

DANMAP 2013

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

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DANMAP 2013

DANMAP 2013 - 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 2013

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This report is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of monitoring the antimicrobial use and antimicrobial resistance in food animals, food and humans in 2013. The report is produced in collaboration between the National Food Institute, Technical University of Denmark and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Science, Innovation and Higher Education and the Ministry of Food, Agriculture and Fisheries.

DANMAP 2013

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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	14
3.	Background information	19
3.1	Populations	20
3.2	Marketed antimicrobial agents	20
4.	Antimicrobial consumption in animals	23
4.1.	Introduction	24
4.2.	Total antimicrobial consumption	25
4.3.	Antimicrobial consumption by animal species	27
5.	Antimicrobial consumption in humans	31
5.1.	Introduction	32
5.2.	Total consumption in both primary healthcare and hospitalcare	32
Textbox 1:	Consumption of antimicrobial agents and incidence of multi-resistant bacteria in Greenland	35
Textbox 2:	Maintained focus on the use of antimicrobial agents in the Faroe Islands	37
Textbox 3:	The Danish Council of Ethics' statement on the use of antibiotics	39
5.3.	Primary healthcare	41
Textbox 4:	Temporal and geographical variation in tetracycline prescription patterns in Denmark	46
5.4.	Hospital care	47
6.	Resistance in zoonotic bacteria	53
6.1.	<i>Salmonella</i>	54
Textbox 5:	Surveillance of <i>Salmonella</i> and <i>Campylobacter</i> in Denmark	59
6.2.	<i>Campylobacter</i>	61
7.	Resistance in indicator bacteria	65
7.1.	Enterococci	66
7.2.	<i>Escherichia coli</i>	70
Textbox 6:	Occurrence of extended spectrum beta-lactamase (ESBL)-producing <i>Escherichia coli</i> in meat and slaughter pigs, but no sign of carbapenemase producing <i>E. coli</i>	74
8.	Resistance in human clinical bacteria	77
8.1	<i>Escherichia coli</i>	78
8.2.	<i>Klebsiella pneumoniae</i>	80
Textbox 7:	Carbapenemase producing bacteria in Denmark	82
8.3.	<i>Pseudomonas aeruginosa</i>	84
8.4.	Streptococci	84
8.5.	Enterococci	85
Textbox 8:	Increased occurrence of vancomycin resistant enterococci in Danish hospitals	86
Textbox 9:	<i>Neisseria gonorrhoeae</i> 2013	87
8.6.	<i>Staphylococcus aureus</i>	89
Textbox 10:	Livestock associated methicillin-resistant <i>Staphylococcus aureus</i> (LA-MRSA) among humans 2013	92

9. Materials and Methods	93
9.1. General information	94
9.2. Data on antimicrobial consumption	94
9.3. Collection of bacterial isolates	97
9.4. Isolation and identification of bacteria	98
9.5. Susceptibility testing	99
9.6. Data handling	100
10. Terminology	103
10.1. List of abbreviations	104
10.2. Glossary	105

1. Introduction

1.1 About DANMAP

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

Humans and animals constitute 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 are presented in Figure 1.1.

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

The objectives of DANMAP are:

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

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

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

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

A web annex presenting minimum inhibitory 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).

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.

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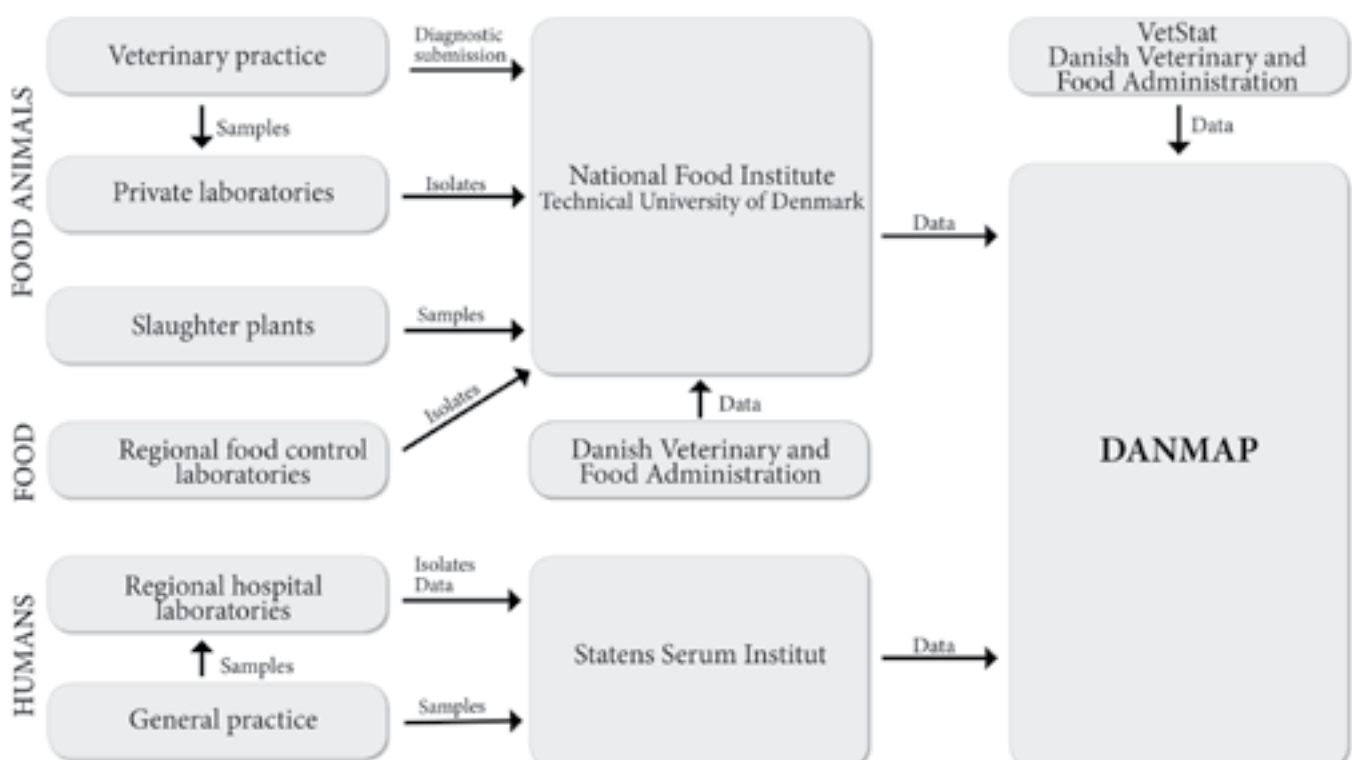
- the meat inspection staff and the personnel at the participating slaughterhouses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin, the results would be less useful;
- the Laboratory of Swine Diseases, the Danish Agriculture and Food Council,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

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- the Departments of Clinical Microbiology in 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.

Finally, we would like to thank all reviewers of the DANMAP report for their careful proofreading and helpful feedback.

Figure 1.1. Organisation of DANMAP



1.3 DANRES

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

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2. Summary

2.1 Sammendrag

DANMAP (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 2013.

Antibiotikaforbrug til dyr

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

I 2013 blev der brugt 116,3 tons antibiotika (aktivt stof) til dyr i Danmark. Fordelt på dyrearter står svin for ca. 78 % af antibiotikaforbruget i 2013, kvæg for 10%, akvakultur for 3 %, fjerkræ for 1 %, pelsdyr for 4 % og kæledyr, heste og andre dyr for de resterende 3 %.

De overordnede ændringer i antibiotikaforbruget til dyr er primært styret af ændringer i forbrugsmønstret til svin. Svineproduktionen står for ca. 84 % af den danske kødproduktion, men kun ca. 40 % af den totale levende biomasse.

Svin: Det totale antibiotikaforbrug til svin i 2012 var på ca. 91 tons aktivt stof. I forhold til forbruget i 2012, er der sket en stigning på 5 % stigning når forbruget udregnes i DAPD og der justeres for eksport. Antibiotikaforbruget til svin faldt drastisk i perioden umiddelbart efter "Gult kort-ordningen" blev bekendtgjort den 1. juli 2010. Ordningen, som har til hensigt at reducere forbruget, er rettet specifikt mod de svinebesætninger, som har det højeste antibiotikaforbrug pr svin. Selvom stigninger blev observeret også i 2013 er forbruget 12 % lavere end i 2009 og på niveau med forbruget i 2008.

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

Kvæg: Det totale antibiotikaforbrug til kvæg har ligget relativt stabilt på ca. 14 tons aktivt stof om året siden 2005, men i 2013 faldt forbruget til ca. 12 tons. Målt i standard doser (DADD) steg forbruget til gouldyrbehandling med 7 %, hvorimod antallet af DADDer til behandling af klinisk mastitis faldt med 9 % i forhold til 2012. Forbruget af beta-laktamase følsomme penicilliner udgjorde som tidligere år størstedelen af forbruget. I relation til antibiotika kritisk vigtige i human behandling har forbruget af fluorkinoloner siden 2003 været stort set nul. Sammenlignet med 2012 faldt forbruget af 3. og 4. generations cefalosporiner til systemisk behandling med 14 %.

Fjerkræ: Antibiotikaforbruget til fjerkræ var cirka 1.270 kg aktivt stof i 2013, hvilket er 57 % højere end i 2012. Den primære årsag til denne stigning var en høj forekomst af luftvejssygdom i kalkunflokkene i starten af 2013, samt en øget forekomst af diarre i slagtekyllingeflokkene i 2013. 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 mere end 10 år.

Fisk: Det totale antibiotikaforbrug til fisk i akvakultur var på 3.582 kg aktivt stof i 2013, en generel stigning på 23 % i forhold til 2012. Antibiotikaforbruget til fisk er meget afhængig af vandtemperaturen, og de ekstraordinære høje temperaturer i juli og august måned i 2013 medførte øget forekomst af bakterielle infektioner i forhold til de foregående år. Industrien har fortsat fokus på brug af vaccine for at begrænse brugen af antibiotika.

Kæledyr og heste: Oplysningerne i VetStat vedrørende antibiotikaforbrug til kæledyr og heste er mindre detaljerede end oplysningerne vedrørende produktionsdyr. Forbrug til kæledyr steg fra 1,449 kg aktivt stof i 2012 til 1,989 kg aktivt stof i 2013. Stigninger forekom for adskillige grupper af antibiotika: aminoglycosider, makrolider, penicilliner (beta-laktamase følsomme), sulfonamider, trimethoprim og tetracykliner. Forbruget af kritisk vigtige antibiotika som cefalosporiner og fluorkinoloner faldt i 2013. Men, forbruget af kritisk vigtige antibiotika til behandling af alvorlige infektionssygdomme hos mennesker er stadig højt i kæledyr sammenlignet med forbruget i produktionsdyr og giver stadig anledning til øget bevågenhed.

Antibiotikaforbruget til dyr fortsatte med at stige i 2013. Det totale forbrug til svin målt i kg aktivt stof steg med 6 % i 2013, svarende til en 5 % stigning i beregnet i DAPD. Brugen af kritisk vigtige antibiotika i den danske svineproduktion er fortsat lav. Derimod er brugen af især kritisk vigtige antibiotika til kæledyr høj sammenlignet med forbruget til produktionsdyr, men forbruget af disse stoffer til kæledyr var lavere i 2013 end 2012.

Antibiotikaforbrug til mennesker

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

Totalforbrug: I 2013 steg det totale forbrug af antibiotika til systemisk brug (primærsektoren og hospitalssektoren sammenlagt) til mennesker med 1,2 %. Forbruget i primærsektoren udgjorde 90 % af det totale forbrug. Forbruget af bredspektrede antibiotika var 5 % højere i 2013 sammenlignet med 2012. Fra 2004 til 2013 er det totale forbrug af antibiotika i Danmark steget med 20 %. For bredspektrede antibiotika var stigningen 72 %.

Primærsektor: Det totale antibiotikaforbrug i primærsektoren steg med 1,2 % sammenlignet med 2012. De mest udtaltstigninger blev observeret for tetracykliner, 'kombinationspenicilliner', beta-laktamase resistente penicilliner og penicilliner med udvidet spektrum. Forbruget af makrolider faldt med 12 % i forhold til 2012 og fortsatte således det fald, der er blevet observeret siden 2011. Forbruget af bredspektrede antibiotika steg 5,7 % fra 2012 til 2013.

I det seneste årti er forbruget af antibiotika i primærsektoren steget med 19 %. Denne stigning skyldes sandsynligvis en stigning i dosis (DDD) per behandlet patient samt en øget dosis per udskrevet medicinpakning. Årsagen til denne ændring kendes ikke.

I **Textbox 4** er forbruget af tetracykliner beskrevet nærmere. Fra 2005 til 2013 er forbruget af tetracykliner (DDD pr. 1.000 indbygger-dage) steget med 54 %. Den største stigning sås for de 10–14 årige (86 %) samt for de 15–19 årige (58 %). Dette betød, at 5.400 flere unge blev behandlet med tetracyclin i 2013 sammenlignet med 2005 (justeret for populationsændringer). Størstedelen af udskrivingerne var sandsynligvis til behandling af akne. Det var oftere praktiserende læger end dermatologer, der udskrev tetracykliner.

Hospitaller: På somatiske hospitaler steg det totale antibiotikaforbrug opgjort i DDD pr. 100 sengedage (DBD) med 1,4 % fra 2012 til 2013.

Fra 2012 til 2013 steg forbruget af 'kombinationspenicilliner', kombinationer af sulfonamid og trimethoprim, beta-laktamase resistente penicilliner, penicilliner med udvidet spektrum og carbapenemer. Der blev observeret et fald for anden generations cefalosporiner, fluorkinoloner, makrolider og tetracykliner.

Fra 2004–2013 steg det totale antibiotikaforbrug med 66 %. Denne stigning skyldtes primært en kombination af stigning i DDD og et fald i antallet af sengedage. I løbet af det seneste årti er forbruget af bredspektret antibiotika på somatiske hospitaler steget med 114 %.

I 2013 steg det totale forbrug af antibiotika til systemisk behandling af mennesker (primær- og hospitalssektoren sammenlagt) med 1,2 % sammenholdt med 2012. Forbruget i primærsektoren udgjorde 90 % af det totale forbrug, mens forbruget på hospitalerne udgjorde de resterende 10 %. Over en 10-årig periode fra 2004 til 2013 steg det totale forbrug af antibiotika til mennesker i Danmark med 20 %.

Resistens i zoonotiske bakterier

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

Salmonella Typhimurium er en af de mest almindelig forekommende serotyper i danske svin, dansk svinekød og i humane *Salmonella* infektioner. Blandt *S. Typhimurium* fra svin var 61–70 % af isolaterne resistente overfor ampicillin, streptomycin, sulfonamid, og tetracyclin, og forekomsten af resistens overfor disse fire antibiotika (ASSuT resistens) er steget over de sidste fem år. Dette kan primært tilskrives den stigende forekomst af monofasiske varianter, som ofte er multiresistente, og i 2013 var 52 % af de undersøgte *S. Typhimurium* isolater fra svin var monofasiske. Der blev også fundet høj forekomst af resistens blandt *S. Typhimurium* isolater fra dansk svinekød. Generelt var forekomsten af multiresistens blandt *S. Typhimurium* isolater fra danske svin (64 %) og svinekød (71 %), højere end i *Salmonella* generelt (*Salmonella* spp.), hvor hhv. 37 % og 36 % var multiresistente. Når resultaterne fra *Salmonella* overvågningsprogrammerne sammenholdes med disse forekomster, estimeres det at 9 % af svinene og 0,5 % af slagtekroppene havde multiresistent *Salmonella*. 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 de monofasiske *S. Typhimurium* varianter blandt isolater fra patienter, som havde erhvervet infektionen i Danmark (både sporadiske og udbrudstilfælde). I 2013 blev der påvist multiresistens i 54 % af *S. Typhimurium* isolater fra sporadiske tilfælde erhvervet i Danmark, og resistensniveauet var generelt på niveau med 2012. Resistensforekomsten var højere for fire ud af de 16 testede stoffer, når forekomsten i isolater fra patienter med rejse-relaterede infektioner sammenlignes med forekomsten i isolater fra sporadiske infektioner erhvervet i Danmark. Det gælder også for fluorkinoloner, som bruges til behandling af patienter med alvorlige mave-tarm infektioner forårsaget af både *Salmonella* og *Campylobacter*. Andelen af multiresistente isolater faldt i 2013 blandt patienter med rejse-relaterede infektioner, og i 2013 var niveauet af multiresistens sammenligneligt med niveauet i isolater fra sporadiske infektioner erhvervet i Danmark.

Forekomsten af monofasiske *S. Typhimurium*, som ofte er multiresistent, er steget i svin, svinekød og blandt humane infektioner de seneste fem år. Blandt de humane infektioner, var resistensforekomsten i 2013 generelt højere, for 4 ud af 16 stoffer i testpanelet (herunder fluorkinoloner), blandt de rejserelaterede humane tilfælde end blandt de hjemligt erhvervede infektioner. Selvom resistens var hyppigst forekommende blandt isolater fra patienter med rejserelaterede infektioner, faldt andelen af multiresistente isolater i 2013 blandt disse, og niveauet af multiresistens var sammenligneligt med niveauet i isolater fra sporadiske infektioner erhvervet i Danmark.

I 2013 var resistensforekomsten i *Campylobacter jejuni* isolater fra kyllinger og kvæg på samme niveau som i 2011, men en anelse højere end i 2012. Selv om disse ændringer ikke var statistisk signifikante, har det fulgt forbrugsmønstret for tetracyclin i fjerkræ.

Set i en europæisk sammenhæng er resistensforekomsten i *C. jejuni* fra danske slagtekyllinger og kyllingekød samt *C. coli* fra svin blandt Europas laveste.

Fluorkinolon resistens i *C. jejuni* var også højere blandt isolater fra importeret kyllingekød (53 %) end fra dansk kyllingekød (20 %).

Som i de foregående år var forekomsten af fluorkinolon resistens i *C. jejuni* isolater fra patienter med rejse-relaterede infektioner (92 %) højere end i isolater fra patienter, hvor infektionen var erhvervet i Danmark (24 %).

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 og denne forskel er mere udtalt i 2013 end i 2012.

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.

I *E. faecalis* fra danske slagtekyllinger var forekomsten af tetracyclin resistens størst (38 %) efterfulgt af resistens overfor erythromycin og salinomycin.

