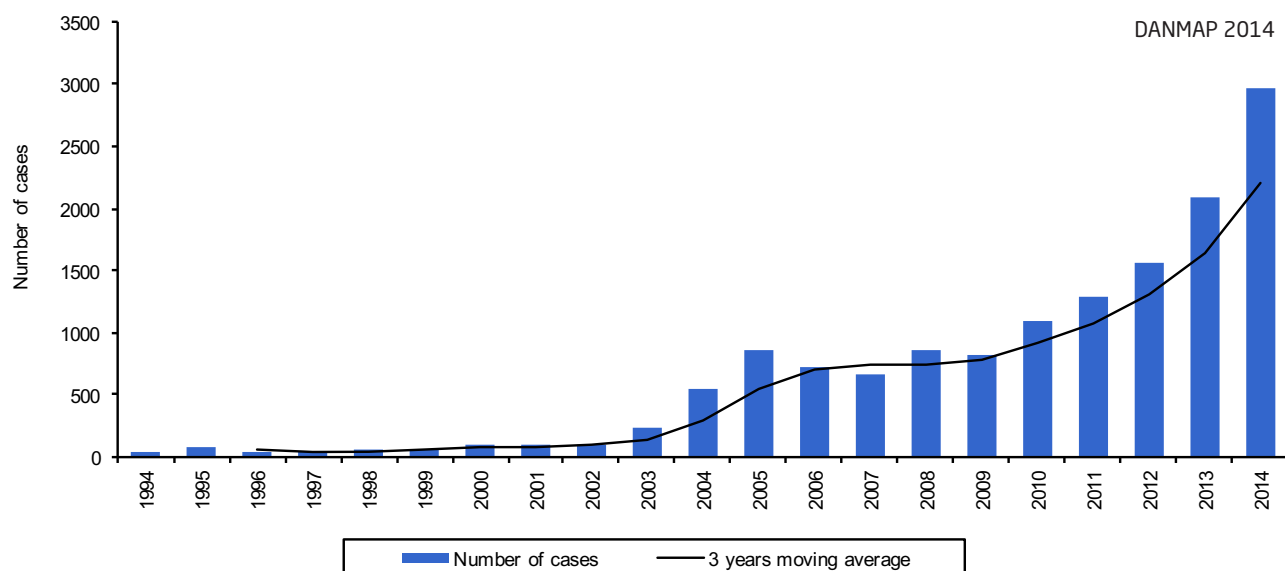
Figure 8.9. Number of MRSA cases, with a three years moving average, Denmark

Table 8.5. Epidemiological classification of new MRSA cases, Denmark

DANMAP 2014

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		411 (14)	230 (56)
Hospital-acquired (HA)		95 (3)	38 (40)
Healthcare associated, community onset (HACO)		187 (6)	
	with known exposure	34	13 (38)
	without known exposure	153	125 (82)
Healthcare worker		50 (2)	13 (26)
Community-acquired (CA)		945 (32)	
	with known exposure	478	86 (18)
	without known exposure	467	370 (79)
CC398		1277 (43)	240 (19)

Note: Numbers shown in bold are totals.

t304/CC8 (n=37), t011/CC398 (n=36), t437/CC59 (n=35), t223/CC22 (n=33) and t032/CC22 (n=31). The PVL encoding gene *lukF-PV* was demonstrated in 32% of the infections and in 7% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n=102), t019 (n=66), t002 (n=41), t437 (n=40) and t044 (n=32).

Resistance among MRSA isolates

Resistance is shown in Table 8.6 and is divided into two categories: CC398 and other. CC398 isolates were typically resistant to tetracycline (99%) and clindamycin (89%) with high levels of resistance to erythromycin and norfloxacin (41% and 28%, respectively). Resistance to fusidic acid and kanamycin was higher among non-CC398 (17% vs 1% and 30% and 7%, respectively). Resistance to at least 1, 2 or 3 other antimicrobials in addition to β -lactam antibiotics (cefoxitin/penicillin) was demonstrated in 70%, 58% and 38% of all of the cases, respectively.

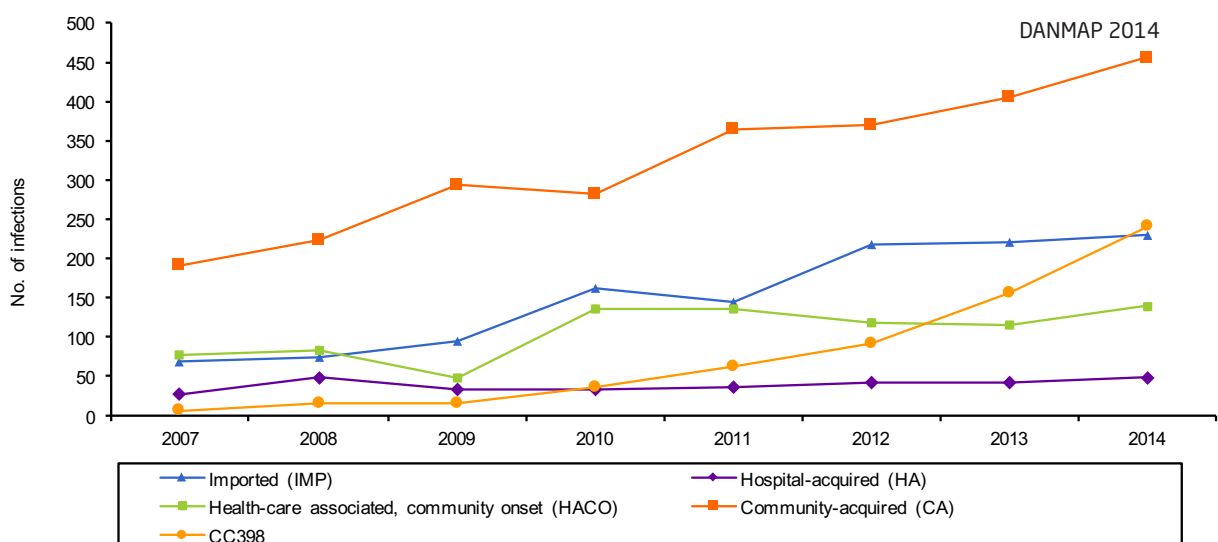
Table 8.6. Resistance (%) in CC398 MRSA and other MRSA cases, Denmark

DANMAP 2014

Clonal complex	CC398 %	other CC %	All cases %
Erythromycin	41	33	35
Clindamycin	89	23	33
Tetracycline	99	21	33
Fusidic acid	1	17	14
Rifampicin	0	<1	<1
Norfloxacin	28	27	27
Kanamycin	7	30	26
Linezolid	0	<1	<1
Mupirocin	0	<1	<1
Trimethoprim-sulfamethoxazole	1	3	3
Number of tested isolates	316	1616	1932

In web annex table A8.2 the distribution of MICs and resistance for all tested antimicrobials are shown.

Andreas Petersen, Robert L. Skov and Anders Rhod Larsen

Figure 8.10. Number of MRSA infections according to epidemiological classification, Denmark

Textbox 8.4

Neisseria gonorrhoeae 2014

Background: *Neisseria gonorrhoeae* is the causative agent of the sexually transmitted infection gonorrhoea, which is usually located in the urethra in males and cervix in females. *N. gonorrhoeae* (gonococci) may sometimes be demonstrated in specimens from the pharynx or rectum in either gender, although infections in these sites are generally asymptomatic. Complications of gonorrhoea include e.g. salpingitis, epididymitis, orchitis, and prostatitis. Further, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct inoculation.

Methods: Through decades, all Departments of Clinical Microbiology in Denmark have submitted their isolates of gonococci to the *Neisseria* and *Streptococcus* Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

At the NSR laboratory ceftriaxone, ciprofloxacin and azithromycin MICs were determined using the Etest® on chocolate agar incubated at 35°C in 5% CO₂. The breakpoints used were those defined by the EUCAST. Both fully and intermediary resistant isolates were categorized as resistant. Penicillinase production was tested for using the Nitrocephin assay.

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, approximately 110 gonococcus isolates are investigated per year for susceptibility to an expanded panel of antimicrobial agents including cefixime and in selected years also spectinomycin, and gentamicin. The latter two drugs were not included on the 2014 survey.

Results and discussion: In 2014, 842 isolates were received. Most of the received isolates were from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

The annual number has increased considerably from 2011 through 2014, presumably because the widespread use of combined PCR-testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* has identified unexpected cases of gonorrhoea (followed by culture), but possibly also due to an increasing incidence of gonorrhoeae.

The ciprofloxacin resistance rate was 46% in 2014, thus showing a steady decline since the peak of 75% in 2009 (Figure 1). The percentage of strains producing penicillinase was 11% in 2014. It has fluctuated between 22% in 2005 and 11% in 2014 and 2013.

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. No cases with such resistance have ever been reported from Denmark. During 2003 through 2009 the proportion of isolates with MIC ≥ 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 2), but during recent years this shift has nearly reversed (44% in 2014). Thus, there is no evidence of emerging ceftriaxone resistance in Denmark.

In 2014, azithromycin resistance including intermediary resistance (MIC > 0.5 mg/L) in gonococci was 36% (Table 1). Several recent guidelines for the treatment of gonorrhoea recommend the combination of high dose ceftriaxone and azithromycin (1 g or 2 g).

Resistance against cefixime (MIC > 0.125 mg/L) was 6% in 2014, i.e. a decrease compared to 2013. Cefixime is an oral cephalosporin that has never been used in Denmark.

Conclusions: The incidence of gonorrhoea seems to be increasing. Although resistance problems are so far not present in Denmark, the emerging ceftriaxone treatment failures in other countries underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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Figure 1. Ciprofloxacin resistance and penicillinase production in gonococci, Denmark, 2005-2014

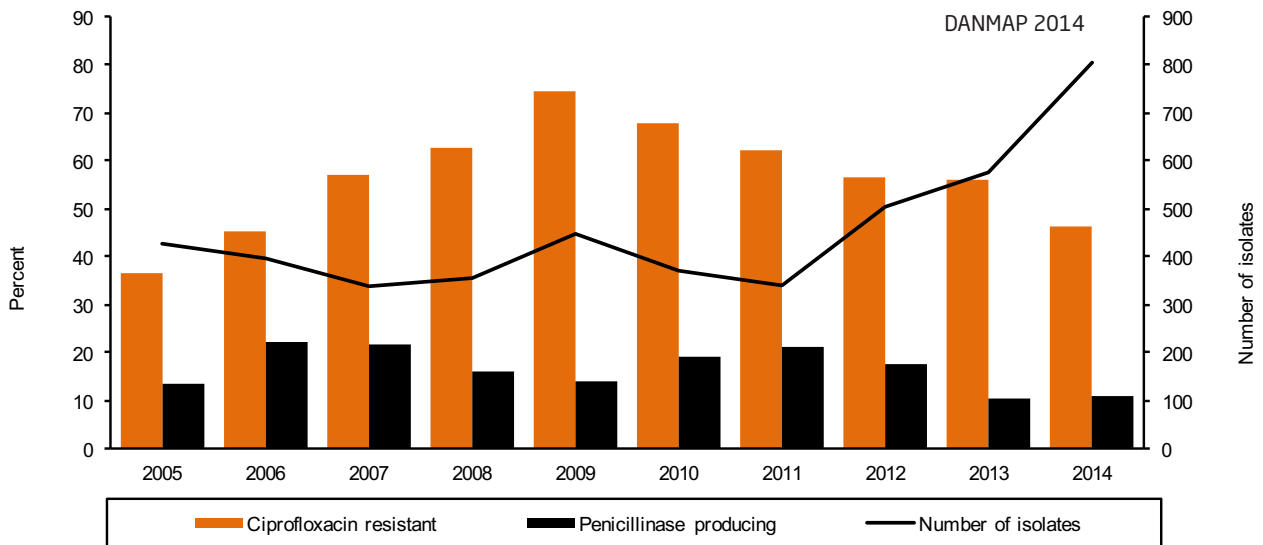
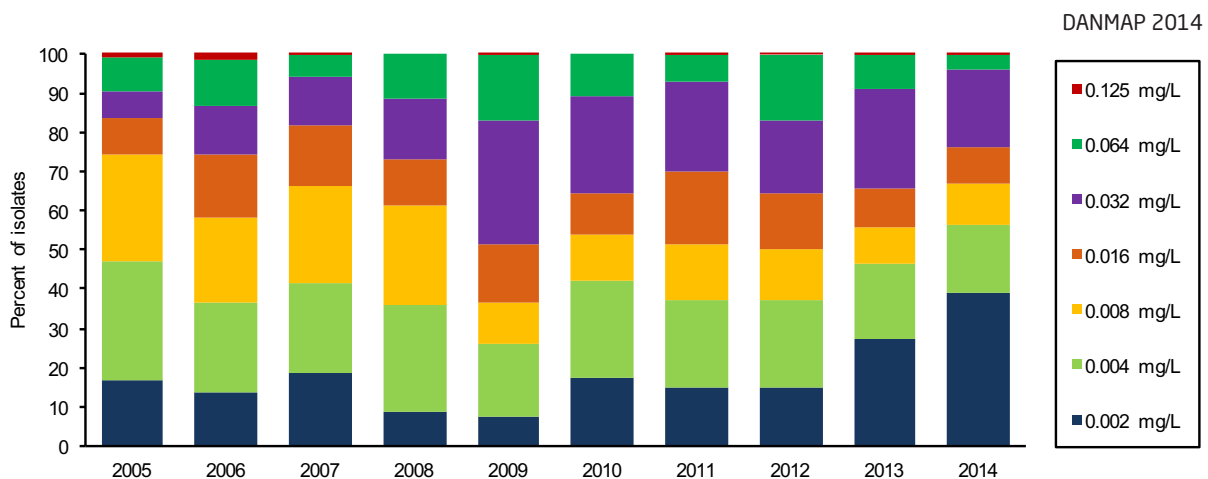