Højeste forekomster af resistens blev fundet blandt *E. faecalis* isoleret fra svin sammenlignet med isolater fra slagtekyllinger, hvilket følger forbrugsmønstret i Danmark til disse dyrearter. Blandt *E. faecalis* isoleret fra svin var forekomsten af tetracyclin højest (91 %). Tetracycliner er og har været de mest anvendte antimikrobielle stoffer til danske svin i en årrække og har primært været brugt til behandling af *E. coli* infektioner. Forekomst af erythromycin resistens blandt *E. faecalis* isoleret fra svin var 45 % og faldt i forhold til forekomsten i 2012. Forekomst af resistens var højere i importeret end i dansk produceret svinekød.

Blandt isolater fra kyllingekød, blev den højeste forekomst af resistens overfor flere stofgrupper fundet i importeret kyllingekød. Ligeledes blev der fundet højere forekomst af multiresistens blandt isolater fra importeret kyllingekød (39 % og 24 % for henholdsvis *E. faecalis* og *E. faecium*) ved sammenligning med forekomster i dansk produceret kød. I alt 72 % af de multiresistente *E. faecalis* fra importeret kyllingekød havde samme resistensprofil (erythromycin, kanamycin, streptomycin og tetracyclin).

Generelt er forekomsten af resistens overfor kritisk vigtige antibiotika lav, men et dansk *E. faecalis* isolat var resistent overfor fluorkinoloner (ciprofloxacin).

Set i et 'One Health' perspektiv er der en direkte sammenhæng mellem antibiotikaforbruget i kyllingeproduktionen og forekomsten af antibiotikaresistente *E. faecalis* i dansk kyllingekød. Denne kobling findes ikke mellem danske svin og dansk svinekød. I dansk svinekød er forekomsten af resistens blandt *E. faecalis* generelt lavere end blandt isolater fra svin. Foruden dette, er resistensforekomsten i svinekød faldende, en tendens som ikke observeres i svin.

Indikator *Escherichia coli* fra slagtekyllinger var ofte resistente overfor sulfonamid og ampicillin (begge 26 %), som typisk bruges til slagtekyllinger. Der blev påvist fluorkinolon (ciprofloxacin) resistens i 6 % af isolaterne, og resistens overfor 3. generations cefalosporiner (ceftiofur) blev påvist i to isolater fra slagtekyllinger. Resistensforekomsten i *E. coli* fra slagtekyllinger og dansk kyllingekød var sammenlignelig. Resistensforekomsten i isolater fra kvæg og dansk oksekød var som foregåede år ganske lav. Resistens i *E. coli* fra svin var den højeste blandt produktionsdyrene og forblev på samme høje niveau som i 2012.

Blandt isolaterne fra kød havde isolaterne fra importeret kyllingekød generelt de højeste resistensforekomster – også når det gjaldt de kritisk vigtige antibiotika. Sammenlignet med dansk kyllingekød havde *E. coli* isolater fra importeret kyllingekød højere resistens overfor 14 af de 16 testede antibiotika. For svinekød havde *E. coli* isolater fra dansk kød en lavere resistensforekomst overfor kinoloner (ciprofloxacin og nalidixin syre) end isolaterne fra importeret svinekød.

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

Seks procent af slagtesvinene havde ESBL-producerende *E. coli*, hvilket var lavere end i 2012 og signifikant lavere end i 2010 og 2009 før landbrugets frivillige stop for brug af cefalosporiner trådte i kraft. Den højeste prævalens af ESBL-producerende *E. coli* i kødprøver blev fundet i importeret kyllingekød (52 %), hvilket er på samme niveau som i 2012 og 2010. Forekomsten af ESBL-producerende *E. coli* i prøver af dansk kyllingekød var signifikant lavere i 2013 sammenlignet med 2012. Desuden var andelen af prøver positive for ESBL producerende *E. coli* i dansk kyllingekød signifikant lavere end forekomsten i det importerede kyllingekød. Dette skyldes sandsynligvis et frivilligt stop af brug af 3. generations cefalosporiner i toppen af avlspyramiden i udlandet, hvilket resulterer i der overføres færre ESBL producerende *E. coli* fra importerede forældredyr til de danske slagtekyllinger. I lighed med sidste år er der stadig ingen fund af carbapenemase producerende *E. coli*.

Det frivillige stop i brugen af cefalosporiner i svineproduktionen resulterer fortsat i en lav forekomst af ESBL producerende *E. coli* i slagtesvin. Ligeledes ses et signifikant fald i ESBL producerende *E. coli* i dansk kyllingekød, hvilket 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 bakterier fra diagnostiske indsendelser fra mennesker er baseret på frivillig indsendelse af data fra DANRES-gruppen, som dækker de Klinisk Mikrobiologiske Afdelinger (KMA) i Danmark. Undtagelser omfatter methicillin-resistente *Staphylococcus aureus* (MRSA) og invasive *Streptococcus pneumoniae*, som er anmeldpligtige. Data vedr. disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt *Escherichia coli* isolater fra blod var forekomsten af 3. generations cefalosporin resistens 8 % i 2013, hvilket er det samme niveau som i 2012, men højere end i de andre nordiske lande i 2012. Ciprofloxacin resistensen faldt til 12 % i 2013 sammenlignet med 14 % i 2012. Aminoglykosid resistensen (gentamicin) var 7 % i 2013, hvilket er samme niveau som i 2012. Resistensdata for piperacillin/tazobactam blev rapporteret for første gang i denne DANMAP rapport. Data omfatter de sidste fem år (2009–2013); i denne periode var forekomsten af resistens på omkring 4 %.

Blandt *E. coli* isolater fra urin fra patienter på hospitalerne var forekomsten af 3. generations cefalosporin resistens 6 % i 2013, hvilket er det samme niveau som i 2012. Forekomsten af fluorkinolon (ciprofloxacin) resistens var 12 % i 2013, hvilket er samme niveau som i 2012, men der har været en stigende forekomst fra 3 % i 2004. Aminoglykosid (gentamicin) resistens var 5 % i 2013 og sulfonamid resistens var 33 %, begge var på samme niveau som i 2012.

Blandt *E. coli* isolater fra urin fra patienter i almen praksis er forekomsten af fluorkinolon (ciprofloxacin) resistens steget støt fra 3 % i 2004 til 10 % i 2013. Forekomsten af 3. generations cefalosporin resistente *E. coli* var 4 %, sulfonamid resistens var 33 % og ampicillin resistens var 40 % i 2013, hvilket for alle tre antibiotika var på samme niveau som i 2012.

Blandt *Klebsiella pneumoniae* isolater fra blod var forekomsten af resistens den samme i 2013 som i 2012. Forekomsten af 3. generations cefalosporin resistens var 9 %, aminoglykosid (gentamicin) resistens var 4 %, og fluorkinolon (ciprofloxacin) resistens var 9 %.

Blandt *K. pneumoniae* isolater fra urin fra patienter på hospitalerne faldt forekomsten af resistens for mecillinam (10 %), sulfonamid (20 %), gentamicin (4 %), 2. generations cefalosporiner (cefuroxim) (9 %) og 3. generations cefalosporiner (7 %) fra 2012 til 2013. Fluorkinolon (ciprofloxacin) resistens var på samme niveau i 2013 som i 2012.

Blandt *K. pneumoniae* isolater fra urin fra patienter i almen praksis var forekomsten af 3. generations cefalosporin resistens 6 %, hvilket er samme niveau som i 2012. Fluorkinolon

(ciprofloxacin) resistens var 7 %, og mecillinam resistens var 10 %, hvilket også er på samme niveau som i 2012. Sulfonamid resistens faldt fra 26 % i 2012 til 22 % i 2013.

Blandt *Pseudomonas aeruginosa* isolater fra blod var niveauet af resistens for alle testede antibiotika ikke signifikant forskelligt fra niveauet i 2012, men der sås en stigende tendens for aminoglykosid (gentamicin) resistens i perioden 2007–2013.

Forekomsten af carbapenemase producerende bakterier i Danmark er beskrevet i **Textbox 7**. I 2013 blev der fundet 18 carbapenemase producerende *Enterobacteriaceae* (CPE) sammenlignet med 19 CPE i hele perioden 2008–2012. I 2013 var der spredning af NDM-1 producerende *Citrobacter freundii* imellem fire patienter på en hospitaletafdeling i Region Nordjylland. Ingen af patienterne havde været ude at rejse for nylig, og kilden til den NDM-1 producerende *C. freundii* var ukendt. To af de fire patienter med *C. freundii* havde også NDM-1 producerende *K. pneumoniae*. Der blev detekteret syv OXA-23 producerende *Acinetobacter baumannii* isolater i 2013.

Der blev i to tilfælde set spredning af OXA-23 producerende *A. baumannii* mellem to patienter. I 2013 blev der fundet tre OXA-40-like producerende *A. baumannii*. Her var der også sket smittespredning fra en patient til en anden. Derudover blev der detekteret to NDM-1 producerende *A. baumannii*. I 2013 blev der fundet tre VIM producerende *P. aeruginosa*, og en NDM og VIM producerende *P. aeruginosa*. For første gang blev der detekteret en IMP producerende *P. aeruginosa* i Danmark. Patienterne med de to sidstnævnte *P. aeruginosa* havde begge forinden været indlagt på udenlandske hospitaler.

I 2013 var forekomsten af resistens for penicillin og makrolider (erythromycin) stadig lav blandt *Streptococcus pneumoniae* og gruppe A, B, C og G streptokokker.

Forekomsten af ampicillin resistens i *Enterococcus faecium* isolater fra blod var 93 % i 2013. Vancomycin resistens var 3,4 % i *E. faecium* og 0,2 % i *Enterococcus faecalis* isolater fra blod. I 2013 modtog Reference laboratoriet for Antibiotikaresistens et øget antal vancomycin resistente enterokokker (VRE) (**Textbox 8**); 258 isolater fra infektioner og 168 fra fækale screeninger. Næsten alle VRE var *vanA E. faecium* isolater. VRE isolaterne var primært fra hospitaler i Region Hovedstaden, men der var også isolater fra hospitaler i Region Sjælland samt Region Midtjylland. Der blev tillige fundet VRE i de to andre regioner, men i meget lavere antal. Pulsfelt gelelektroforese typning viste, at der var sket spredning af flere forskellige typer *vanA* VRE både inden for hospitalerne samt imellem hospitalerne.

Forekomsten af fluorkinolon (ciprofloxacin) resistens i *Neisseria gonorrhoeae* steg støt fra 30 % i 2003 til 75 % i 2009, efterfulgt af et fald til 56 % i 2013. Penicillinase produktion blandt gonokok isolater svingede imellem 24 % i 2003 og 11 % i 2013. Der blev ikke rapporteret hverken ceftriaxon resistente isolater eller tilfælde af ceftriaxon behandlingssvigt i denne periode. I 2013 var forekomsten af makrolid (azithromycin) resistens 45 %, cefixim resistens var 9 %, og der var ingen spectinomycin resistente isolater (**Textbox 9**).

I 2013 blev der indrapporteret 1.769 tilfælde af *Staphylococcus aureus* bakteriemier svarende til en incidens på 32,9 tilfælde per 100.000 indbyggere. Antallet af methicillin-resistente *S. aureus* (MRSA) fra bakteriemier var 30 (1,7 %), hvilket er på

samme niveau som tidligere år og blandt de laveste incidenser observeret i Europa. Den højeste forekomst af resistens ud over penicillin resistens var resistens for fusidinsyre (15 %), erythromycin (7 %), clindamycin (6 %) og norfloxacin (5 %). Niveaulet af resistens for de testede antibiotika var det samme som i 2012.

Antallet af nye MRSA tilfælde (både koloniserede og inficerede personer) steg i 2013 til 2.094 sammenlignet med 1.556 i 2012. Stigningen blev primært set for MRSA tilhørende klonal kompleks CC398 (LA-MRSA), hvor der blev observeret 643 tilfælde i 2013 sammenlignet med 232 tilfælde i 2012. CC398 udgjorde 31 % af alle nye MRSA tilfælde i 2013. En stor del af stigningen skyldes formentlig inklusionen af kontakt til svin som en risikofaktor medførende screening ved indlæggelse på hospitalerne. Størstedelen (87 %) af de personer, som havde CC398 (både infektion og kolonisation), havde haft kontakt med svin eller boede sammen med personer, der havde kontakt med svin. Der var dog ingen tegn på betydelig spredning af CC398 til byområder, hvilket indikerer, at fødevarer ikke udgør en væsentlig smittekilde.

Blandt alle MRSA tilfælde var forekomsten af infektioner lavere i 2013 end i 2012 (45 % versus 57 %). Antallet af hospitals-erhvervede tilfælde var fortsat lavt i 2013 og udgjorde kun 2 %. I 2013 er der ikke foretaget undersøgelser for MRSA i dyr og fødevarer.

I 2013 blev der fundet 18 carbapenemase producerende *Enterobacteriaceae* (CPE) sammenlignet med 19 CPE i hele perioden 2008–2012. I 2013 var der spredning af NDM-1 producerende *Citrobacter freundii* imellem fire patienter på en hospitalsafdeling i Region Nordjylland. Ingen af patienterne havde været ude at rejse for nylig, og kilden til NDM-1 producerende *C. freundii* var ukendt.

Et stigende antal vancomycin resistente enterokokker blev modtaget på SSI i 2013; 258 isolater fra infektioner og 168 fra fækale screeninger. Næsten alle VRE isolater var *vanA E. faecium*. VRE isolaterne var primært fra hospitaler i Region Hovedstaden, men der var også isolater fra hospitaler i Region Sjælland samt Region Midtjylland.

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2.2 Summary

DANMAP (Danish Antimicrobial Resistance Monitoring and Research Program) has monitored antimicrobial resistance and consumption of antimicrobial agents in food animals and in humans in Denmark since 1995. This report describes changes and trends in 2013.

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 2013 amounted to 116.3 tonnes of active compounds, an increase of 4% increase compared with 2012. Pigs accounted for approximately 78%, cattle for approximately 10%, fur animals for 4%, aquaculture for 3%, and poultry for 1% of the total veterinary consumption of antimicrobials measured in kg active compounds. The remaining 3% was used in pets, horses and others.

The overall changes in veterinary consumption are generally driven by changes in consumption in pig production. Pigs account for approximately 84% of the meat production in Denmark, but only about 40% of the total live biomass.

Pigs: The total consumption of veterinary antimicrobial agents in Danish pig production was approximately 91 tonnes. Measured in DAPD, we observed a 5% increase from 2012 to 2013. This follows a decrease in 2010–2011, which was probably the result of the introduction of legislation to reduce overuse of antimicrobials in the pig production. Even though the antimicrobial consumption increased in 2013, it remained approximately 12% lower than in 2009 and was at the same level as in 2008.

In 2013, the increase in consumption (measured in DAPD) was attributed mainly to an increase in pleuromutilins and tetracyclines and to a lesser extent, to the use of penicillins and sulfonamides/trimethoprim. Pleuromutilins and tetracyclines are mainly used in feed or water medication for gastrointestinal disease. Some types of antimicrobials including fluoroquinolones and 3rd and 4th generation cephalosporins are considered critically important for treatment of severe infections in humans. The use of 3rd and 4th generation cephalosporins in pigs remained very low (3 kg), as a result of a voluntary ban on cephalosporins introduced by the Danish pig industry in 2010. The use of fluoroquinolones remained at the same low level as has been observed since legal restrictions were enforced in 2003.

Cattle: Overall, the antimicrobial consumption in cattle has remained stable at around 14 tonnes since 2005, but in 2013 it declined to about 12 tonnes. Measured in standard doses (DADD) the consumption for drying-off treatment increased by 7% whereas, the number of DADDs for treatment of clinical mastitis decreased by 9% compared to 2012. The beta-lactamase sensitive penicillins account for the majority of the consumption. For critically important antimicrobials, the use of fluoroquinolones has been close to zero since 2003. The use of 3rd and 4th generation cephalosporins for systemic treatment decreased by 14% compared with 2012.

Poultry: In 2013, the overall consumption of antimicrobial agents in poultry was approximately 1,270 kg active compound, which represents a 57% increase compared with 2012. The main reason for this appears to be widespread problems with respiratory disease in turkey flocks produced in early 2013 and an increased occurrence of diarrhea in broiler flocks. The reported use of fluoroquinolones in poultry has been low since 2006, and they were not used in the poultry production in 2013. Furthermore, use of cephalosporins has not been reported in Danish poultry production for more than a decade.

Aquaculture: The overall antimicrobial consumption in aquaculture was 3,582 kg in 2013, an overall increase of 23%. The relatively large increase in consumption in 2013 is explained mainly by extraordinary high temperatures in July and August 2013, leading to higher water temperatures and an increase in the occurrence of bacteriological infections. There is, however, in the aquaculture industry, still focus on vaccination to reduce the risk of diseases that may require antibiotic treatment.

Pets and horses: The information available on antimicrobial consumption in pet animals and horses is less detailed as for production animals. The overall antimicrobial consumption for pets increased from 1,449 kg in 2012 to 1,989 kg active compound in 2013. The increase was seen for several antimicrobial classes; aminoglycosides, macrolides and penicillins (b-lactamase sensitive), sulfonamides and trimethoprim, as well as for tetracyclines. The consumption of antimicrobials critical for human treatment such as cephalosporins and fluoroquinolones decreased in 2013. 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 both production animals and is a matter of concern.

The antimicrobial consumption in animals continued to increase in 2013. The total consumption (measured in kg active compound) in pigs increased by 6% in 2013, corresponding to a 5% increase in DAPD. Use of critically important antimicrobials in the pig production remains low. However, the use of critically important antimicrobials in pets remains high compared with other species, but decreased from 2012 to 2013.

Antimicrobial consumption in humans

In Denmark, the Department of Data Delivery and Medicinal Product Statistics at Statens Serum Institut register the consumption of antimicrobial agents to humans.

Total consumption: In 2013, the total consumption of antimicrobial agents for systemic use (primary healthcare and hospital care) was 1.2% higher than in 2012. The consumption in primary healthcare accounts for 90% of the total antimicrobial consumption. The proportion of broad-spectrum agents was 5% higher in 2013 compared with 2012. Since 2004, the overall consumption of antimicrobial agents in humans has increased by 20%. For broad-spectrum agents, the increase has been 72%.

Primary healthcare: In 2013, the total consumption of antimicrobial agents for systemic use in the primary sector was 1.2% higher than observed in 2012. Particular increases were observed for tetracyclines, 'combination penicillins', beta-lactamase resistant penicillins and penicillins with extended

spectrum. The consumption of macrolides was 12% lower in 2013 compared with 2012, continuing the decrease observed since 2011. The consumption of broad-spectrum agents increased by 5.7% from 2012 to 2013.

During the past decade, the consumption of antimicrobial agents in the Danish primary sector has increased by 19%. This seems to be caused by the fact that the dosage prescribed for each patient and in each package has increased significantly over the years, however, the underlying medical reasons for this (if any) are unclear.