Figure 2. Distribution of ceftriaxon MIC values (mg/L) in gonococci, Denmark, 2005-2014



Textbox 8.5

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) among humans in and in pig herds 2014

Background: Livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) was first recognized in 2005 and have since disseminated worldwide. In Europe, LA-MRSA primarily belongs to clonal complex 398 (CC398) and is especially associated with pigs from where it spreads to humans, primarily to persons with contact to pigs.

Livestock associated MRSA in humans: The number of persons positive for MRSA CC398 continued to increase to 1277 cases in 2014 (42 in 2009, 111 in 2010, 164 in 2011, 232 in 2012 and 643 in 2013). A significant part of the increase from 2012 and onwards was associated with a change in the MRSA screening policy of patients admitted to hospital as contact to pigs was included as a risk factor requiring screening for MRSA in December 2012.

[<https://sundhedsstyrelsen.dk/en/health/infectious-diseases/mrsa/guidelines>].

The most frequent spa types related to CC398 were t034 (n = 990), t011 (n = 180) and t571 (n = 20). The majority of CC398 cases (1140, 89%) were in persons with documented close contact to pigs or in members of their household. This resembles the proportion from previous years. In actual numbers, cases without documented contact to livestock increased from 83 cases in 2013 to 137 in 2014. The majority of these cases are from areas of high pig density and there is still no sign of significant spread of CC398 to urban areas.

Two hundred and forty of the 1,277 CC398 cases (19%) presented with infections. This is considerably lower than for community acquired MRSA (CA-MRSA) in general, and reflects that the majority of cases are found through screening programmes. In 2014, eight bacteraemia cases were MRSA CC398. Two of the patients died within 30 days. The patients did not have any direct contact to pigs.

MRSA in animals: In 2014, a national survey was carried out to estimate the prevalence of LA-MRSA in Danish pig herds. Samples, consisting of five pools of five nasal swabs per herd were collected in 70 breeding herds and 205 randomly selected slaughter pig herds. All samples were collected at the farm.

The results are shown in Table 1. The overall prevalence of MRSA was high in both breeding herds, 63 %, and slaughter pig herds, 68 %, which is a marked increase compared to previous surveys, where 16 % of the herds, tested at the farm were found positive for MRSA (DANMAP 2010 and 2011). Differences between regions were not significant.

Table 1: LA-MRSA in 275 Danish pig herds, Denmark

DANMAP 2014

	No. tested herds	Prevalence
Breeding herds	70	63 %
Slaughter pigs - Denmark	205	68 %
Slaughter pigs - Jutland	147	70 %
Slaughter pigs - Funen	39	69 %
Slaughter pigs - Zealand	19	53 %

Conclusions: In 2014, a steep increase in CC398 cases was detected and CC398 continued to be the most common CC group among human MRSA cases. An increasing number of CC398 cases had no documented contact to pigs but lived in areas with high density of pigs. Likewise, an increase since 2010 in the percentage of infected pig herds was evident.

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9

MATERIALS AND METHODS



9. Materials and methods

9.1 General information

For the DANMAP 2014 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined as the individual farm, meaning that only one isolate per bacterial species per farm was included in the report. The same epidemiological unit was applied for broilers, except for *Salmonella* where the individual flock of broilers was defined as the epidemiological unit. For food, the epidemiological unit was defined as the individual meat sample. For humans, the epidemiological unit was defined as the individual patient and the first isolate per patient per year was included.

Unless stated specifically, all differences and temporal trends noted in the text are statistically significant ($p < 0.05$) using either Chi-square tests or linear logistic regression (see Section 9.6.3).

9.2 Data on antimicrobial consumption

Antimicrobial agents used for humans and animals in Denmark are presented in Table 3.2.

9.2.1 Data on antimicrobial consumption in animals

Since 2001, consumption data presented in this report have been obtained from the national monitoring programme VetStat, which is a database hosted by the Danish Veterinary and Food Administration. Prior to 2001, data were based on national sales figures from the pharmaceutical industry.

Data registration

In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals, except in a few instances when medicines are prescribed on special license (i.e. medicines not approved for marketing in Denmark). In addition, data on consumption of coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) are collected by VetStat.

Until 2007, antimicrobial agents could only be purchased at the pharmacy or in medicated feed from the feed mills. From April 2007, the monopoly was suspended and private companies (four in 2014) were permitted - on certain conditions identical conditions as for pharmacies - to sell prescribed veterinary medical products for animals. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration costs related to sale of large quantities to the veterinarians.

The pharmacy or company either sells the medicines to veterinarians for own use in practice or for re-sale to farmers, or sells the medicines directly to the animal holder on presentation of a prescription. By law, the profit that veterinarians may make on the sale of medicine is very small (5%), thereby limiting the economic incentive to sell medicine.

In 2014, 93% of antimicrobial agents were purchased through pharmacies and the drug trading companies, while 4% were purchased from the feed mills. These percentages do not include prescribed zinc oxide from the feeding mills for the pig production. In cattle, the majority of antimicrobial agents are now purchased from pharmacies, whereas 10 years ago where more than 80% of the antimicrobial agents used in cattle was purchased through the veterinarian. In aquaculture, more than 80% is purchased through the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to VetStat. Veterinarians are required by law to report to VetStat all use and prescriptions for production animals (monthly submissions). For most veterinarians, the registration of data is linked to the writing of invoices. However, errors in the veterinarians invoice system sometimes cause errors in amounts reported, and these data are not validated at entry to VetStat. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and identity of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at SSI and on to VetStat also daily.