The increased consumption of tetracyclines has been further described in **Textbox 4**. From 2005 to 2013, the consumption (DDDs per 1,000 inhabitant-days) of tetracyclines for all ages increased by 54% with large increases observed for the 10–14 year olds (86%) and 15–19 year olds (58%). When adjusted for population increases, approximately 5,400 more persons in these age groups were treated with tetracyclines in 2013 than in 2005. General Practitioners (GPs) rather than dermatologists prescribed the majority of the prescriptions, presumably for acne.

Hospitals: In 2013, the consumption of antimicrobial agents in somatic hospitals (expressed as DBDs, i.e. DDDs per 100 occupied bed-days) was 1.4% higher than in 2012.

From 2012 to 2013, a higher consumption was observed for 'combination penicillins', combinations of sulfonamide and trimethoprim, beta-lactamase resistant penicillins, penicillins with extended spectrum and carbapenems, while decreased consumption was seen for 2nd generation cephalosporins, fluoroquinolones, macrolides and tetracyclines.

The consumption in hospitals has steadily increased by 66% in the past decade, primarily caused by an increase in DDDs and a decrease in the number of hospital bed-days. This has especially been seen in the consumption of broad-spectrum antimicrobial agents which increased by 114%.

In humans, the overall consumption of antimicrobial agents for systemic use increased slightly (1.2 %) from 2012 to 2013. Antimicrobial consumption in the primary healthcare sector represented 90% of the total consumption and the hospital sector accounted for the remaining 10%. From 2004 to 2013, the total consumption of antimicrobial agents by humans in Denmark increased by 20%.

Resistance in zoonotic bacteria

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

Resistance in zoonotic bacteria

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

Salmonella Typhimurium is one of the most common serovars in Danish pigs and pork as well as in human infections. Among *S. Typhimurium* from pigs, 61–70% of the isolates were resistant to ampicillin, streptomycin, sulfonamide, and tetracycline; and the occurrence of resistance to these four 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 2013, 52% of the *S. Typhimurium* isolates from pigs were of the monophasic 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 Danish pigs (64%) and pork (71%) compared to other *Salmonella* spp. isolates from pigs and pork (37% and 36%, respectively). Based on the relative occurrence of resistance from DANMAP samples and the *Salmonella* prevalence from the national control programmes, it was estimated that 9% of Danish pigs and 0.5% of the pig carcasses were positive with multi-resistant *Salmonella*. As in previous years, resistance to cephalosporins (ceftiofur or cefotaxim) 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 2013, multi-resistant isolates were recovered from 54% of the domestic sporadic cases, however, generally the levels of resistance was comparable to 2012. Resistance to 4 of the 16 tested compounds was higher among travel-associated cases than domestic human cases, including resistance to fluoroquinolones, which are used for empiric treatment of adults with severe bacterial gastroenteritis caused by both *Salmonella* and *Campylobacter*. Among the travel-associated cases, the occurrence of multi-resistance decreased in 2013 to a level comparable to the occurrence among the sporadic domestic cases.

The occurrence of monophasic *S. Typhimurium*, which are often multi-resistant, has increased in pigs, pork and among isolates from human infections the past five years. Among isolates from human infections, the resistance levels were higher for four out of 16 compounds tested (including fluoroquinolones) among the travel related human cases compared to the domestically acquired cases. Although resistance was most common among isolates from travel related human cases the amount of multi-resistance isolates decreased in 2013 to a level comparable to isolates from domestically sporadic cases.

In 2013, *Campylobacter jejuni* isolates from Danish broilers and cattle were similar to the observed levels in 2011, and slightly higher than in 2012. The observed changes in resistance to tetracycline were not statistically significant; nonetheless they correspond well to changes in consumption of tetracycline in the broiler production.

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

For several years, the level of fluoroquinolone resistance in *C. jejuni* has been higher among isolates from imported broiler meat (53%), compared with isolates from Danish broiler meat (20%).

As observed in previous years, the *C. jejuni* isolates from the travel-associated cases continued to have a significantly higher level of fluoroquinolone resistance (92%) compared with domestic cases (24%).

The level of fluoroquinolone (ciprofloxacin) resistance in *C. 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 and this difference became more pronounced from 2012 to 2013.

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 Danish broilers, resistance to tetracycline (38%) was the most dominant, followed by resistance to erythromycin and salinomycin.

Higher prevalence of resistance to antimicrobials was observed among *E. faecalis* isolated from pigs compared to poultry, which reflects the usage pattern of antimicrobials in these animal species. Among *E. faecalis* from pigs, the highest occurrence of resistance was to tetracycline (91%). Tetracycline has been the most widely used antimicrobial agent in the Danish pig production for more than a decade and has primary been used for treatment of *E. coli* infections. Erythromycin resistance in *E. faecalis* from pigs was 45% a decline since 2012. Occurrences of resistance were higher in imported pork when compared to Danish produced pork.

Among isolates from broiler meat, the highest level of resistance to several compounds was observed in imported broiler meat, similar to previous years. Moreover, higher prevalences of multi-resistance was observed in isolates from imported broiler meat (39% and 34% for *E. faecalis* and *E. faecium*, respectively) compared to broiler meat produced in Denmark. Among the multi-resistant *E. faecalis* isolates from imported broiler meat, 72% had an identical resistance profile (erythromycin, kanamycin, streptomycin and tetracycline). In general, resistance to antimicrobial agents of critical importance for human treatment was low, but fluoroquinolone (ciprofloxacin) resistance was observed in one *E. faecalis* isolate from a Danish pig.

In a One Health perspective, there appears to be a direct link between the antimicrobial consumption in the broiler production and the level of resistance in *E. faecalis* isolates from Danish broilers. A similar link between Danish pork and pigs has not been observed. *E. faecalis* isolates from pork were generally more susceptible when compared with isolates from pigs. Furthermore, resistance in Danish pork has been declining, a trend not seen in Danish pigs.

The voluntary stop in usage of cephalosporins in the Danish pig production continuously results in a low occurrence of ESBL producing *E. coli* in pigs at slaughter. Also a significant decrease in the occurrence of ESBL producing *E. coli* in Danish produced broiler meat was observed, which is most likely due to a voluntary stop in usage in cephalosporins in the top of the breeding pyramid abroad.

Indicator *Escherichia coli* from broilers were most often resistant to sulfonamide and ampicillin (both 26%), which can be explained by the usage pattern. Resistance to fluoroquinolones was observed in 6% of the isolates and ceftiofur (3rd generation cephalosporin) resistance was observed in two *E. coli* isolates from broilers. Resistance in isolates from Danish broiler meat was similar to the findings in broilers. Resistance in isolates from cattle and beef was generally low. The highest occurrence among production animals was observed in pigs, and resistance was at the same high level as in 2012.

In isolates from meat, the highest occurrence of resistance, including resistance to critically important antimicrobials, was found in imported broiler meat. Compared with Danish broiler meat, we found higher levels of resistance for 14 of 16 tested antimicrobial agents from imported broiler meat. For *E. coli* from pork of domestic origin, resistance to quinolones (ciprofloxacin and nalidixic acid) was significantly lower than in imported pork.

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

In 2013, six percent of pigs at slaughter had ESBL-producing *E. coli*, which was lower than in 2012 and 2010 and 2009. From meat samples, the highest prevalence of ESBL producing *E. coli* was found among imported broiler meat (52%), similar level as in 2010 to 2012. The occurrence of meat samples positive for ESBL producing *E. coli* from Danish broiler meat was significantly lower (25%) than in 2012 (36%). The occurrence of ESBL producing *E. coli* in meat samples was significantly higher in imported broiler meat when compared to Danish 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 no carbapenemase producing *E. coli* were found.

Resistance in human clinical bacteria

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

In *Escherichia coli* blood isolates, resistance to 3rd generation cephalosporins was 8% in 2013, the same level as reported in 2012, but above the 2012 level reported in the other Nordic countries. The occurrence of ciprofloxacin resistance decreased from 14% in 2012 to 12% in 2013. Aminoglycoside (gentamicin) resistance was 7% and at the same level as reported in 2012. Resistance data on piperacillin/tazobactam was reported for the first time in the present DANMAP report. Data were obtained for five years (2009–2013). For all five years, the resistance was at the same level (around 4%).

In *E. coli* urine isolates from hospital patients, 3rd generation cephalosporin resistance was 6% - the same level as in 2012. The occurrence of ciprofloxacin resistance (12%) was at the same level as in 2012, but a steady increase has been seen in ciprofloxacin resistance from 3% in 2004. Aminoglycoside (gentamicin) resistance was 5% and sulfonamide resistance was 33%, which are at the same levels as in 2012.

In *E. coli* urine isolates from primary healthcare, ciprofloxacin resistance has increased steadily from 3% in 2004 to 10% in 2013. The occurrence of 3rd generation cephalosporin resistance was 4%, sulfonamide resistance was 33% and ampicillin resistance was 40% in 2013, all at same levels as reported in 2012.

In *Klebsiella pneumoniae* blood isolates, the resistance levels were the same as reported in 2012. Resistance to 3rd generation cephalosporins was 9%, aminoglycoside (gentamicin) resistance was 4%, and ciprofloxacin resistance was 9%.

In *K. pneumoniae* urine isolates from hospital patients, resistance to mecillinam (10%), sulfonamide (20%), gentamicin (4%), 2nd generation cephalosporins (cefuroxime) (9%) and to 3rd generation cephalosporins (7%) decreased from 2012 to 2013. Ciprofloxacin resistance was at the same level as reported in 2012.

In *K. pneumoniae* urine isolates from primary healthcare, resistance to 3rd generation cephalosporins was 6%, which is similar to the level reported in 2012. Resistance to ciprofloxacin was 7% and resistance to mecillinam was 10%, which were similar to the levels reported in 2012. Sulfonamide resistance decreased from 26% in 2012 to 22% in 2013.

In *Pseudomonas aeruginosa* blood isolates, resistance to all the tested antimicrobial agents was not significantly different from the levels reported in 2012, but an increasing trend has been observed for gentamicin resistance during 2007–2013.

The occurrence of carbapenemase producing bacteria in Denmark is described in **Textbox 7**. In 2013, 18 carbapenemase producing *Enterobacteriaceae* (CPE) were detected compared to 19 during 2008–2012. In 2013, spread of NDM-1 producing *Citrobacter freundii* was detected between four patients at a hospital ward in the North Denmark Region. None of the four patients had been travelling recently and the origin of the NDM-1 producing *C. freundii* was unknown. Besides the NDM-1 producing *C. freundii*, two of the four patients had NDM-1 producing *K. pneumoniae*. During 2013, seven OXA-23 producing *A. baumannii* isolates were detected. Spread of OXA-23 producing *A. baumannii* was detected twice between patients. Furthermore, OXA-40-like producing *A. baumannii* (n = 3) was detected, two of these isolates were part of the same transmission chain. Two NDM-1 producing *A. baumannii* isolates were detected. In 2013, three VIM producing *P. aeruginosa* isolates were detected. Furthermore, an NDM and VIM producing *P. aeruginosa* was detected. For the first time, an IMP producing *P. aeruginosa* isolate was detected in Denmark. Both patients had been hospitalized abroad prior to detection of these isolates.

Resistance to penicillin and erythromycin in *Streptococcus pneumoniae* and in group A, B, C and G streptococci remained low in 2013.

In 2013, resistance to ampicillin was 93% in *Enterococcus faecium* isolates from blood. Vancomycin resistance was 3.4% in *E. faecium* and 0.2% in *Enterococcus faecalis* blood isolates. During 2013, an increasing number of vancomycin resistant enterococci were referred to the Antimicrobial Resistance Reference Laboratory at SSI (**Textbox 8**). Of the VRE isolates, 248 were from clinical infections and 168 were faecal screening isolates. Nearly all VRE isolates were *vanA E. faecium* isolates. The VRE isolates were primarily from 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. Pulsed-field gel electrophoresis typing showed spread of several *vanA E. faecium* types both within hospitals and between hospitals.

Ciprofloxacin resistance in *Neisseria gonorrhoeae* increased steadily from 30% in 2003 to 75% in 2009, followed by a decrease to 56% in 2013. Penicillinase production among gonococcus isolates fluctuated between 24% in 2003 and 11% in 2013, and no ceftriaxone resistant isolates or cases of ceftriaxone treatment failure, were reported in this period. In 2013, azithromycin resistance was 45%, cefixime resistance was 9%, and no spectinomycin resistant isolates were detected (**Textbox 9**).

In 2013, 1,769 cases of *Staphylococcus aureus* bacteraemia were reported, corresponding to 32.9 cases per 100,000 inhabitants. The number of methicillin-resistant *S. aureus* (MRSA) from bacteraemia was 30 (1.7%), a level similar to previous years and among the lowest incidences recorded in Europe. The highest frequency of resistance in addition to penicillin was observed for fusidic acid (15%), erythromycin (7%), clindamycin (6%) and norfloxacin (5%). Susceptibility to the tested antimicrobial agents was at the same level as in 2012.

The number of new cases of MRSA (both infected and colonized persons) increased in 2013 to 2,094 compared to 1,556 in 2012. The increase was primarily seen in livestock associated MRSA, belonging to clonal complex 398 (CC398), with 643 cases in 2013 vs 232 in 2012. In 2013, CC398 constituted 31% of all new MRSA cases in Denmark. A significant part of the increase was associated with inclusion of contact to pigs as a risk factor requiring screening for MRSA when being admitted to hospitals. The majority (87%) 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. Among all MRSA cases, the proportion of cases presenting with infection was lower in 2013 compared to 2012 (45% vs. 57%, respectively). The number of hospital-acquired (HA) cases continued to be low and constituted only 2% of the total number of MRSA cases in 2013. In 2013, no monitoring of MRSA in animals and meat was performed.

In 2013, 18 carbapenemase producing *Enterobacteriaceae* (CPE) were detected compared to 19 CPE during 2008–2012. Spread of NDM-1 producing *C. freundii* was detected between four patients at a hospital ward in the North Denmark Region. None of the four patients had been travelling recently and the origin of the NDM-1 producing *C. freundii* was unknown.

An increasing number of vancomycin resistant enterococci were received at SSI during 2013. Two-hundred-four-eight of the VRE isolates were from clinical infections and 168 were faecal screening isolates. Nearly all VRE isolates were *vanA E. faecium*. They were primarily detected at hospitals in the Capital Region, but also from the Zealand Region and the Central Denmark Region.

The number of new cases of MRSA (both infected and colonized persons) increased in 2013 to 2,094 compared to 1,556 in 2012. The increase was primarily seen in livestock associated MRSA, belonging to clonal complex 398 (CC398), with 643 cases in 2013 vs 232 in 2012. CC398 constituted 31% of all new MRSA cases in Denmark in 2013. The number of hospital-acquired MRSA cases continued to be low and constituted only 2% of the total number of MRSA cases in 2013.



3. Background information

The following section presents general information about the human population in Denmark in 2013, and the production of food animals 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 2013.

3.1 Populations

The distribution of the Danish human population, which could potentially have received antimicrobial treatment in 2013, is displayed in Figure 3.1, together with the 5 healthcare regions and the 11 Departments

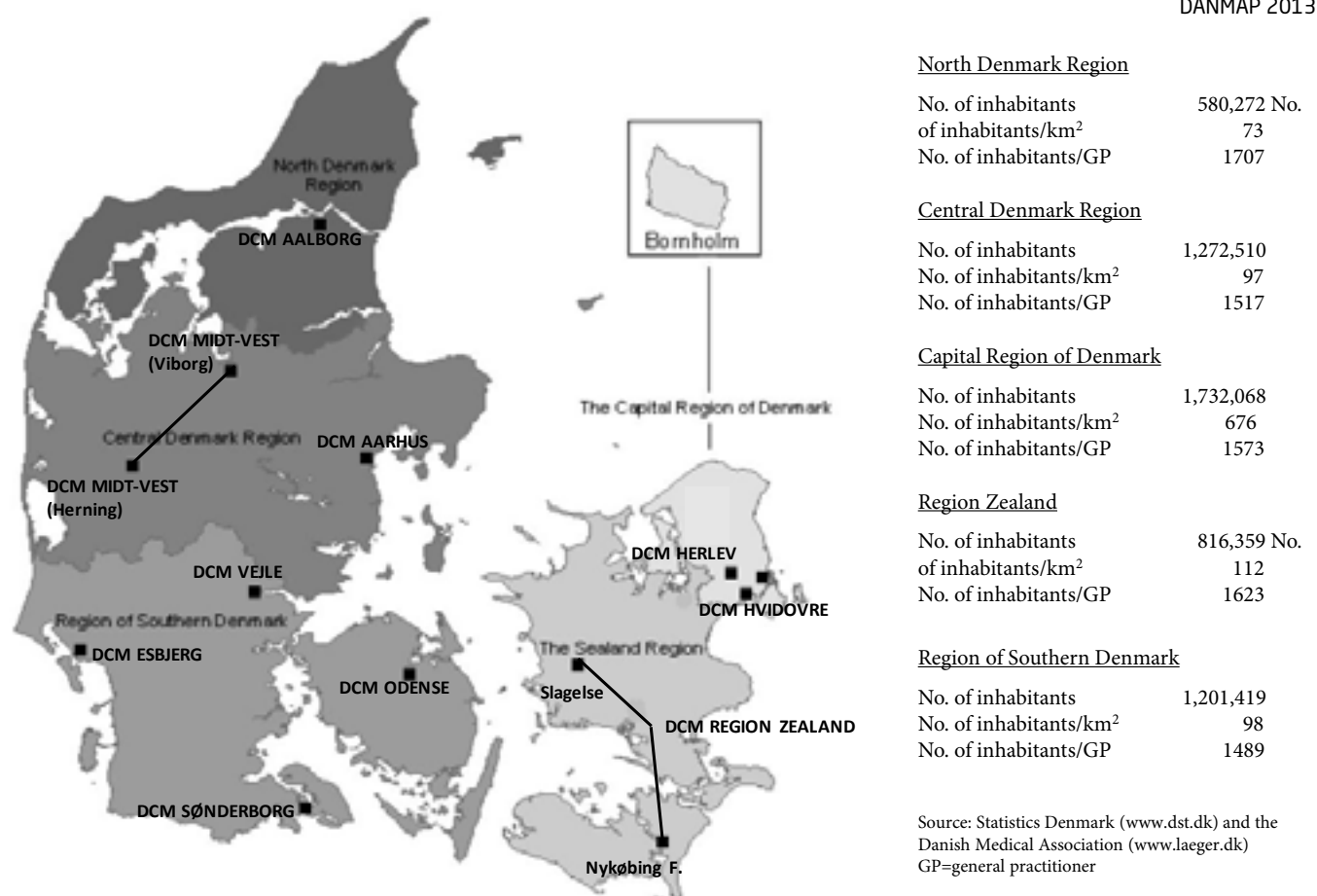
The production of food animals and the production of meat and milk is presented in Table 3.1. While the number pigs was approximately 1% lower than in 2012, the number of fattening pigs (15–50 kg) exported increased by 5%, and the export has increased by more than five-fold since 2004. As in the previous years, the amount of milk produced increased (2%).

There was a 6% increase in the Danish broiler production from 2012 to 2013 (Table 3.1). Approximately 8% of the broilers produced in 2013 were exported for slaughter, a slight decrease compared to 2012, but a marked increase from 0.1% of the production in 2003. The annual production of turkeys has fluctuated considerably over the last decade. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus all turkey meat available in Denmark is listed as imported.

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

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



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

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

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

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			DANMAP 2013 Farmed fish ^(a)	
												Fresh water	Marine
	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	mill. kg	mill. kg
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	139	574	5025	28996	9224	1896	30	13
Increase ^(d)	6%	5%	-37%	-33%	2%	1%	-1%	2%	<1%	5%	0%	8%	1%

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Production data for farmed fish was not available for 2013. Live animals exported prior to slaughter are included in number of animals and amount of meat produced. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg live pigs from Danish Agriculture and Food

a) The numbers for 2013 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 2012 to 2013

Table 3.2. Antimicrobial agents marketed for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2013

DANMAP 2013

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, bacampicillin, pivmecillinam, piperacillin, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin, benzathine benzynpenicillin, procaine penicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin, cloxacillin, methicillin
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, cefalotin
J01DC	Second-generation cephalosporins		Cefuroxime, cefoxitine
J01DD / QJ01DD,QJ51DD	Third-generation cephalosporins	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, cefpodoxime
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, imipenem/cilastatin, doripenem
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb. of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxazol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine, spiramycine
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, norfloxacin, fleroxacin, grepafloxacin, trovafloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	Nalidixic acid
QJ01MQ ^(b)	Quinoxalines	(Carbadox, olaquinox)	
J01XA,A07AA / Not in ATCvet ^(b,c)	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
J01XB / QA07AA ^(b)	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB ^(c)	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet ^(b)	Oligosaccharides	(Avilamycin)	
Not in ATCvet ^(b)	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes starts with a Q

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

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



4. Antimicrobial consumption in animals

4.1 Introduction

The use of antimicrobial agents in humans and animals has been monitored by the DANMAP programme since 1995. Since the early 1990s, there has been both political and public focus on the use of antimicrobial agents in the Danish animal production. This resulted in discontinued use of several antimicrobial agents used for growth promotion from 1994-1999, and more recently, a voluntary ban of use of cephalosporins in the pig production, as well as 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. The increase in veterinary consumption can partly be explained by the increase in pork production, which constitutes approximately 84% 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.

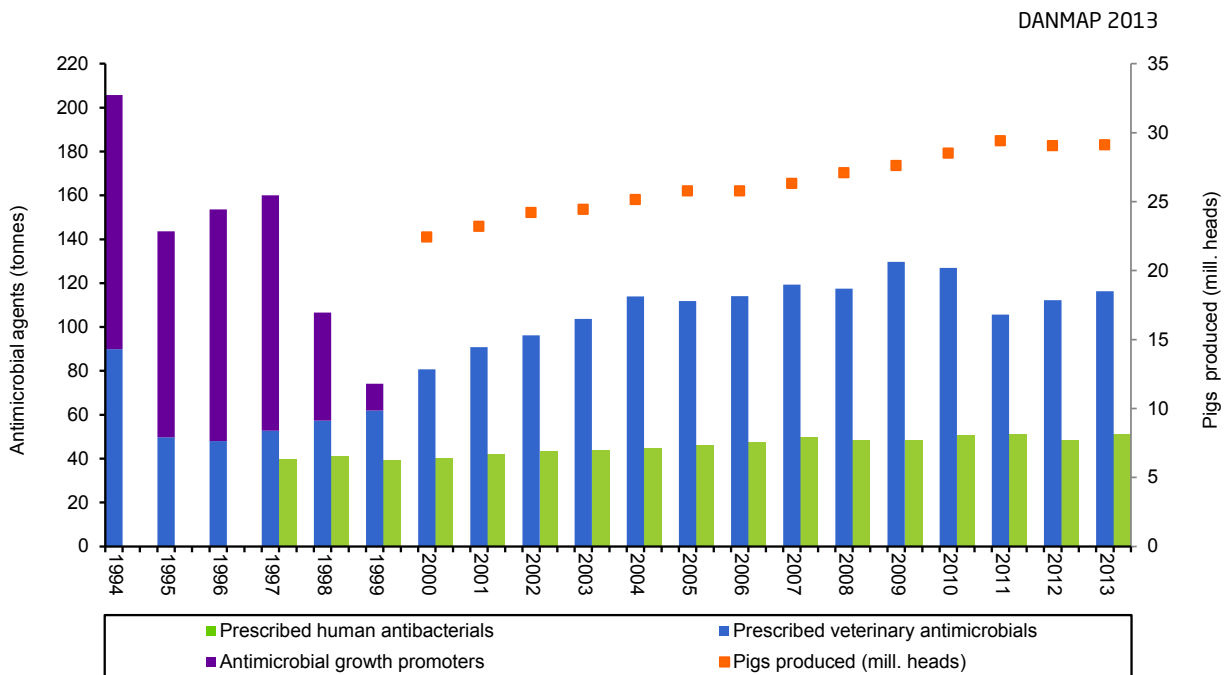
The prescription pattern has been clearly influenced by implemented legislation. For example, the decrease in antimicrobial consumption after 1994 was likely the result of 1) limitation of veterinarians profit from sales of medicine 2) implementation of preventive veterinary strategies with herd health contracts 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, due to concerns regarding extended beta-lactamase resistance (ESBL).

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

Official guidelines for the selection of antimicrobial agents that veterinarians may choose from for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for the selection of the appropriate antimicrobial agents for treatment of all common indications in major production animal species. Initially, guidelines were developed by the National Veterinary 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

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 2001-2013 originate from VetStat

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 2013 - for use in DANMAP - were extracted from VetStat by the Danish Veterinary and Food Administration (DVFA) on 11 June 2014. The 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 2013 are presented in the web annex.

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

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

DAPD or estimated treatment proportion is a statistical measure, providing a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population (e.g. broiler population), on average, receives a certain treatment on a given day (Section 4.3 and Chapter 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 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.

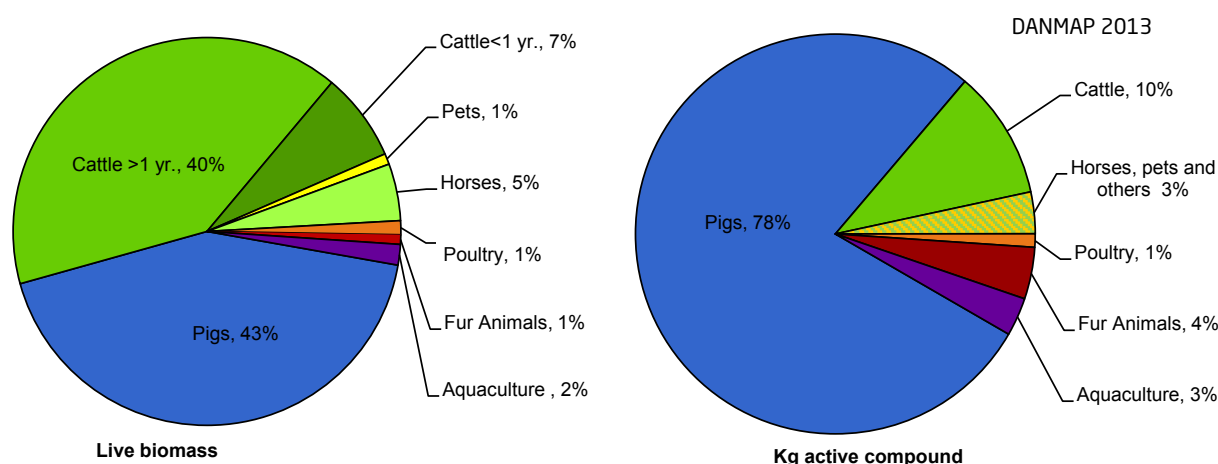
In the text below, unless otherwise stated, DAPD will be used to describe patterns in veterinary antimicrobial consumption.

4.2 Total antimicrobial consumption

In 2013, the total veterinary consumption of antimicrobial agents, including agents used for companion animals, amounted to 116.3 tonnes active compound (Table 4.1), representing a 4% increase compared with 2012. The increase was mainly attributed to a 6% increase 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 2013, the antimicrobial consumption in pigs, cattle and poultry comprised 78%, ~10%, and ~1% of the total veterinary consumption, respectively (Figure 4.2).

4. ANTIMICROBIAL CONSUMPTION IN ANIMALS

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



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

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

DANMAP 2013

ATCvet code	QJ01B	QJ01G	QJ01D	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XQ	QJ01CE	QJ01CA	QJ01E	QJ01AA		
Therapeutic group	Amphenicols	Aminoglycosides	Cephalosporins	Fluoroquinolones	Other quinolones	Lincosamides	Macrolides	Pleuromutilins	Penicillin's, b-lactamase sensitive	Penicillin's, others ^(a)	Sulfonamides and trimethoprim ^(b)	Tetracyclines	Others	Total
Pigs, total^(c)	263	4606	3	11	-	2173	11118	8958	16631	7740	8908	29797	398	90606
-Sows and piglets	229	1818	2	11	-	475	565	845	8935	3584	6181	2330	58	25033
-Weaners	26	2516	1	-	-	787	7166	3303	1639	2938	2296	16807	338	37817
-Finishers	8	272	0	0	-	911	3387	4810	6057	1218	431	10660	2	27756
Cattle, total	503	534	116	0	0	6	195	1	7090	923	1105	1617	17	12106
-Intramammary	-	19	76	-	-	2	-	-	192	162	5	-	2	458
-Calves < 12 mdr	484	233	2	0	-	3	65	-	365	191	183	425	15	1967
-Cows and bulls	13	286	113	0	-	3	127	1	6636	719	910	1147	2	9957
-Heifers and steers	5	15	1	-	-	0	3	-	89	12	12	45	0	182
Poultry, total	9	36	-	0	0	18	280	3	152	190	60	519	1	1270
Poultry incl. broilers, layers and other poultry	1	1	-	0	0	0	37	3	110	168	59	110	1	491
Turkeys	8	35	-	-	-	17	244	-	42	22	1	409	0	779
Other production animal species	185	381	0	0	881	187	632	-	2	1947	3653	578	1	8447
Aquaculture	185	-	-	-	881	-	-	-	-	9	2506	2	-	3582
Fur animals	0	381	0	0	-	187	632	-	2	1938	1147	576	1	4866
Companion animals^(d)	0	150	234	17	0	72	50	11	850	833	1459	192	43	3911
Horses	0	6	5	6	0	0	1	0	813	151	913	27	0	1922
Pets	0	144	229	11	0	72	49	11	37	682	546	165	43	1989
Total	959	5707	353	28	881	2456	12275	8973	24725	11633	15186	32703	461	116339

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

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

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

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

d) 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

Historically, the overall consumption – measured as kg active compound - was 43% lower in 2013 compared with 1994 - while the total meat production increased by 13% during this period (Table 3.1 and Figure 4.1). A major part of the decrease in consumption can be explained by discontinued use of growth promoters (1994-1999).

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

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

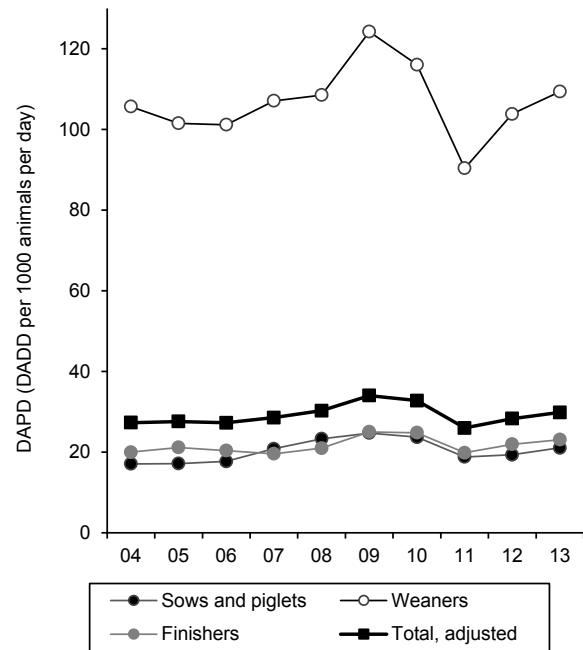
In 2013, the total antimicrobial consumption in pigs was 90.6 tonnes active compound (Table 4.1), an increase of 5.5 tonnes (6%) compared with 2012. The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.4 and in the web annex (Table A4.1).

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, e.g. increased export of live pigs at 30 kg. The increase in the export would in itself cause an increase in DAPD for the remaining population, because the DAPD for finishers is relatively low. Thus, a true impression of the antimicrobial consumption pattern and selection pressure in the pig production requires that changes in export and productivity are taken into account. Overall, the antimicrobial consumption in pigs increased by 5% to approximately 30 DAPD (Figure 4.3) when adjusted for changes in export. Overall the number of pigs produced in 2013 was similar to 2012; however the number of pigs exported increased by 5% (Table 3.1).

Within the different age groups, the DAPD increased in all age groups; 9% in sow herds, and 5% in both finishers and weaners. The increase in consumption was associated primarily with the use of pleuromutilins and tetracyclines, and to a lesser extent, to the use of penicillins and sulfonamides/trimethoprim (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

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



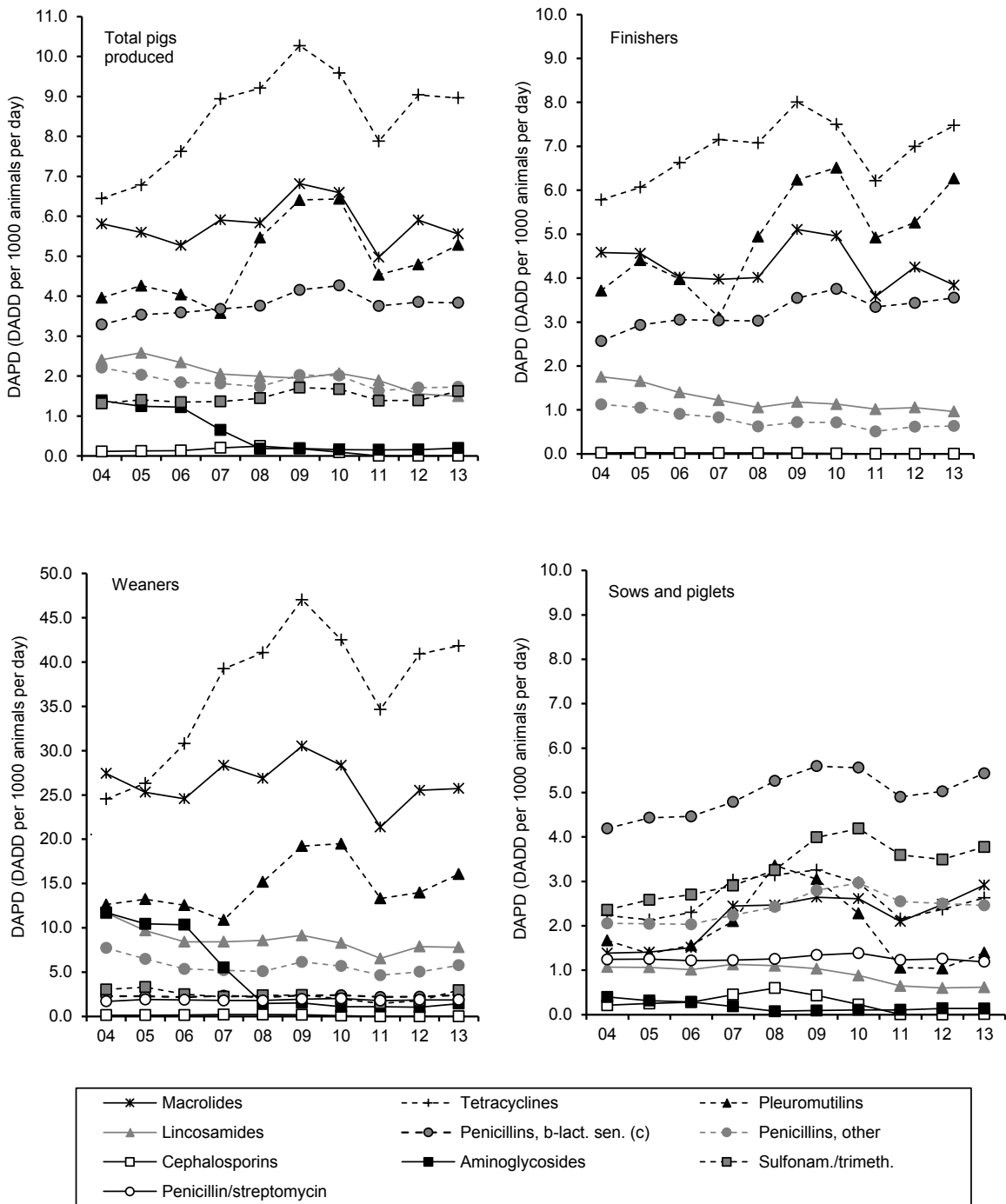
Note: The "adjusted total" is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in piglets pre-weaning. ^a The DAPD is calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group at the total population (in tonnes)

administered orally, and particularly used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall treatment proportion (DAPD) of pleuromutilins increased by 18%, while the use of tetracyclines increased by 4%.

For the critically important antimicrobial agents, the use of flouroquinolones increased for the second year running, however remains at a very low level and constitutes less than 1 per mille of the total consumption in pigs. The increase in 2013 is largely explained by a clinical trial approved by the DVFA. The use of cephalosporins in pigs was low (3 kg), however, increased from 1 kg in 2012 (Figure 4.5).

Over the last decade, the treatment proportion (DAPD) increased from 2004 to 2009. However, in 2010 and 2011, a decrease in DAPD compared with 2009 was observed, probably as a response to the DFVA's implementation of the "yellow card initiative" – a special provision for reduction of antimicrobial consumption in pig production (See DANMAP 2010 for further details). The reductions in antimicrobial use were associated with increasing use of vaccines and a slight temporary decrease in productivity in some herds, but disease outbreaks did not increase [Danish Veterinary Bulletin no. 6, 2012]. With the increase in antimicrobial consumption in pigs in 2013, the consumption is still 12% lower than in 2009, and similar to the 2008 level (Figure 4.3).