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age-group, disease category and code for farm-identity (CHR - Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" - a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no].

Methods

In DANMAP we want to compare consumption of antimicrobials between different animal populations and between veterinary and human sectors. In order to do this we need to take into account the quantity of antimicrobials used, their potency, their formulation, the route of administration and - sometimes - the age of the animals in which they are used. We also need to know the size of the populations to which the antimicrobials are administered.

In DANMAP 2014, we use defined animal daily doses (DADDs) that are harmonised so products with the same active compound, strength and dispensing form all have the same dose, even if higher doses listed in the approved Summary of Product Characteristics (SPC). Thus the animal daily doses currently listed in the VetStat database (ADDs) and the DADDs used in DANMAP 2014 are not in full compliance; however adjustments of the VetStat ADDs are being implemented. See Textbox 9.1 for further description of this issue. DADDs are applied for estimation antimicrobial consumption in pigs and cattle, and listed in the web annex.

DADD - Defined animal daily dose

DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species and when appropriate, also age group. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The basic principles for the DADD are similar to the principles previously described for the ADD. The designation of the DADD is based on the VetStat ADDs, but re-defined for each group of antimicrobial agents, i.e. for each combination of active compound, administration route, formulation, considering the following principles:

1. Minor inconsistencies, e.g. due to rounding of numbers, have been corrected;
2. Approved dosage for the most widely used antimicrobial products were given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group are maintained as the common DADD even if a new product is approved with a higher dosage;
4. In determining the dosage for a group with large variation between approved dosages of the products, the dosages in accordance with the dose given in "The Veterinary Formulary" [British Veterinary Association, 2005, 6th edition] are applied;

5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age group/indication or formulation.

When principle 2 and 3 are conflicting, principle 4 is applied.

Denominator

Trends in antimicrobial consumption in pigs are presented in DADD per 1,000 animals per day - DAPD. The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live body-weight and the average life-span in each age group. In 2014, DAPD calculations were carried out for pigs only.

DAPD - DADD per 1,000 animals per day

The number of DADDs administered to a specific animal species during a year (in thousands) divided by the number of standard animals at risk per day. The number of standard animals at risk per day takes into account species differences in average body-mass and life-span. When relevant, the numbers of DADDs and standard animals at risk are estimated for specific age groups, or simply as number of doses (DADDs) used to treat one kg of animal divided with the total estimated biomass (in tonnes).

DAPD is a statistical measure, providing a rough estimate of the proportion (in thousands) of animals treated daily with an average maintenance dose of a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. The DAPD is also referred to as the treatment proportion.

In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, Defined daily dose per 1,000 inhabitants per day (DID), see Section 9.2.3.

Due to a relative high number of pigs exported around 30 kg weight (32% of pigs produced in 2014, Table 3.1), an adjusted measure of consumption per pig was calculated. The adjustment is based on the assumption that pigs exported at 30 kg, on average, received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg.

Antimicrobial use per pig produced (adjusted) = $[DADDs + DADDw + (1+Q)*DADDf] / (\text{biomass-days-total} + Nw*5800(\text{kg*days}))$, where DADDs = amount of antimicrobial agents used in sows; DADDw = amount of antimicrobial agents used in weaners; DADDf = amount of antimicrobial agents used in finishers; Q is the proportion of weaning pigs exported around 30 kg. Nw = number of pigs exported at 30 kg body-weight, and Nw*5800 is the number of biomass days the

exported pigs would have contributed to the live biomass if not exported.

9.2.2 Estimation of live biomass of animals

The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species.

Pig production. The estimation was based on the number of pigs produced, including exports at different ages [Statistics Denmark; Danish Agriculture and Food Council], productivity data for each year [Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) were estimated from the productivity number. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this could not be accounted for.

Cattle production. The live biomass of the cattle population is estimated from census data [Statistics Denmark] and the average live weight of the different age groups. The Danish cattle

population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Turkey production. The live biomass is estimated based on the number of turkeys produced [Statistics Denmark; Danish Agriculture and Food Council] and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Fur animals. The live biomass of mink is estimated from production data [Statistics Denmark, Copenhagen Fur] and the average weight at pelting was 2.45 kg [Kopenhagen Fur]. The progeny live for approximately 7 months. The biomass for the breeding animals (female) was estimated based on census data and an assumed average live weight of 2 kg.

Pet animals. Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The population is based on census

Textbox 9.1 Revised method for calculating the ADD in Vetstat

When Vetstat was introduced in 2000, the Animal Daily Doses (ADDs) was calculated based on the approved Summary of Product Characteristics (SPC). The intention was that products with the same active compound, same strength and same dispensing form should have the same ADD. For products with dose ranges, the ADD was calculated as the average value. In cases where different indications required different doses, the ADD was calculated on the basis of the most frequent indication. These principles are similar to those used for antimicrobial products used for humans. Most products for veterinary use were registered through a national procedure, making it possible to apply the principles described above. Furthermore, the ADDs used in Vetstat had no legislative implication at the time.

In 2011, an increasing number of veterinary products were registered by the central registration procedure by the European Medicines Agency (EMA). In some cases, registration is based in information from studies carried out in the other European countries, where the average doses for same indication are somewhat higher than in Denmark. As a consequence of this, several products came on the Denmark market with approved ADDs that were double up compared to similar products already on the market, but approved through the national procedure. This issue was addressed in DANMAP 2012.

In 2010 the yellow card was introduced [DANMAP 2010], and as a consequence of this, the ADDs defined in Vetstat became of considerable legislative importance. Therefore, it was unacceptable if products with the same active compound, strength and dispensing form had different ADDs in Vetstat. Consequently, it was decided that Vetstat, by announcement, should be able to regulate ADD on its own and not necessarily follow the approved SPC (BKG nr. 178 af26/2 2014).

The following shows the principles for calculating ADD (only for pig and cattle). The main points are as follows:

- Products with same active compound, same strength and same dispensing form should have same ADD
- If a product is brought on the market with two or more different strengths, the ADDs for each strength are calculated proportionally.
- If one or more products with same active compound and dispensing form are approved for two or more indications and with different doses, the ADD will be calculated on basis of the indication for which it is more frequently used.

The next step will be a parallel ADD (DDDA) based on the active ingredient. The DDDA will at this stage be used only for calculation of number of doses used of every active ingredient. This will not affect the yellow card system.

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data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture. The estimation is based on data from the Danish AgriFish Agency (NaturErhvervstyrelsen) on produced amounts in each subtype of production, and information on the typical lifespan and entrance and exit body weights, and were calculated in cooperation with Danish Aquaculture [NH Henriksen, Danish Aquaculture].

9.2.3 Data on antimicrobial consumption in humans

Data on consumption of antibacterial agents in humans were obtained from The Register of Medical the Department of Data Delivery and Medicinal Product Statistics, at Statens Serum Institut. The Register receives monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies. Data from the primary health care sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

Only somatic hospitals were included, when the consumption was measured by occupied bed-days and admissions. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

In the primary health care in Denmark, all antibacterial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code, which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDDs), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and pharmacy of the transaction, and information regarding reimbursement of cost, if applicable. For hospital data we have information of the type of department and hospital but no information on the individual consumption level.

Before 2012 data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. From 2013 onwards all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at SSI:

The present report includes data on the consumption of "antimicrobials for systemic use", or group J01, of the 2014 update of the ATC classification, in primary health care and in hospitals

as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 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 with primary health care, and DBDs, the number of DDDs per 100 occupied beds 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 DDDs per 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].

9.3. Collection of bacterial isolates

9.3.1 Animals

Samples from healthy pigs and cattle and broilers were collected for the DANMAP programme at slaughter by meat inspection staff or abattoir personnel.

Salmonella. DANMAP 2014 only includes *Salmonella* isolates from caecum samples originating from random sampling of healthy pigs. Samples were sent for examination at the Danish Veterinary and Food Administration (DVFA) laboratory in Ringsted. Serotyping was performed at DTU National Food Institute. Data from previous years also include isolates from the national *Salmonella* surveillance programme where the results of a serological surveillance at the slaughterhouses and in all breeding herds appoint risk herds to be further examined by microbiological analysis of pen-faecal samples.

No *Salmonella* data from layers, broilers and cattle are presented in DANMAP 2014 due to a low number of isolates available from the national surveillance. Further details on the sampling procedures and the findings of the Danish surveillance programs are presented in the Annual Report on Zoonoses in Denmark, 2014 [www.food.dtu.dk].

Campylobacter, indicator E. coli and enterococci. For broilers, caecal samples were collected at slaughter throughout 2014, covering 99.8% of all broilers slaughtered in Denmark

(Sampling according to Commission decision 2013/652/EU). From each flock, four intact caeca were pooled and sent for examination at the DVFA laboratory (*Campylobacter* and *E. coli*) and DTU National Food Institute (*Enterococcus*). From broilers, isolates of *Campylobacter jejuni*, indicator *E. coli* and *Enterococcus faecalis* were collected.

Random sampling of healthy pigs (same caecum samples as for *Salmonella*) and cattle (rectum samples) were collected once a month throughout 2014. The slaughter plants included in the DANMAP programme accounted for 99% and 95% of the total number of pig and cattle slaughtered in Denmark during 2014, respectively. The number of pigs and cattle samples from each slaughter plant was proportional to the annual number of animals slaughtered at the plant. Samples were sent for examination at DTU National Food Institute. Indicator *E. coli* and *Enterococcus faecium* were isolated from pigs, whereas *Campylobacter jejuni* and indicator *E. coli* were isolated from cattle.

9.3.2 Meat

Salmonella. The *Salmonella* isolates from Danish pork originated from the national *Salmonella* surveillance programme (carcass swabs taken at the slaughterhouse after cooling). Samples were sent for examination at the Industry laboratories. Serotyping was performed at DTU National Food Institute. No *Salmonella* data from broiler meat and beef, as well as imported meat are presented in DANMAP 2014 due to a low number of isolates available from the national surveillance and control programmes.

Campylobacter. The *Campylobacter jejuni* isolates originated from the national control program: Intensified control of *Salmonella* and *Campylobacter* in fresh meat based on a case-by-case risk assessment. Sampling was carried out by the regional DVFA officers, and included Danish and imported fresh broiler meat ready for retail, e.g. located in cold stores, slaughterhouses, cutting and processing facilities, but also at catering companies or at border control posts. The Samples were examined at the DVFA laboratory.

Indicator *E. coli* and enterococci. Approximately a thousand meat samples were collected at wholesale and retail outlets in all regions of Denmark throughout 2014. Samples were collected by the DVFA Food Control Offices during routine inspection or on specific request for the DANMAP programme and examined at the DVFA laboratory. Indicator *E. coli*, *Enterococcus faecium* and *Enterococcus faecalis* were isolated from Danish and imported broiler meat, beef and pork.

9.3.3 Humans

S. Typhimurium and C. jejuni. Antimicrobial susceptibility was performed on human faecal isolates submitted to Statens Serum Institut (SSI). *Campylobacter* isolates were submitted from Departments of Clinical Microbiology (DCM) covering three geographical regions: Northern Jutland, Funen and Zealand. In-

formation on travel history was obtained for the patients. *Salmonella* isolates were submitted from all DCM in Denmark. Exact figures of the proportion tested and the sampling strategy for the different species can be found in Sections 6.1 and 6.2.

Staphylococcus aureus. All blood isolates were referred to the *Staphylococcus* reference laboratory at SSI on a voluntary basis. In November 2006, methicillin-resistant *S. aureus* (MRSA) became a notifiable disease in Denmark and it became mandatory to submit all MRSA isolates to the reference laboratory.

Invasive Streptococcus pneumoniae, Streptococcus pyogenes (group A streptococci), group B, C and G streptococci. Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all invasive isolates nationwide are sent to SSI for identification or confirmation as well as susceptibility testing and typing. Invasive group A, B, C and G streptococcal isolates are referred to SSI on a voluntary basis. Traditionally, only isolates from blood and spinal fluid are included in the DANMAP report.

E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. faecium and E. faecalis. Data were provided on all clinical isolates recorded from either blood samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecium* and *E. faecalis*) or urine samples (*E. coli* and *K. pneumoniae*) submitted for susceptibility testing to the DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev, Region Zealand, Odense, Esbjerg, Vejle, Herning/Viborg, Aarhus and Aalborg.

No samples were collected from healthy humans.