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 1.7 million in 2004 to 9.2 million in 2013. 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

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 2013, the total antimicrobial consumption in poultry (all poultry) was 1,270 kg active compound, an increase of 57% compared with 2012. In poultry (other than turkeys), the increase was mainly in the use of simple penicillins (from 10 kg in 2012 to 110 kg in 2013), but also in the use of tetracyclines (36 kg in 2012 and 110 kg in 2013). In turkeys there was also an increase in the use of simple penicillins, but the most significant increase was seen in the use of tetracyclines, which increased from 127 kg to 409 kg in 2013. The main reason appears to be widespread problems with respiratory disease in turkey flocks produced early in 2013. Furthermore, an increased occurrence of diarrhea in broiler flocks in 2013 may be the reason for the increased consumption of penicillins.

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 2010-2013.

4.3.3 Antimicrobial consumption in cattle

In DANMAP 2012 we expressed reservations about data on antimicrobial consumption in cattle. However, analyses of this issue in collaboration with representatives from the cattle industry and the veterinary authorities has shown that the data quality of reporting of use in cattle has increased markedly in recent years and is now as high as that for other major production animal species.

The data shows that use of antimicrobials in cattle has declined to a total of about 12 tonnes active compound in 2013. During this period, the veal and beef production has remained at the same level, although with minor fluctuation from year to year, and the milk production has increased slightly.

In general, the majority of the parenteral use in cattle is for cows (Table 4.1), and is mainly prescribed for mastitis. The systemic use of fluoroquinolones in cattle was close to zero in 2013, and has been at a low level since 2003. In 2013, systemic use of cephalosporins in cattle declined to 40 kg from 47 kg in 2012 (Figure 4.5). Use of 3rd and 4th generation cephalosporins in intramammaries has declined steadily from a peak at 27 kg in 2007 to 7 kg in 2013 (Table 4.2). This is mainly a result of increased focus by the Danish Cattle Association, and veterinary practitioners, on the public health aspects of antimicrobial resistance caused by high consumption of this group of compounds.

Data on intramammary use show a slight reduction in overall level of intramammary treatment from 2005 to 2013 (Table 4.2). However, drying-off treatment has increased steadily during the same period, while therapeutic treatment has decreased (Table 4.3). A “milk quality campaign” conducted by the Danish Cattle Association (Agriculture and Food Council)

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

DANMAP 2013

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

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)

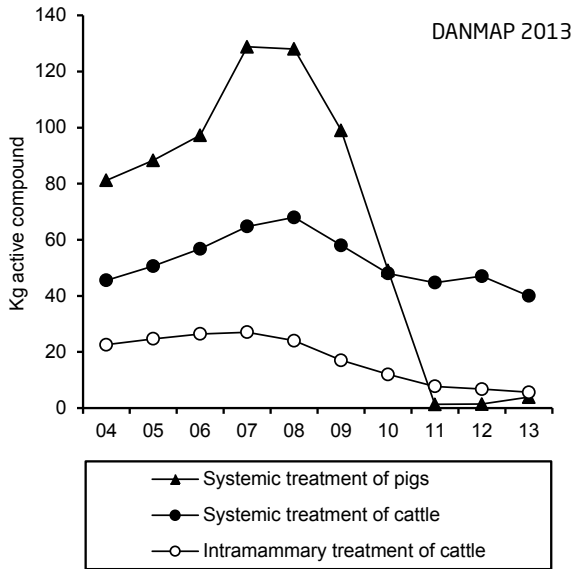
c) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

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

DANMAP 2013

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

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

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.

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

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The antimicrobial consumption in aquaculture increased by 23% to 3,582 kg in 2013 compared with 2012 (Table 4.1). Measured in kg active compound sulfonamide/trimethoprim comprised 70%, quinolones 25% and amphenicols 5%. The antimicrobial consumption in both 2012 and 2011 was very low compared to previous years, probably due to cold summers in 2011–2012, and the relatively large increase in consumption in 2013 is explained mainly by extraordinary high temperatures in July and August 2013, leading to higher water temperatures and an increase in the occurrence of bacteriological infections [personal communication: N.H. Henriksen, Danish Aquaculture]. There is, however, in the aquaculture industry, increased focus on vaccination to reduce the risk of diseases that may require antibiotic treatment.

In 2013, the production of mink increased slightly to 17.5 million mink from 17 million in 2012 (Source: Copenhagen Fur). Antimicrobial consumption in fur animals decreased by approximately 10% to 4.9 tonnes, kg active compound compared with 2012 (Table 4.1). This reverses the continuous increase in consumption for fur animals seen over the past five years (from 2008–2009 consumption increased by 60%). Use of fluoroquinolones and cephalosporins in fur animal production is close to zero.

The information available on antimicrobial consumption in companion animals is not as detailed as for production animals. In 2013 the overall antimicrobial consumption in pets amounted to 1,989 kg active compound (1,449 kg in 2012). The increase was seen for several antimicrobial classes; aminoglycosides, macrolides and penicillins (b-lactamase sensitive), sulfonamides and trimethoprim, as well as for tetracyclines (Table 4.1).

The use of fluoroquinolones for use in pets was 11 kg in 2013. This means that the total use of fluoroquinolones constitutes nearly 40% the total veterinary use (kg) of fluoroquinolones. Similarly, they account for a significant proportion of the use of cephalosporins. In a One Health perspective, this is a cause for concern, because the close contact with their owners poses a risk for transmission of extended spectrum beta lactamase resistance to humans. Presently, however, there is no information available concerning the prevalence of antimicrobial resistance in pet animals.

A large proportion of antimicrobials used for pet animals are prescribed for treatment chronic or recurrent disease, mainly dermatitis. Particularly the consumption of critically important antimicrobial agents in pet animals could pose an important risk to owners of diseased dogs that are frequently treated.

Acknowledgement

The methodology for calculating veterinary consumption in DAPD, as first applied in DANMAP 2012, was developed and described by Vibeke Frøkjær Jensen.

Leonardo de Knegt, Birgitte Borck Høg and Flemming Bager



5. Antimicrobial consumptions in humans

5.1 Introduction

In Denmark, the use of antimicrobial agents for humans has been monitored since 1995 and presently all data on consumption in both the primary healthcare and hospital care is registered by the Department of Data Delivery and Medicinal Product Statistics at Statens Serum Institut.

In this section, the term ‘antimicrobial agents’ covers only antibacterial agents for systemic use in humans, agents used for treatment of infections caused by virus or fungi are not included. Currently available antimicrobial agents for systemic treatment in humans (and in animals) are listed in Table 3.2. Antimicrobial agents have been classified as either narrow-spectrum or broad-spectrum agents according to the spectrum of the activity (Table 5.1).

5.2 Total consumption (primary healthcare and hospital care)

The overall consumption of antimicrobial agents in Denmark is calculated as a combined measure of the consumption in the primary healthcare and hospital care. This measure is presented in DID and is intended for comparison between sectors and for illustration of the consumption in hospital care without taking account of hospital activity (discharges) (Figure 5.1). Since the first publication of DANMAP, the consumption in primary healthcare has accounted for 90% of the total antimicrobial consumption in Denmark.

In 2013, the total consumption of antimicrobial agents for systemic use (primary healthcare and hospital care) was 1.2% higher than the previous year (Figure 5.1). Since 2004, the overall consumption of antimicrobial agents has increased by 20%.

The proportion of broad-spectrum agents was 5% higher than in 2012 (Figure 5.2). This continues the trend from the past decade where broad-spectrum agents increased by 72%, comprising 32% of the total consumption in 2004 and 46% in 2013.

The distribution of DIDs between primary healthcare and hospital care differed between antimicrobial agents (Figure 5.3). For most of the antimicrobial agents, the consumption was higher in primary healthcare, with exception of cephalosporins and other β -lactams, aminoglycosides and imidazole derivatives.

In 2013, 51,500 kg of antimicrobial agents for systemic use were used in humans in Denmark. This is the highest level observed since 1995, representing an increase of 6,500 kg (14%) since 2004 (Table A5.1 in web annex).

Table 5.1. Classification of antimicrobial agents for systemic use in humans into narrow-spectrum and broad-spectrum agents, Denmark DANMAP 2013

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

a) From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.1. Total consumption of antimicrobial agents (J01) in humans in primary healthcare vs hospital care, Denmark

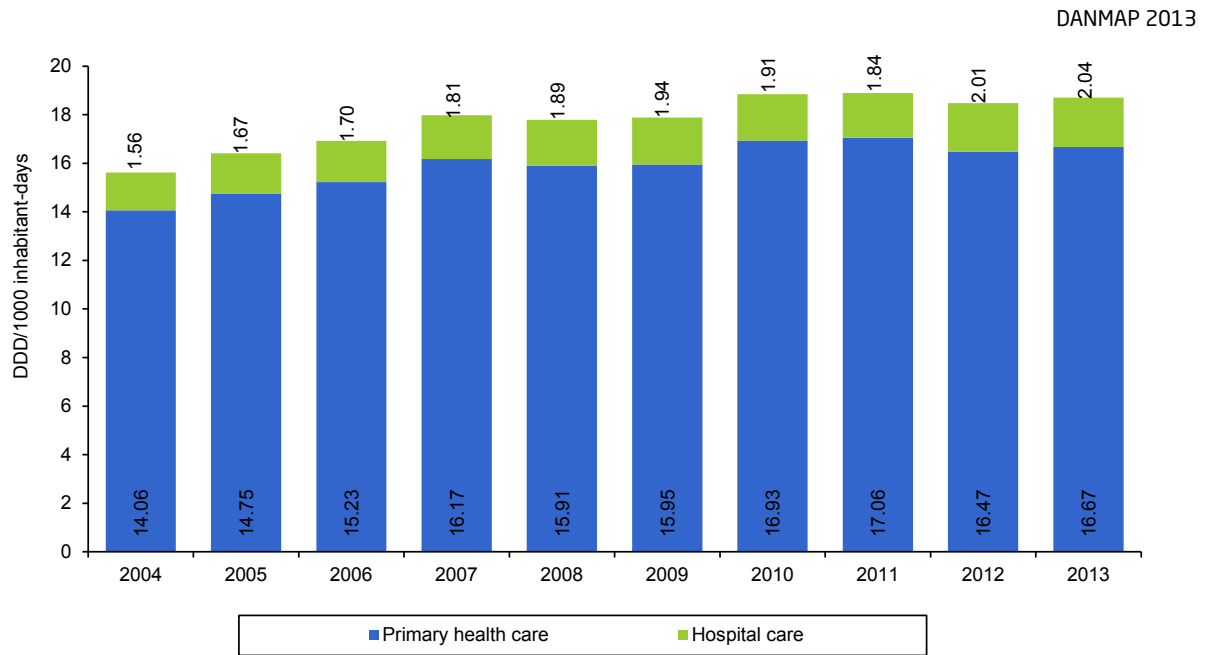
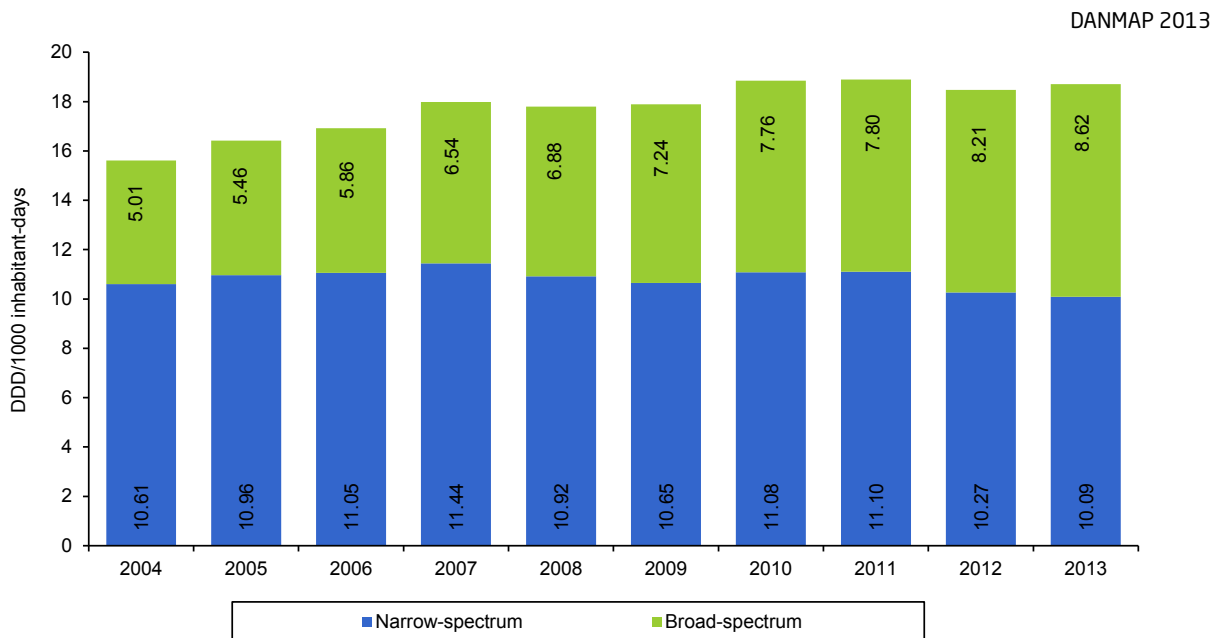


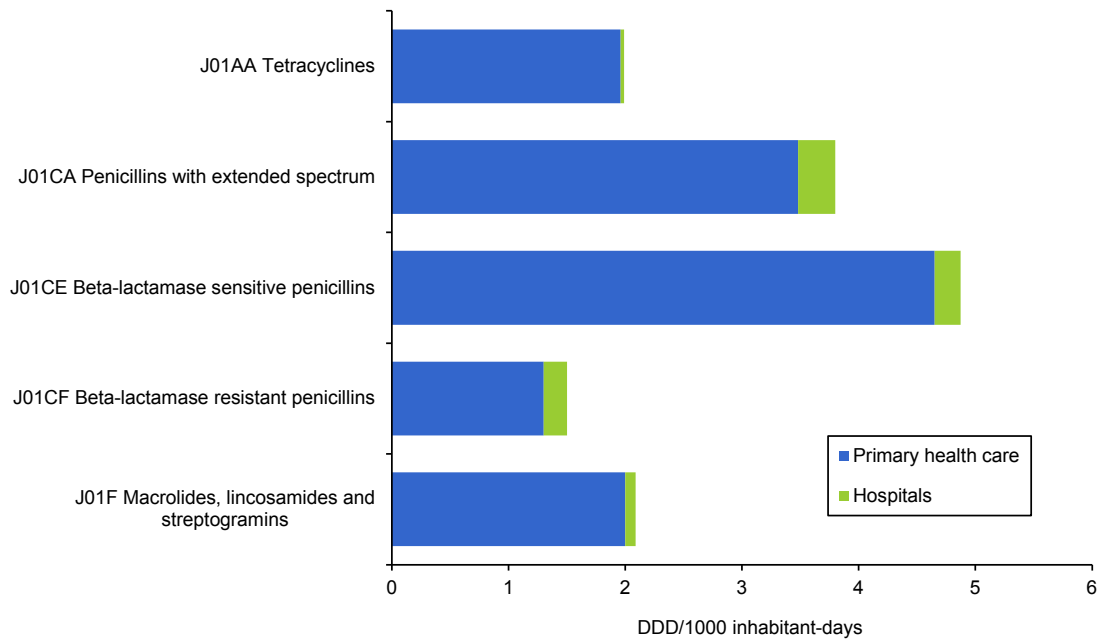
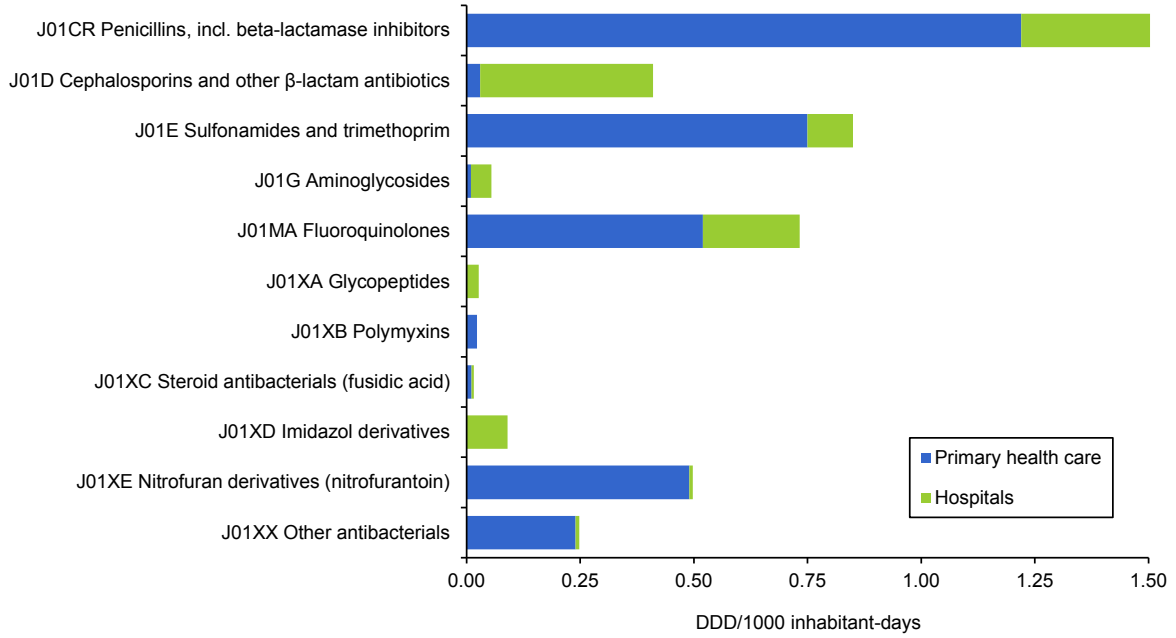
Figure 5.2. Total consumption of antimicrobial agents (J01) in humans by narrow-spectrum and broad-spectrum agents, Denmark



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

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

DANMAP 2013



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

Background: Greenland has a population of 56.370 inhabitants (January 2013) and Nuuk is the capital with around 16.000 inhabitants. Greenland has its own Ministry of Health and the country is divided into five health regions: Qeqqa (the former health districts Sisimiut and Maniitsoq), Disko (the former health districts Aasiaat, Kangaatsiaq, Qeqertarsuaq and Qasigiannnguit), Avannaa (the former health districts 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 Ingrids Hospital, is situated in Nuuk (185 beds). There are several smaller hospitals and healthcare centers in the five health regions. Around 15-16.000 persons are admitted to hospital once or several times a year. The primary healthcare is organized different from that in Denmark; there are no general practitioners with private practice, and the hospital clinics are used for patients from the primary healthcare. In Nuuk, a large healthcare center has combined function as medical clinic, emergency room and primary healthcare with doctors and nurses. The settlements have nursing stations (supervised by doctors via phone or telemedicine and doctors visiting three to four times a year). Medication on prescription is free of charge. Patients with specific/serious diseases are transferred to Denmark or Iceland for further treatment (e.g. hemodialysis, cancer treatment, brain surgery etc.).

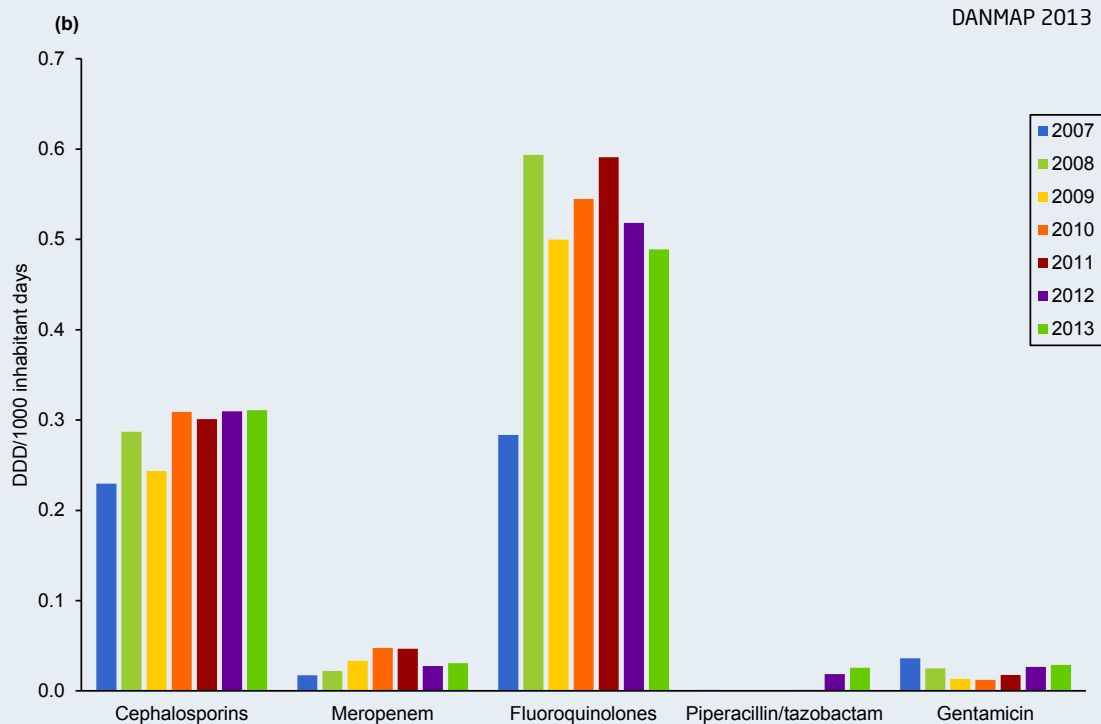
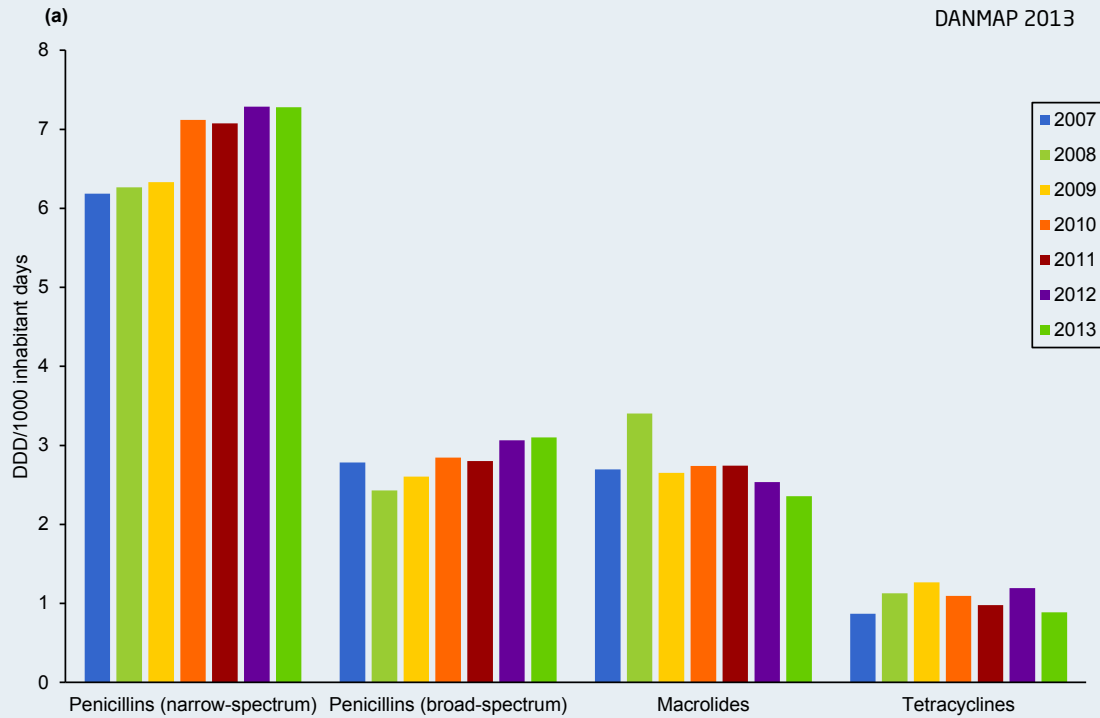
Resistant bacteria: From 2004 to 2013, 12 patients have been diagnosed with MRSA, 33 patients with ESBL-producing *Enterobacteriaceae*, and 29 out of 85 patients with *Clostridium difficile* infection had the 027 type. Most of these resistant bacteria were imported from Denmark or abroad, but in some 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* infections (mainly type 027) 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 is planned for the near future.

Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and disseminated from the National Pharmacy. The total purchase of selected antimicrobial agents from 2007 to 2013 are shown in Figure 1. From 2007–2013, an increase of narrow-spectrum (18%) and broad-spectrum penicillins (12%) has been seen. From 2012 to 2013, an increase in piperacillin-tazobactam (37%) and a decrease in broad-spectrum antimicrobial agents such as tetracyclines (26%), macrolides (7%), and fluoroquinolones (6%) have been seen. Meropenem has increased with 11% whereas cephalosporins (mainly ceftriaxone) have been at the same level from 2012 to 2013.

Conclusion: Frequent change in workforce and hospitalization abroad are challenges for maintaining a restrictive antibiotic policy and of these reasons continued focus on the use of broad-spectrum antimicrobial agents - both in hospitals and in primary healthcare - and the incidence of multi-resistant bacteria in Greenland is very important in the future.

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Figure 1. Consumption of selected antimicrobial agents in humans in Greenland (DDD/1,000 inhabitants/day) 2007-2013: (a) consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines; and (b) consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin. (Note: Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin and dicloxacillin, and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor).



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

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

Data and data sources: Data for antimicrobial consumption for FI and for LS were supplied by the Chief Pharmaceutical Office, beginning from 2007. Bed-days and data on MRSA and ESBL-producing bacteria were obtained from LS.

Antimicrobial consumption in primary healthcare: Total antimicrobial consumption outside the hospitals was 14.23 DDD/1000 inhabitants/day; this is unchanged since 2010, but represents a 16.6% decrease compared to 2007. The use of tetracyclines has increased by 32.8% compared to 2007 whereas the consumption of macrolides decreased with 31.1% from 2007 to 2013. The reason for this shift remains to be elucidated. Regarding antimicrobial agents used for urinary tract infections, marked changes have been seen mainly reflecting identification of a potentially fatal carnitine transporter gene defect in 1/3.600 inhabitants of the FI [Joensen *et al.* 2006. Ugeskr Laeg 168: 667-670] which led to an almost total stop in the use of pivampicillin and pivmecillinam in 2012. In 2013, pivampicillin was still not used, whereas pivmecillinam use outside hospitals increased to 13.3% of the 2007 level compared to 3.6% in 2012 (Figure 1). Data from hospitals show the same trend. This increase in consumption, although still at a low level, is probably due to increased screening for the gene defect. The decreased use of pivmecillinam has led to an increase in the use of sulfamethizole (43.0%), trimethoprim (40.2%), and ciprofloxacin (46.7%) compared to 2007.

Antimicrobial consumption in hospital care: The consumption of antimicrobial agents in 2013 was 39.70 DBD (DDD/100 bed-days). This is a decline from 2012 (41.45 DBD) and the consumption is now back at the same level as in 2007 (Table 1). However, whereas the total consumption of antimicrobial agents at LS is at the 2007 level, the use of three antimicrobials, which are well known for their effect on selection of virulent binary-toxin positive *C. difficile* - cefuroxime, ciprofloxacin, and meropenem - has increased to 137.4%, 424.5%, and 175.0% of the 2007 level (Table 1).

The consumption of cefuroxime, ciprofloxacin, and meropenem and piperacillin with enzyme inhibitor, in a comparison between LS and Danish hospitals for 2007 to 2013 are shown in Figure 2. It is thus evident that LS has been on the same curve of increase as Danish hospitals in general, only with some 7-8 years' delay. This is also illustrated by the fact that the consumption of the three above antimicrobials together increased from 8.99 DBD in 2007 to 14.54 in 2013 or from 39.8 to 58.8% of the use in Danish hospitals. However, it seems that the use in LS is now at least stabilizing.

Resistant microorganisms. Since the first case of MRSA in 2004, a total of 36 cases of MRSA have been identified (22 with infection and 14 carriers). From 2006–2013, 15 ESBL-producing *Escherichia coli* and four ESBL-producing *Klebsiella pneumoniae* have been detected, furthermore one patient had both an ESBL-producing *E. coli* and an ESBL-producing *K. pneumoniae*.

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

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Figure 1. Consumption of antimicrobial agents used prophylaxis and treatment of urinary tract infections in primary healthcare (2007-2013)

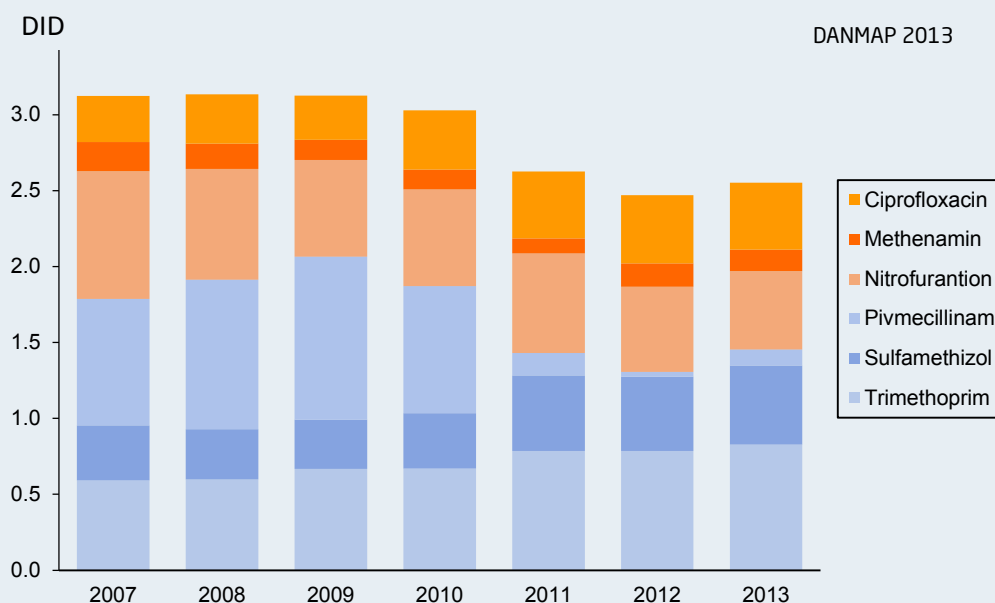
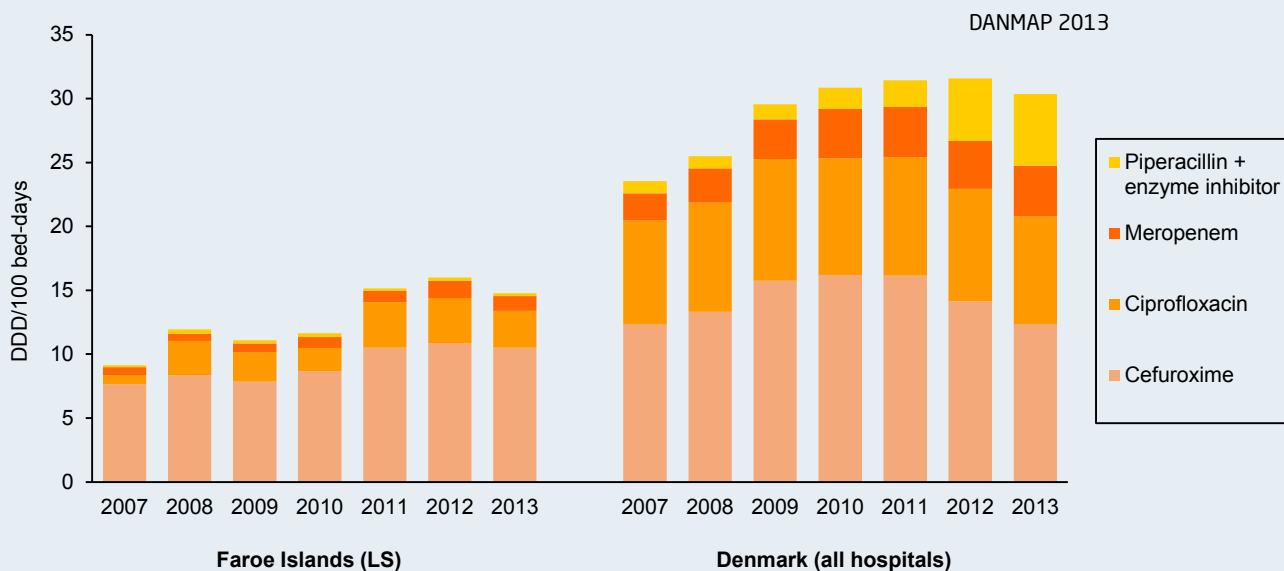


Table 1. Total consumption of antimicrobials and consumption of cefuroxime, ciprofloxacin, meropenem, and piperacillin w. enzyme inhibitor at LS 2007-2013, shown as DBD (DDD/100 bed-days) and as % of 2007 level DANMAP 2013

	2007	2008	2009	2010	2011	2012	2013
Cefuroxime	7.65 (100.0)	8.36 (109.2)	7.87 (102.8)	8.66 (113.2)	10.52 (137.5)	10.86 (141.9)	10.51 (137.4)
Ciprofloxacin	0.68 (100.0)	2.68 (396.4)	2.30 (339.3)	1.83 (271.0)	3.54 (523.6)	3.54 (522.8)	2.87 (424.5)
Meropenem	0.66 (100.0)	0.55 (83.7)	0.67 (101.0)	0.85 (128.4)	0.89 (135.2)	1.35 (204.9)	1.15 (175.0)
Piperacillin + inhibitor	0.14 (100.0)	0.34 (237.0)	0.26 (182.4)	0.29 (199.5)	0.20 (141.0)	0.26 (180.1)	0.23 (159.0)
Total	39.20 (100.0)	40.52 (103.4)	40.56 (103.5)	40.79 (104.0)	46.37 (118.3)	41.45 (105.7)	39.70 (101.3)

Figure 2. Consumption of cefuroxime, ciprofloxacin, meropenem, and piperacillin with enzyme inhibitor at the LS hospital and in Danish hospitals (2007-2013)



The Danish Council of Ethics' statement on the use of antibiotics

Background: In January 2014, The Danish Council of Ethics published a set of recommendations on the ethical use of antibiotics in a Danish context.

The recommendations set out to prescribe action as a response to two specific ethical dilemmas. These are introduced below.

Despite many years of efforts to the contrary, antibiotics use and antimicrobial resistance (AMR) problems have been in a steady increase in Denmark and elsewhere. In the view of the Danish Council of Ethics, the presence of ethical dilemmas associated with combating AMR is likely to be an important factor behind the challenges encountered.

Ethical dilemmas

Dilemma 1: Rationing antibiotics

The need to prescribe human antibiotics reflects the general living condition of a population, including hygiene conditions in homes, in public spaces and at workplaces, day-care facilities and hospitals. The consumption of antibiotics in farming is largely a result of the production methods chosen.

Owing to the great mobility of people, animals and foodstuffs, the effect of a cautious approach to using antibiotics on the local resistance situation is uncertain.

However, the extent of resistance is inextricably bound up with the consumption of antibiotics. Even well-justified use promotes resistance development. Within a fairly short span of years, increasing resistance to antibiotics will reduce patients' access to potent and effective antibiotics.

It is crucial, therefore, to exercise reticence regarding the use of antibiotics, in spite of the fact that greater reticence will involve a greater risk for patients, animals and livestock. Examples of obviously unjustifiable use - that is to say where antibiotics are prescribed even though the treatment is known to be ineffective, and where reticence is therefore bound to have no impact - are probably rare.

How should the doctor, veterinarian and farmer balance consideration for the patient, animal or herd with regard to future patients?

Dilemma 2: Preventing infection

Being a carrier of antibiotic-resistant bacteria entails a risk of infection. Infection can entail medical risks and social strains and stresses.

Those not infected should be protected against infection. That may call for the use of isolation, restraining measures etc. Those not infected should also have the option of deciding which infection risks they want to expose themselves to. That advocates openness around infection sources, e.g. by informing the public about infected animal herds or having a duty to report knowledge of infected individuals.

For carriers of resistant bacteria, however, such initiatives can be stigmatizing, offensive and involve interfering in the individual's freedom. Furthermore, experience has shown that carrier status, as a consequence of stigma, has been concealed to a greater extent, thus causing the risk of infection to rise.