9.4 Isolation and identification of bacteria

9.4.1 Animals

Salmonella from pigs was isolated according to the open reference methods for detection in foods, faeces and materials from primary animal production using MSRV [NMKL No. 187, 2007] or Annex D, ISO 6579 [ISO6579:2002/Amd 1:2007]. Serotyping of suspect colonies by slide agglutination according to the White-Kauffmann-Le Minor Scheme in combination with "The molecular approach developed by CDC [Fitzgerald *et al.* 2007. J Clin Microbiol. 45:3323-34; McQuiston *et al.* 2011. J Clin Microbiol. 49: 565-573]. Only one isolate per herd of each serotype was selected.

Campylobacter. For samples from broilers, isolation was performed according to the guidelines for microbiological examination of food [NMKL No. 119, 2007]. Identification was performed by microscopy and by oxidase activity, catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. For samples from cattle, selective enrichment in Preston broth at a ratio of 1:10 incubated in microaerophilic atmosphere for 24 h at 41.5°C was performed followed by inoculation of 10 µl of the enrichment broth onto mCCD agar and incubated as above.

Campylobacter-like colonies were verified by microscopy and species identification of *C. jejuni* was performed by a real-time PCR assay [Mayr *et al.* 2010. J Food Prot. 72(2):241-50]. Only one *C. jejuni* isolate per broiler flock or cattle herd was selected.

Indicator *E. coli*. The material from pigs and cattle was inoculated directly onto Drigalski agar (SSI Diagnostica, Denmark) and incubated o/n at 37°C. Yellow colonies were inoculated onto BBL CHROM agar Orientation Medium (Becton Dickinson, Germany) and red colonies were collected as *E. coli* after o/n incubation at 37°C. For broilers, four caecum samples was pooled and added 10 ml of BPW, stomached 30 sec. followed by streaking (1 µl) onto violet red bile (RVG) agar and incubated for 24 h at 44°C. Presumptive *E. coli* was identified using TBX agar incubated o/n at 44°C. Only one *E. coli* isolate per broiler flock, pig or cattle herd was selected.

Indicator enterococci. An adequate amount of material suspended in 2 ml of sodium chloride (0.9%) was inoculated on Slanetz Bartley agar and incubated two days at 42°C. Three colonies resembling typical *E. faecalis* were sub-cultivated on blood agar. *E. faecalis* were identified by motility- and arginine dihydrolase tests and the ability to ferment mannitol, sorbitol, arabinose and raffinose. Only one *E. faecalis* isolate per broiler flock or pig herd was selected.

9.4.2 Meat

Salmonella was isolated according to the open reference methods issued by the NMKL [NMKL No. 187, 2007 or NMKL No. 71, 1999], the ISO 6579:2002 or alternative methods validated against the reference method according to ISO 16140:2001. Serotyping of for *Salmonella* isolates from animals. Only one isolate per positive swab sample of each serotype was included.

Campylobacter was isolated according to the guidelines for microbiological examination of food [NMKL No. 119, 2007]. Identification was performed by microscopy and by oxidase activity, catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. Only one *C. jejuni* isolate per batch of fresh meat was selected.

Indicator *E. coli* was isolated by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulphate-broth, which was incubated o/n at 44°C, subsequently streaked onto violet red bile agar and incubated for 24 h at 44°C. Presumptive *E. coli* was identified using TBX agar TBX agar incubated o/n at 44°C. Only one *E. coli* isolate per meat sample was selected.

Indicator enterococci were isolated by adding 5 g of the sample to 45 ml azide dextrose broth, incubated o/n at 44°C and subsequently streaked onto Slanetz-Bartley agar and incubated 48 h at 44°C. Colonies typically for *E. faecium* and *E. faecalis* were identified by a real-time PCR assay. Only one isolate of of *E. faecium* and *E. faecalis* per meat sample was selected.

9.4.3 Humans

Salmonella isolates were serotyped by slide agglutination according to the Kauffman-White Scheme.

***Campylobacter*.** Species identification was performed using a species-specific PCR assay [Klena *et al.* 2004. J Clin Microbiol. 42: 5549-5557].

***Staphylococcus aureus*.** Species confirmation and typing was performed by sequencing of the *S. aureus* specific *spa* gene [Harmsen *et al.* 41: 5442-5448, J Clin Microbiol., 2003]. *Spa*-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the *spa* type and known association with MLST typing, each isolate was assigned to a clonal complex (CC). For MRSA isolates, presence of the *mecA* or *mecC* methicillin resistance genes was confirmed by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611-614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395-400]. For all isolates, presence of *lukF-PV* gene (PVL) was demonstrated by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611-614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395-400].

Invasive *Streptococcus pneumoniae*. Serotype identification of invasive *S. pneumoniae* was performed by latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Hillerød, Denmark) and further with factor specific antisera by the Neufeld Quellung test (SSI Diagnostica, Hillerød, Denmark).

Invasive *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. Identification of groups were performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Roskilde, Denmark).

9.5 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter*, indicator *E. coli*, *Enterococcus* and *Staphylococcus aureus* was performed as microbroth dilution MIC with Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

The relevant 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 were susceptibility tested partly at DTU National Food Institute (pigs - *E. coli* and *E. faecalis*; cattle - *E. coli* and *Campylobacter*; poultry - *E. faecalis*) and the DVFA laboratory (poultry - *E. coli* and *Campylobacter*). Isolates from meat were generally tested at the DVFA laboratory, except for *Salmonella* from the surveillance of fresh meat that were tested at DTU National Food Institute. Isolates were stored at -80°C until susceptibility testing. MIC-testing at DTU National

Table 9.1. Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values (blue fields) and the corresponding EUCAST clinical breakpoints (white fields)

DANMAP 2014

Antimicrobial agent	<i>Salmonella</i> ^(a)		<i>E. coli</i>		<i>E. faecium</i>		<i>E. faecalis</i>		<i>C. jejuni</i>	
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8*	>8*	>8*	>8*	>4*	>8*	>4*	>8*		
Apramycin	>16		>16							
Cefotaxime	>0,5*	>2*	>0,25*	>2*						
Ceftazidime	>2*		>0,5*							
Ceftiofur	>2*		>1*							
Chloramphenicol	>16*	>8*	>16*	>8*	>32*		>32*			
Ciprofloxacin	>0,064*	>0.064*	>0,064*	>1*	>4*	>4*	>4*	>4*	>0,5*	>0.5*
Colistin	>2*/8 ^(b)	>2*	>2*	>2*						
Daptomycin					>4*		>4*			
Erythromycin					>4*		>4*		>4*	>4*
Florfenicol	>16*		>16*							
Gentamicin	>2*	>4*	>2*	>4*	>32*		>32*		>2*	
Linezolid					>4*	>4*	>4*	>4*		
Meropenem	>0,125*	>8*	>0,125*	>8*						
Nalidixic acid	>16*		>16*						>16*	
Neomycin	>4*		>8*							
Quinupristin/dalfopristin					>4 ^(d)	>4*				
Spectinomycin	>64									
Streptomycin	>16*									
Sulfonamide	>256 ^(c)		>64*							
Teicoplanin					>2*	>2*	>2*	>2*		
Tetracycline	>8*		>8*		>4*	>0.5*	>4*	>0.5*	>1*	>2*
Tigecycline	>1*	>2*	>1*	>2*	>0,25*		>0,25*			
Trimethoprim	>2*	>4*	>2*	>4*						
Vancomycin					>4*	>4*	>4*	>4*		
Trimethoprim										
Vancomycin										

Note: * indicates EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints. ECOFFs marked in orange indicate antimicrobial agent included in the new EU test panels, not present in the DANMAP test panel used in previous years. The following compounds are not included in the EU test panels for *Salmonella* and *E. coli*: apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin (new compounds are ceftazidime, meropenem and tigecycline); for *Enterococcus*: kanamycin, penicillin, salinomycin and streptomycin (new compound is daptomycin); for *Campylobacter*: chloramphenicol. In addition, azithromycin is included in the EU test panel for *Salmonella* and *E. coli* but are not included in the DANMAP 2014 as no EUCAST ECOFFs were available.

a) In 2014, only the *Salmonella* isolates from Danish pork were tested for susceptibility to apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin.

b) The EUCAST ECOFF (>2) for colistin was applied for *S. Typhimurium* and other serotypes, except for *S. Enteritidis* and *S. Dublin* where ECOFF >8 was applied according to investigations presented in DANMAP 2011.

c) No current EUCAST ECOFF is available for sulfamethoxazole, so the previous cutoff (>256) was maintained.

d) The EUCAST ECOFF (>1) was not applied for quinupristin/dalfopristin (tradename synercid) according to investigations presented in DANMAP 2006.

Food Institute and the DVFA laboratory in Ringsted is accredited by DANAK (the national body for accreditation).

Salmonella, *Campylobacter* and *Staphylococcus aureus* isolates of human origin were tested at SSI.

Table 9.1 presents the interpretation of MIC-values used for any combination of bacteria and antimicrobial agent. Since 2007, data are interpreted by EUCAST epidemiological cut-off values (ECOFFs) with a few exceptions described in Table 9.1.

The corresponding clinical breakpoints validated by EUCAST are presented both in Table 9.1 and in the MIC-distribution tables to visualize the impact of the use of ECOFFs contra clinical breakpoints. In general, if ECOFFs were re-validated and changed by EUCAST during the past year, all data presented from previous years were interpreted using the changed ECOFFs. Data from susceptibility testing of *Staphylococcus aureus* were interpreted using EUCAST clinical breakpoints. All MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the

Table 9.2. Definitions of antimicrobial classes for calculation of multi-resistance (MR) in zoonotic and indicator bacteria, DANMAP

DANMAP 2014

Antimicrobial classes	<i>Salmonella</i> and <i>E. coli</i> ^(a)	<i>Campylobacter</i> ^(b)	<i>Enterococcus</i> ^(c)
Tetracyclins	Tetracycline	Tetracycline	Tetracycline
Phenicols	Chloramphenicol		Chloramphenicol
Penicillins	Ampicillin		Ampicillin
Cephalosporins	Cefotaxime and/or ceftazidime		
Carbapenems	Meropenem		
Sulfonamides	Sulfonamides		
Trimethoprim	Trimethoprim		
Aminoglycosides I	Gentamicin	Gentamicin	Gentamicin
Aminoglycosides II		Streptomycin	
Quinolones	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin
Polymyxins	Colistin		
Macrolides		Erythromycin	Erythromycin
Glycopeptids			Vancomycin and/or teicoplanin
Oxazolidinones			Linezolid
Glycylcyclines	Tigecycline		Tigecycline
Lipopeptide			Daptomycin

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the panel for the selected bacterial species

a) An isolate is considered multi-resistant if resistant to three or more of the eleven antimicrobial classes

b) An isolate is considered multi-resistant if resistant to three or more of the five antimicrobial classes

c) An isolate is considered multi-resistant if resistant to three or more of the ten antimicrobial classes

IMPORTANT: The following compounds are not included in the EU test panels for *Salmonella* and *E. coli*: apramycin, ceftiofur, florfenicol, neomycin, streptomycin and spectinomycin (new compounds are ceftazidime, meropenem and tigecycline); for *Enterococcus*: kanamycin, penicillin, salinomycin and streptomycin (new compound is daptomycin); for *Campylobacter*: chloramphenicol. In addition, azithromycin is included in the EU test panel for *Salmonella* and *E. coli* but are not included in the DANMAP 2014 as no EUCAST ECOFFs were available. Thus calculations of multi-resistance and fully sensitive isolates in 2014 is not comparable to results presented in previous years DANMAP reports.

number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals.

Multi-resistance was defined as resistance to three or more of the antimicrobial classes listed in Table 9.2. Isolates were considered fully sensitive if susceptible to all the antimicrobial agents included in the test panels.

Invasive *Streptococcus pneumoniae* from humans. Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK) as recommended by the manufacturer. All breakpoints used were as defined by the EUCAST. Both fully and intermediary resistant isolates were defined as resistant.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans. Screening for penicillin-, erythromycin- and clindamycin-resistant streptococci was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Roskilde, Denmark), respectively, on Müller-Hinton agar (Müller-Hinton plate, 5%

blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Isolates were simultaneously tested for inducible clindamycin resistance. Non-sensitive streptococci were tested further with the respective E-tests (Biomérieux), either benzylpenicillin, erythromycin or clindamycin on Müller-Hinton agar. The breakpoints used were as defined by the EUCAST. Both fully and intermediary resistant isolates were defined as resistant.

***E. coli*, *K. pneumoniae*, invasive *P. aeruginosa*, invasive *E. faecium* and *E. faecalis* from humans.** The DCM performed either disk (Oxoid, Basingstoke, UK) or tablet (Neo-Sensitabs®, A/S Rosco) diffusion susceptibility testing on a number of media. Since 2013, all DCM have used breakpoints defined by EUCAST.