Recommendations

- In guidelines the authorities should acknowledge the ethical dilemmas in which doctors, veterinarians, farmers and ordinary citizens are placed as a result of endeavours to ration antibiotics and prevent the spread of antibiotic-resistant bacteria
- The authorities' efforts to combat antibiotic resistance should be intensified in the health sector with a view to reducing the problem both nationally and internationally. Particular heed should be paid to specialist guidelines for the use of antibiotics and their implementation

- The authorities should minimize the stigmatization, isolation and discrimination that may attach to being a carrier of antibiotic-resistant bacteria. First and foremost, the risk of infection should be limited by improving general hygiene
- Use of antibiotics “to be on the safe side” or to reduce discomfort should be avoided in interaction between doctors and patients
- The authorities should redouble their efforts to combat antibiotic consumption in farming. This can be done, for example, by promoting and demanding stricter criteria for healthier forms of production and by limiting the use of herd medication
- Nationally and internationally, the authorities should work to promote consumers’ scope for choosing products made with limited consumption of antibiotics
- Statement, working papers, an interview and more (in Danish): <http://www.dketik.dk/Projekter/Antibiotika.aspx>

**On behalf of the Danish Council of Ethics, professor Gorm Greisen,
chairman of the antibiotics working group**



5.3 Primary healthcare

5.3.1 Total consumption in primary healthcare

In 2013, the consumption of antimicrobial agents in primary healthcare was 1.2% higher than that observed in 2012 (Table 5.2) and thus reversed a large part of the positive decline seen in 2012. During the past decade, the consumption of antimicrobial agents in Danish primary healthcare has increased by 2.61 DID (19%).

The proportion of broad-spectrum agents in 2013 was 0.39 DID (5.7%) higher than in 2012 (Figure 5.4). Since 2004, the proportion of broad-spectrum agents has increased by 72%.

As observed in previous years, beta-lactamase sensitive penicillins represented the largest therapeutic group of antimicrobial agents consumed (28%) – and penicillins in general accounted for 64% of the total consumption in 2013 (Figure 5.5). For the other therapeutic groups, several changes in consumption were observed between 2012 and 2013 (Table 5.2). Especially noteworthy are an 11% increase in tetracyclines (see Textbox 4) and a 16% increase in ‘combination penicillins’. The consumption of macrolides was 12% lower in 2013 compared to 2012, upholding the decrease observed since 2011 (Figure 5.6).

5.3.2 Measures at treated patient level

In 2013, each treated patient received 21.3 DDDs (Table 5.3) – an increase of 3.4% compared to 2012. The number of patients treated was, however, 2.3% less (Table A5.2 and A5.3 in web annex), which means that in 2013, more DDDs were prescribed for each package. During the past decade, the 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 significantly since 2004 while the number of packages per patient has remained relatively constant (Table 5.3).

Considering the leading antimicrobial groups, each treated patient received 11.8 – 22.6 DDDs in 1.4 – 1.7 packages with the exception of tetracyclines (Table 5.3). For tetracyclines, both the number of DDDs per patient and DDDs per package are significantly higher than any other antimicrobial group, primarily due to the fact that tetracyclines are commonly used for acne treatment; there higher dosages are given for longer periods.

When examining three different indicators at the treated patient level (Figure 5.7), a marked increase is observed for DIDs while the number of packages and treated patients has remained relatively constant since 2004. In other words, the dosage prescribed for each patient and in each package has increased significantly during the past years. The reasons for these changes are as yet unclear.

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

		DANMAP 2013									
ATC group ^{a)}	Therapeutic group	Year									
		2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	1.17	1.28	1.38	1.48	1.54	1.61	1.69	1.64	1.76	1.96
J01CA	Penicillins with extended spectrum	2.63	2.79	2.95	3.25	3.26	3.29	3.47	3.41	3.40	3.48
J01CE	Beta-lactamase sensitive penicillins	5.20	5.28	5.40	5.67	5.30	5.12	5.25	5.31	4.68	4.65
J01CF	Beta-lactamase resistant penicillins	0.92	0.97	1.05	1.09	1.12	1.13	1.17	1.14	1.21	1.30
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.06	0.08	0.12	0.19	0.27	0.45	0.68	0.89	1.05	1.22
J01D	Cephalosporins and other β -lactam antibiotics	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.41	0.44	0.47	0.49	0.49	0.48	0.51	0.50	0.52	0.53
J01EB	Short-acting sulfonamides	0.36	0.35	0.35	0.31	0.28	0.27	0.26	0.24	0.22	0.22
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
J01FA	Macrolides	2.23	2.41	2.31	2.42	2.28	2.21	2.44	2.47	2.19	1.93
J01FF	Lincosamides	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.04	0.05
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
J01MA	Fluoroquinolones	0.28	0.33	0.37	0.44	0.51	0.52	0.57	0.57	0.55	0.52
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01
J01XD	Imidazole derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.43	0.45	0.46	0.47	0.47	0.49	0.51	0.50	0.49	0.49
J01XX	Other antibacterials (methenamine >99%)	0.30	0.28	0.27	0.26	0.27	0.26	0.27	0.26	0.25	0.24
J01	Antibacterial agents for systemic use (total)	14.06	14.75	15.23	16.17	15.91	15.95	16.93	17.06	16.47	16.67

a) From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system

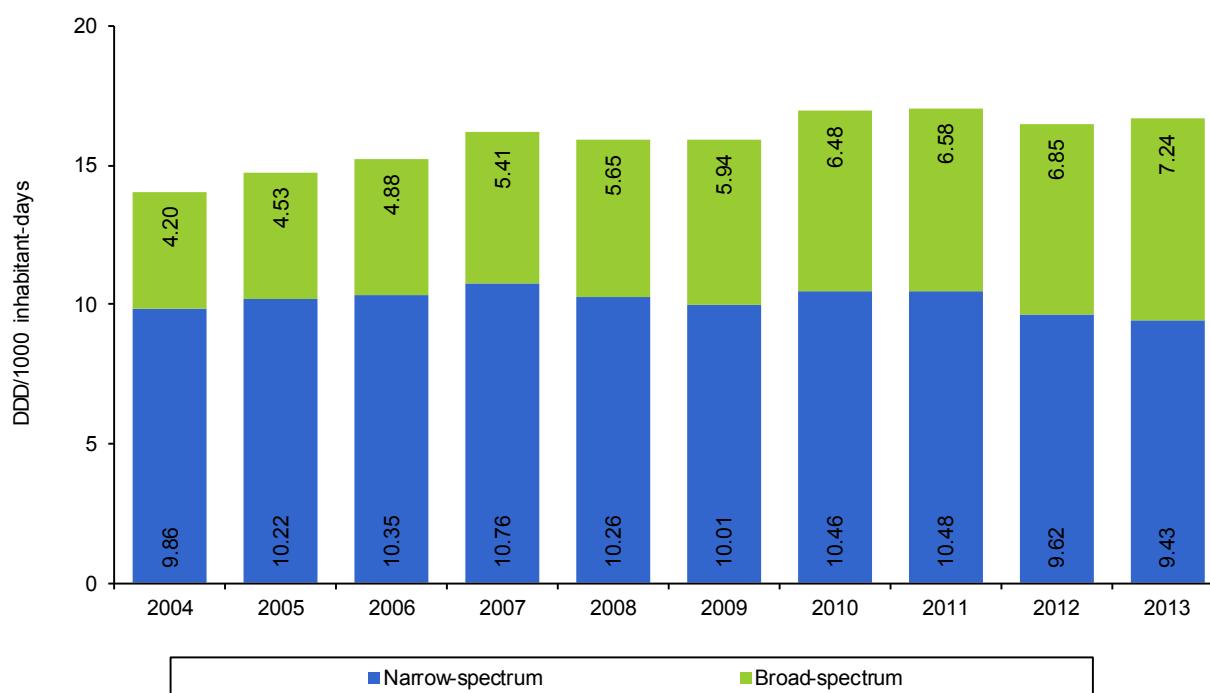
Table 5.3. Number of DDDs and packages per treated patient among leading groups of antimicrobial agents in primary healthcare, Denmark

			DANMAP 2013									
ATC group ^(a)	Therapeutic group	Indicator	Year									
			2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	DDD's / patient	36.9	39.0	40.9	43.0	44.4	45.2	45.9	44.0	47.6	51.6
		Packages / patient	1.9	2.0	1.9	2.0	2.0	2.0	2.0	1.9	2.1	2.1
		DDD's / package	19.0	19.6	21.0	22.0	22.7	22.7	22.7	22.6	23.1	25.5
J01CA	Penicillins with extended spectrum	DDD's / patient	13.6	13.9	14.2	14.4	14.7	14.8	14.9	14.8	16.1	16.7
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7
		DDD's / package	8.4	8.5	8.9	9.0	9.2	9.2	9.0	9.2	9.7	10.0
J01CE	Beta-lactamase sensitive penicillins	DDD's / patient	11.1	11.3	11.5	11.7	11.8	11.8	11.8	11.8	11.8	12.0
		Packages / patient	1.5	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
		DDD's / package	7.5	7.7	8.0	8.2	8.2	8.4	8.4	8.4	8.4	8.5
J01CF	Beta-lactamase resistant penicillins	DDD's / patient	12.4	12.7	13.0	13.4	13.7	13.9	14.2	13.8	15.5	16.4
		Packages / patient	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.4	1.6	1.7
		DDD's / package	7.8	8.0	8.6	8.7	9.0	9.1	9.3	9.6	9.7	9.4
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDD's / patient	17.2	16.8	19.3	19.1	19.9	20.4	21.1	21.9	22.3	22.6
		Packages / patient	2.0	2.0	1.8	1.6	1.6	1.5	1.5	1.6	1.6	1.6
		DDD's / package	9.1	9.3	10.7	11.7	12.4	13.3	13.7	14.1	14.3	14.3
J01FA	Macrolides	DDD's / patient	12.4	12.4	12.6	12.4	12.5	12.5	12.2	11.5	12.4	12.6
		Packages / patient	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.6
		DDD's / package	7.9	8.0	8.3	8.1	8.1	8.1	8.1	7.9	8.0	8.0
J01MA	Fluoroquinolones	DDD's / patient	9.5	9.6	10.3	10.6	11.0	11.2	11.2	11.5	11.7	11.8
		Packages / patient	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDD's / package	6.4	6.5	6.9	7.0	7.5	7.6	7.6	7.7	7.8	7.8
J01	Antibacterial agents for systemic use (total)	DDD's / patient	17.0	17.5	17.9	17.3	18.9	19.2	19.6	19.4	20.6	21.3
		Packages / patient	2.1	2.1	2.0	1.9	2.1	2.1	2.1	2.1	2.1	2.1
		DDD's / package	8.1	8.3	8.7	8.9	9.1	9.3	9.3	9.3	9.7	9.9

a) From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system

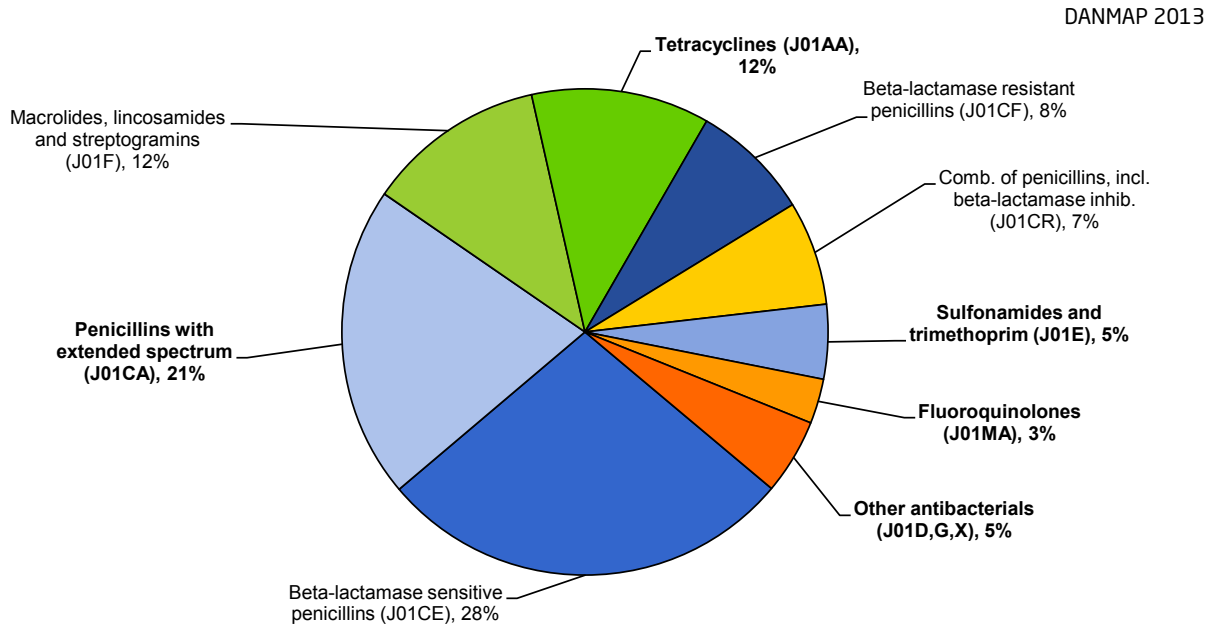
Figure 5.4. Consumption of antimicrobial agents (J01) in primary healthcare by narrow-spectrum and broad-spectrum agents, Denmark

DANMAP 2013



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

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



Note: Bold highlights indicate broad-spectrum antimicrobial agents

Figure 5.6. Consumption of leading antimicrobial groups for systemic use in primary healthcare, Denmark

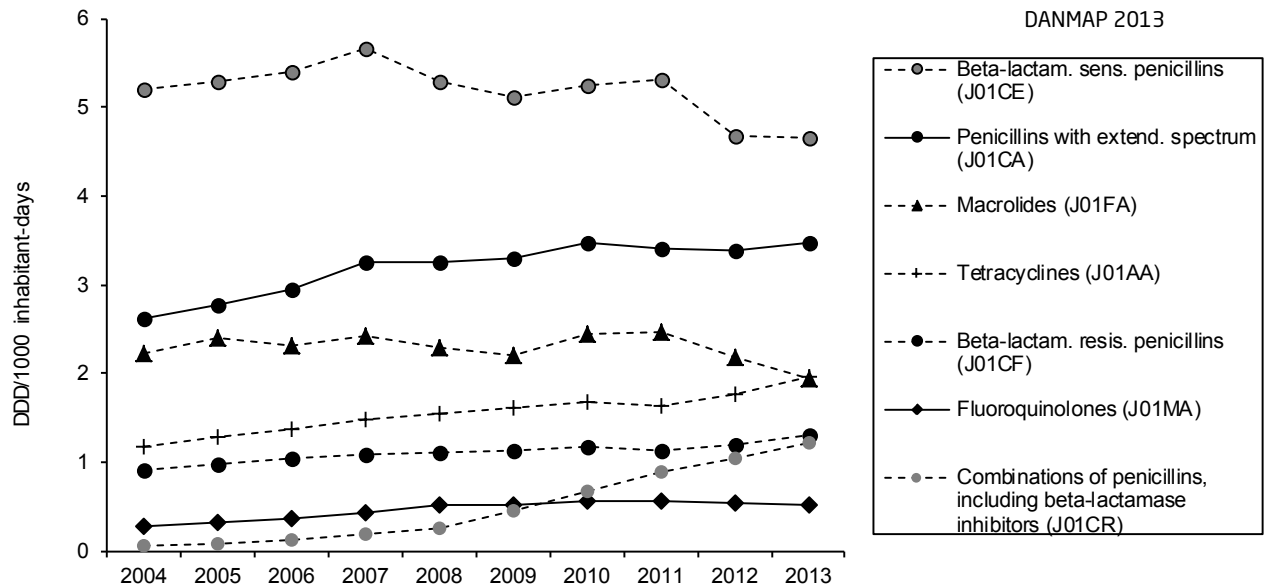
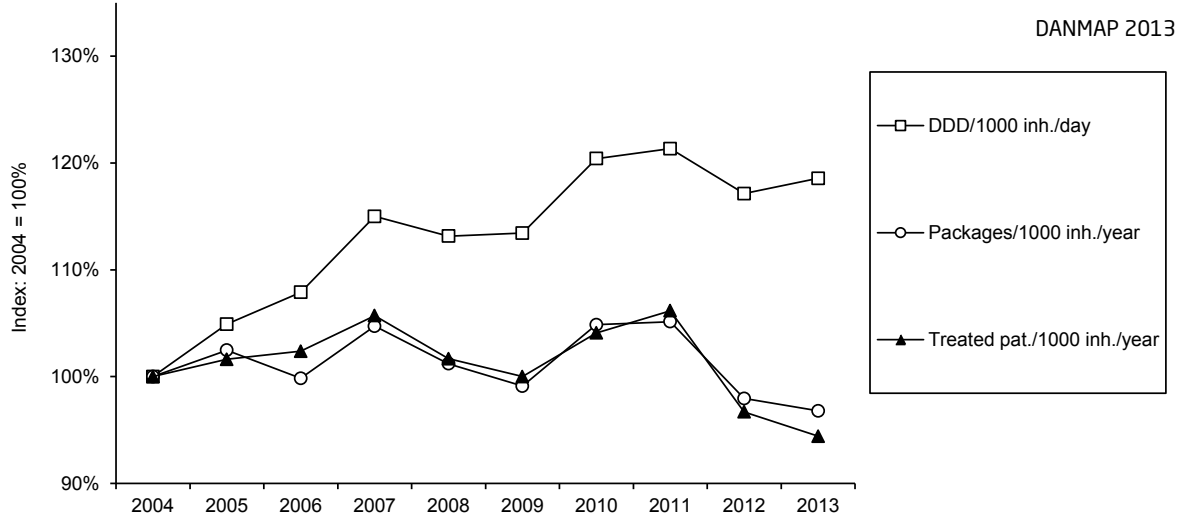


Figure 5.7. Indicators of antimicrobial consumption (J01) in primary healthcare, Denmark



5.3.3 Tetracyclines

From 2012 to 2013, the overall consumption of tetracyclines increased by 11.4%, primarily due to increase in use of doxycycline which is now the most used substance (Table 5.2). Since 2004, the consumption of tetracyclines has increased by 68%, primarily driven by significant increases in both doxycycline and lymecycline and a more conservative increase in tetracycline consumption (Figure 5.8). See also Textbox 4 about tetracycline prescription patterns in Denmark.

5.3.4 Penicillins

The overall consumption of penicillins in 2013 was 3% higher than that observed in 2012 (Table 5.2), continuing the generally increasing trend observed during the past decade with an increased consumption of 21% from 2004–2013.