Data on antimicrobial resistance from private hospitals and clinics and from psychiatric hospitals were excluded. All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

9.6 Data handling

9.6.1 Animals and meat

The results from the analysis of the bacteria isolated and the susceptibility were harmonised and validated using SAS, and the isolate-based data were stored in a database contain-

ing all antimicrobial resistance data used for DANMAP and/or reported to EFSA since 2007. The Oracle database (9i Enterprise Edition®) is maintained at DTU National Food Institute. The susceptibility data were stored as continuous values as well as categorised as susceptible or resistant as defined by the relevant ECOFF. Each animal isolate was identified by the bacterial species, the subtype as applicable and by the date of sampling and species of animal. Information on the farm of origin was also recorded when available. For each meat isolate, information was available on food type, bacterial species, date and place of sampling, date of examination and country of origin was recorded whenever possible. Identification numbers were also included, which makes it possible to obtain further information about the isolate from the relevant authorities or laboratories.

All handling and evaluation of results were carried out using SAS®Software, SAS Enterprise Guide 6.1.

9.6.2 Human

Salmonella and Campylobacter. Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

Staphylococcus aureus. For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonization/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonization), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 12 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalizations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to

CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

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 linked to 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. Ten out of eleven DCM in Denmark provided data on resistance levels in *E. coli*, *K. pneumoniae*, *invasive P. aeruginosa*, *invasive E. faecium* and *invasive E. faecalis* isolates. Data were extracted from the following laboratory information systems:

- ADBakt (Autonik AB, Skoldinge, Sweden) for the DCM at Hvidovre, Herlev and Aalborg Hospitals.
- MADS (DCM Skejby Hospital, Aarhus, Denmark) for the DCM at Rigshospitalet, Slagelse/Region Zealand, Odense, Esbjerg, Vejle, Herning/Viborg, and Aarhus (Skejby) Hospitals.

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.

9.6.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 6.1 or StatCalc in EpiInfo™ v. 6. Difference in pair-wise comparisons were tested using Chi-square, or Fisher's Exact Test when the number of samples is low. When appropriate, significance of temporal trends is tested using linear logistic regression using Proc LOGISTIC procedure in SAS (Likelihood ratio test).

In the text, commented differences imply statistically significant differences where $p < 0.05$.

When comparing proportions between years, the EUCAST epidemiological cut-off values for 2014 were also used for interpretation of previous years MICs.

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10

TERMINOLOGY



List of abbreviations

AGP	Antimicrobial growth promoter
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
CA	Community-acquired
CC	Clonal complex
CDI	<i>Clostridium difficile</i> infections
CHR	Central Husbandry Register
CI	Confidence interval
CNS	Central nervous system
COHA	Community onset hospital-acquired
CPE	Carbapenemase producing Enterobacteriaceae
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined animal daily dose
DAPD	Defined animal daily dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1000 inhabitant days)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	Extended spectrum beta-lactamase
GI	Gastrointestinal
GP	General practitioner
HAI	Hospital-acquired infections
HAIBA	Hospital-acquired infections database
HLGR	High-level gentamicin resistance
HOHA	Hospital onset hospital-acquired
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
RFCA	Regional Veterinary and Food Control Authorities
SSI	Statens Serum Institut
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin resistant enterococci
WGS	Whole genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification.

International classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) (www.whocc.no/atcddd/indexdatabase/).

The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whocc.no/atcvet/database/).

Antibacterial agents. Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. Antimycobacterial agents are not included. In the section of human consumption, 'antibacterial agents' are referred to as 'antimicrobial agents' (see below).

Antimicrobial agents. The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is usually used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only). In the section of human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system).

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.66 kg.

Central Husbandry Register (CHR). This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined animal daily dose (DADD). DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC). In contrast to the ADD previously used in DANMAP, the DADD not has been defined for each antimicrobial agent, administration route and animal species but at product level. In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which has been used since DANMAP 2003. For more details, see Chapter 10, Materials and Methods. The DADDs used in DANMAP 2012 are presented the web annex.

DADD per 1,000 animals per day (DAPD). Trends in veterinary consumption, both within and across species, are presented in DAPD, allowing for comparison between sectors and adjusting for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight and lifetime. This may also be referred to as the 'standard-animals-at-risk' and takes into account species differences in body-mass and life-span. DAPD is a statistical measure, providing an estimate of the proportion of animals (in thousands) treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day (Section 4.3 and Chapter 10, Materials and Methods).

Defined Daily Dose (DDD). This is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whocc.no/atcddd/indexdatabase/).

DDD per 1,000 inhabitants per day (DID). Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, allowing for comparison between sectors and for illustration of the consumption in hospital care without taking account of hospital activity (discharges). Data presented in DID provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DIDs indicates that 1% of the population on average gets a certain treatment daily. In figure presented as DDD/1,000 inhabitant-days.

ESBL. In the DANMAP report, 'ESBL' describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC and OXA-ESBLs [Giske et al. 2009. J. Antimicrob. Chemother. 63: 1-4].

Finishers. Pigs from 30-100 kg live weight from after the weaner stage to time of slaughter.

Fully sensitive. An isolate will be referred to as fully sensitive if susceptible to all antimicrobial agents included in the test panel for the specific bacteria.

Intramammaria. Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Layer. A hen raised to produce eggs for consumption.

Minimum inhibitory concentration (MIC). This is the lowest concentration of antimicrobial agent in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

Multi-resistant. A Salmonella, Campylobacter, Enterococcus or E. coli isolate is assumed multi-resistant if it is resistant to three or more of the antimicrobial classes. The number of antimicrobial classes and antimicrobial agent included therein depend on the test panel for each bacterium (See Table 10.3, Materials and methods).

Pet animals. Dogs, cats, birds, mice, guinea pigs and more exotic species kept at home for pleasure, rather than one kept for work or food. Horses are not included as pet animals. The live biomasses of Danish pets used for estimating veterinary consumption only include dogs and cat.

Piglet. The newborn pig is called a piglet from birth till they are permanently separated from the sow at 3-4 weeks of age. The weight of the piglet at weaning is approximately 7 kg.

Poultry. The major production species are fowl - Gallus gallus (broilers, layers, including breeding and rearing) and turkey. Regarding antimicrobial consumption, 'poultry' also includes domesticated ducks, geese, game birds and pigeons.

Sow. Any breeding female pig on the farm.

Weaner. Any pig of 7-30 kg live weight after it has been weaned.



DANMAP 2014