For individual penicillin groups, the consumption of narrow spectrum penicillins (i.e. phenoxymethylpenicillin) continued to decrease whereas increases in consumption were observed for ‘combination penicillins’ (16%), beta-lactamase resistant penicillins (7.4%) and penicillins with extended spectrum (2.4%). The consumption of these three groups in particular has also increased significantly over the past decade replacing the use of narrow spectrum penicillins (Table 5.2).

At the substance-level in 2013, phenoxymethylpenicillin continues to be the most commonly consumed penicillin despite decreasing usage. The consumption of flucloxacillin, amoxicillin and enzyme inhibitor, and pivmecillinam increased (Figure 5.9). The consumption of ampicillin decreased by 28%.

‘Combination penicillins’ (primarily amoxicillin/clavulanic acid) are currently advocated for broad treatment of respiratory infections, including patients with exacerbation of chronic obstructive pulmonary disease (COPD). The highest consumption of these drugs is observed in people aged 65 and older, suggesting that the increase may be related to an increased rate of diagnosis and treatment of respiratory infections and COPD in this age group. Further, the increase may also partly be explained by an increasing tendency of GPs to prescribe the sweet-tasting amoxicillin mixture for respiratory infections in children rather than benzylpenicillin (beta-lactamase sensitive penicillins).

5.3.5 Macrolides

From 2012–2013, the consumption of macrolides decreased by 12% (Table 5.2), and this decrease was apparent for all substances (Figure 5.10). The high consumption of macrolides in 2010 and 2011 may be explained by two epidemic waves of *Mycoplasma pneumoniae* infection (DANMAP 2010 & 2011) and the return to a generally lower consumption in 2012 and onwards is most likely related to the absence of significant disease outbreaks (DANMAP 2012).

From 2004–2013, the consumption of roxithromycin, clarithromycin and azithromycin increased (Figure 5.10). The consumption of erythromycin decreased substantially, most likely in response to changes in national guidelines which in 2004 substituted the first-choice macrolide in primary care from erythromycin to roxithromycin and subsequently also to clarithromycin in 2007.

Figure 5.8. Consumption of tetracyclines in primary healthcare, Denmark

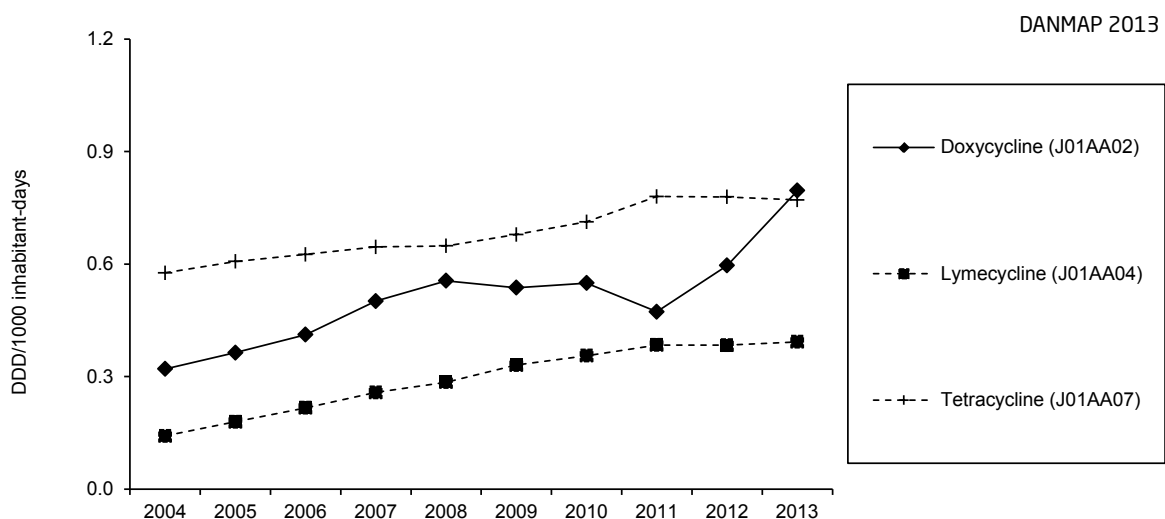


Figure 5.9. Consumption of leading penicillins in primary healthcare, Denmark

DANMAP 2013

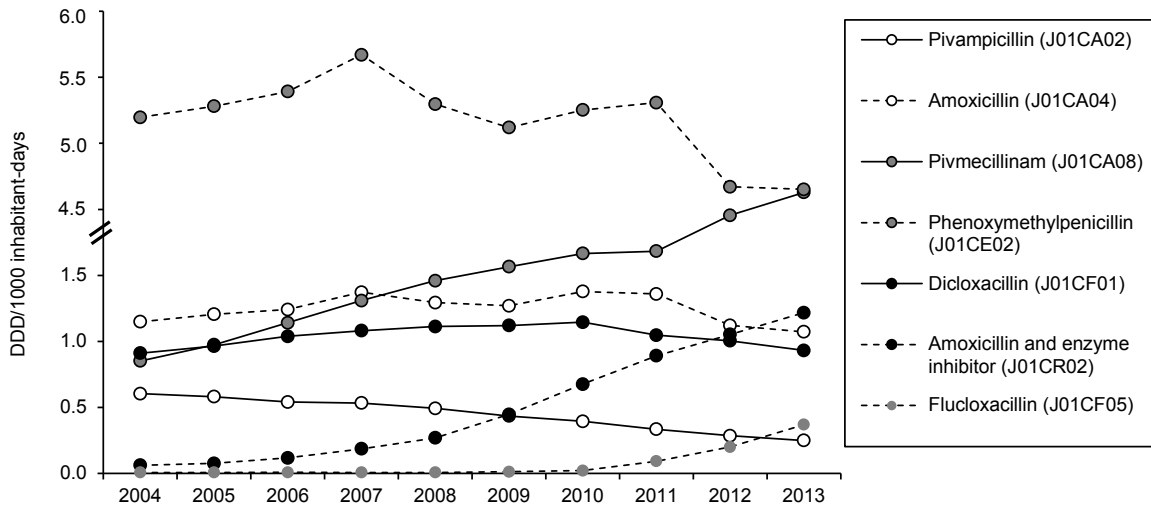
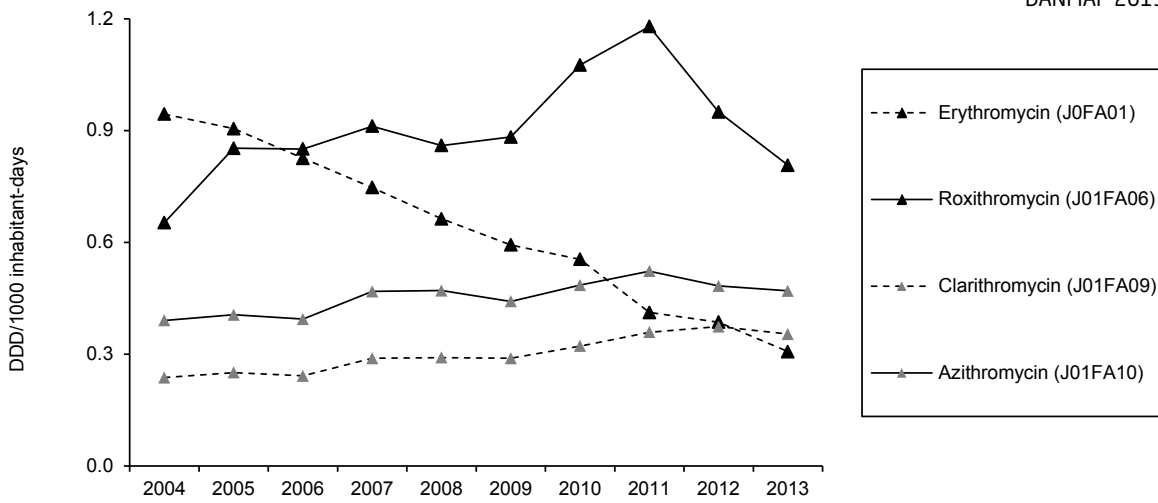


Figure 5.10. Consumption of macrolides in primary healthcare, Denmark

DANMAP 2013



Temporal and geographical variation in tetracycline prescription patterns in Denmark

Background: In Denmark, tetracyclines are used as treatment for acne vulgaris in young adults. In DANMAP 2011, it was shown that a large increase in the consumption of antimicrobial agents in primary healthcare was driven by 15–19 year olds with particular increases for tetracyclines. In this age group, tetracyclines are prescribed almost as often as penicillins and the level of consumption is steadily increasing [DANMAP 2012].

In the present study, Danish tetracycline prescriptions were investigated more closely with particular focus on the variation through time (2005–2013) and at the geographical level for different age groups.

Methods: Data on consumption of tetracyclines (DID, number of persons treated and number of prescriptions) from 2005 to 2013, the type (GP versus dermatologist) and geographical location of the prescriber were obtained from Data Delivery and Medicinal Product Statistics at Statens Serum Institut. Data were analysed by descriptive statistics and two-tailed t-tests using the STATA™ software 11.0 (Statacorp., Lakeway, TX, USA).

Results: From 2005 to 2013, the consumption (DID) of tetracyclines for all ages increased by 54% with large increases observed for 10–14 year olds (86%) and 15–19 year olds (58%). When adjusted for population increases, approximately 5.400 more persons in these age groups were treated with tetracyclines in 2013 than in 2005.

A large difference at the municipality level (kommuner) in tetracycline consumption was seen for 10 – 19 year olds; adolescents in six Danish municipalities had a consumption which was 170 to 200% higher than the national average (Figure 1) while the lowest consuming municipalities were 70 to 40% below average.

Discussion: The current results show that that the observed increases in tetracycline consumption are mainly driven by Danish adolescents. In the two age groups 10–14 and 15–19 years, an extra 5.400 persons were treated with tetracyclines from 2005 to 2013. We further show that the majority of prescriptions, presumably for acne [DANMAP 2012], are prescribed by GPs rather than dermatologists, highlighting the ease of access to GPs in comparison to dermatologists where a referral is needed.

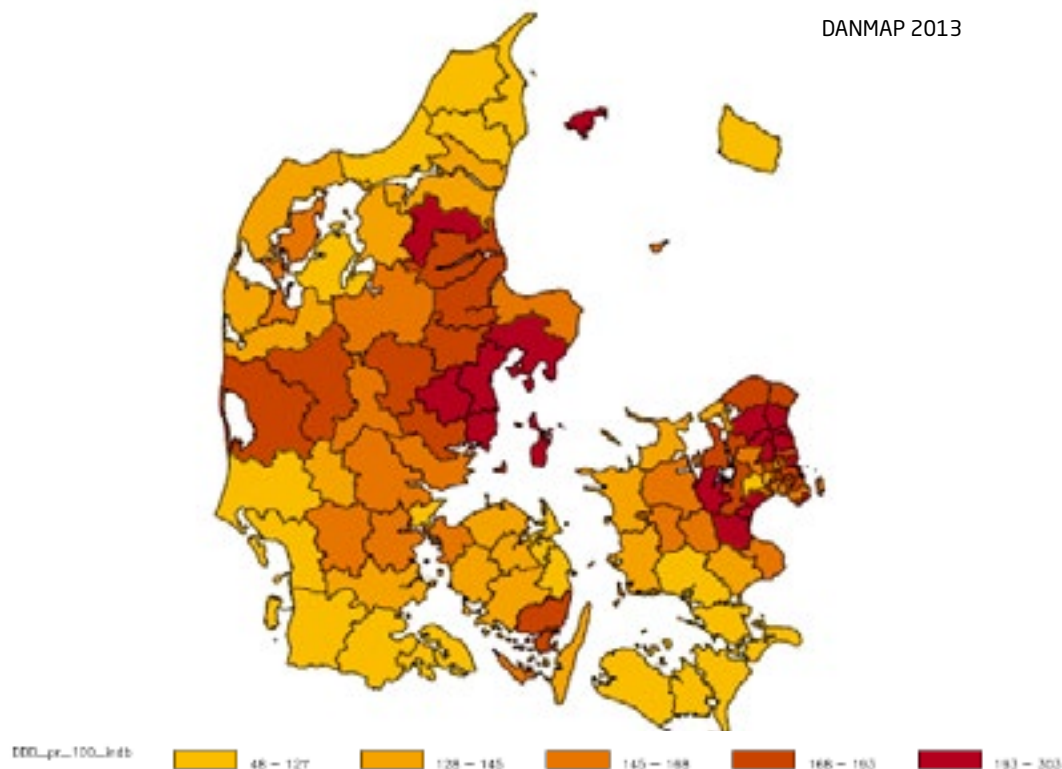
The geographical pattern of tetracycline consumption is highly varied with some municipalities showing a consistently high consumption in the adolescent age groups and other municipalities having a below-average consumption through time.

Resistance to broad-spectrum antimicrobial agents is an important problem in Denmark. There is currently a strong need to firstly investigate the extent of tetracycline resistance in bacteria from young adults and secondly to establish more firm treatment guidelines for adolescents presenting to a GP with acne problems.

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Figure 1. The consumption of (DDD/1000 inhabitants) of tetracyclines in 13-19 year olds in Danish municipalities



5.4 Hospital care

5.4.1 Introduction

Hospital care includes all hospitals (i.e. rehabilitation centres, hospices, private-, psychiatric-, specialized-, and somatic hospitals). Somatic hospitals account for approximately 97% of the antimicrobial consumption within hospital care. As specialized hospitals (psychiatric hospitals, hospices and rehabilitation centres) contribute a large proportion of bed-days and admissions but only a small proportion of antimicrobial consumption, the antimicrobial consumption for hospital care in Denmark is related only to bed-days and admissions in somatic hospitals.

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. Further, data are also presented as DID purely to enable comparison with primary healthcare.

During the past decade, the hospitalization pattern in Denmark has changed notably: more people are admitted to somatic hospitals but the average length of stay has been shortened (Figure 5.11, Table A5.4 in web annex) and outpatient treatment has increased significantly. Selection pressure for the emergence of antimicrobial resistance increases with increasing hospital activity (admissions) and thus the selection pressure has increased considerably from 2004 to 2013.

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

In 2013, the consumption of antimicrobial agents in somatic hospitals was 1.4% higher than in 2012 (Table 5.4). In the past decade the consumption has increased by 66%. This was caused by a combination of an increase in DDDs and a decrease in the number of hospital bed-days. This was particularly seen for broad spectrum agents which increased by 114%.

Extended spectrum penicillins represented the largest group of antimicrobial agents consumed and penicillins in general accounted for 51% of the total consumption (Figure 5.12). Cephalosporins (14%) and fluoroquinolones (10%) were also among the most commonly consumed antimicrobial agents in hospital care.

From 2012 to 2013, a higher consumption was observed for combinations of sulfonamide and trimethoprim (26%), 'combination penicillins' (14%), beta-lactamase resistant penicillins (8.4%), carbapenems (4.1%) and penicillins with extended spectrum (1.1%). A lower consumption was observed for 2nd generation cephalosporins (13%), macrolides (4.8%), tetracyclines (3.8%) and fluoroquinolones (2.5%) (Table 5.4).

The changes in leading groups of antimicrobial agents used in somatic hospitals during 2004–2013 are shown in Figure 5.13. Large increases were observed for 'combinations of penicillins' (1520%), carbapenems (370%) and fluoroquinolones (100%). The consumption of beta-lactamase sensitive penicillins, however, decreased 16% during the past decade.

The observed increases in 'combination penicillins' is most likely attributable to a change in empiric treatment from cephalosporins to piperacillin-tazobactam for severe infections and an increasing usage of amoxicillin-clavulanic acid for treatment of acute exacerbation of chronic obstructive pulmonary diseases (COPD).

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 antimicrobial agents 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 was 1.6% higher in 2013 compared to 2012 (Table 5.5). During the past decade, DAD increased by 19.3%; 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', combinations of sulfonamide and trimethoprim, beta-lactamase resistant penicillins and carbapenems. As observed in 2012, the consumption of 2nd generation cephalosporins and fluoroquinolones also decreased in 2013 by 13% and 2.2%, respectively (Table 5.5). The same trends were seen when consumption was expressed as DBD (Table 5.4).

DDD per 1,000 inhabitants per day (DID)

The consumption of antimicrobial agents in somatic hospitals was 1.5% higher in 2013 compared to 2012. In the past decade, the DID consumed in hospitals have increased by 31% (Table A5.5 in web annex). During the same time, broad-spectrum agents have increased by 70%, comprising 68% of the total consumption in 2013 compared to 52% in 2004 (Figure 5.14).

Katrin Gaardbo Kuhn and Maja Laursen

Figure 5.11. Number of bed-days and admissions in somatic hospitals, Denmark

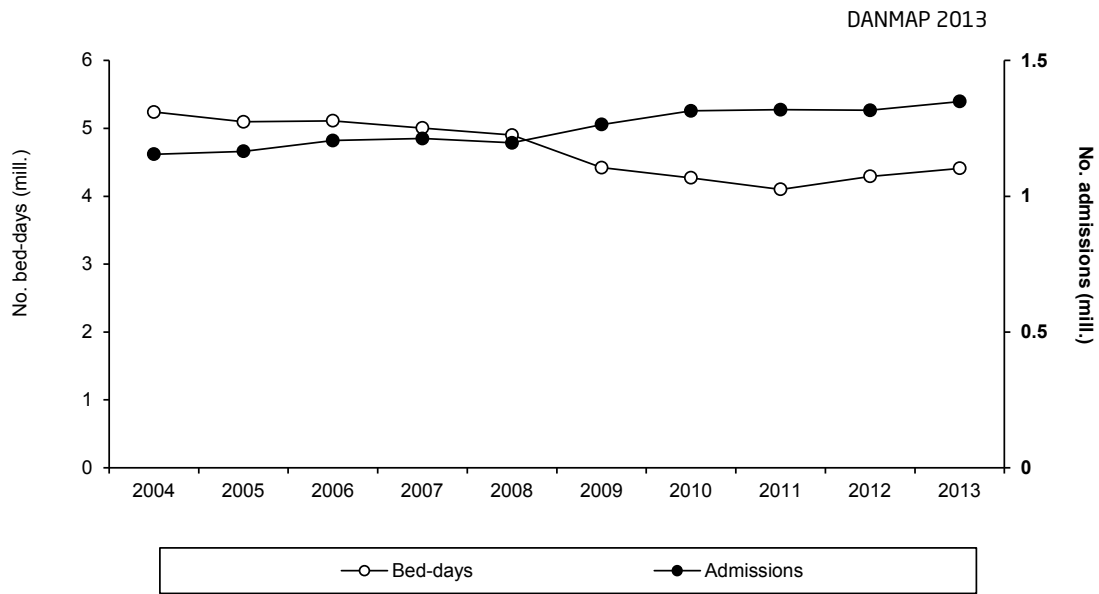
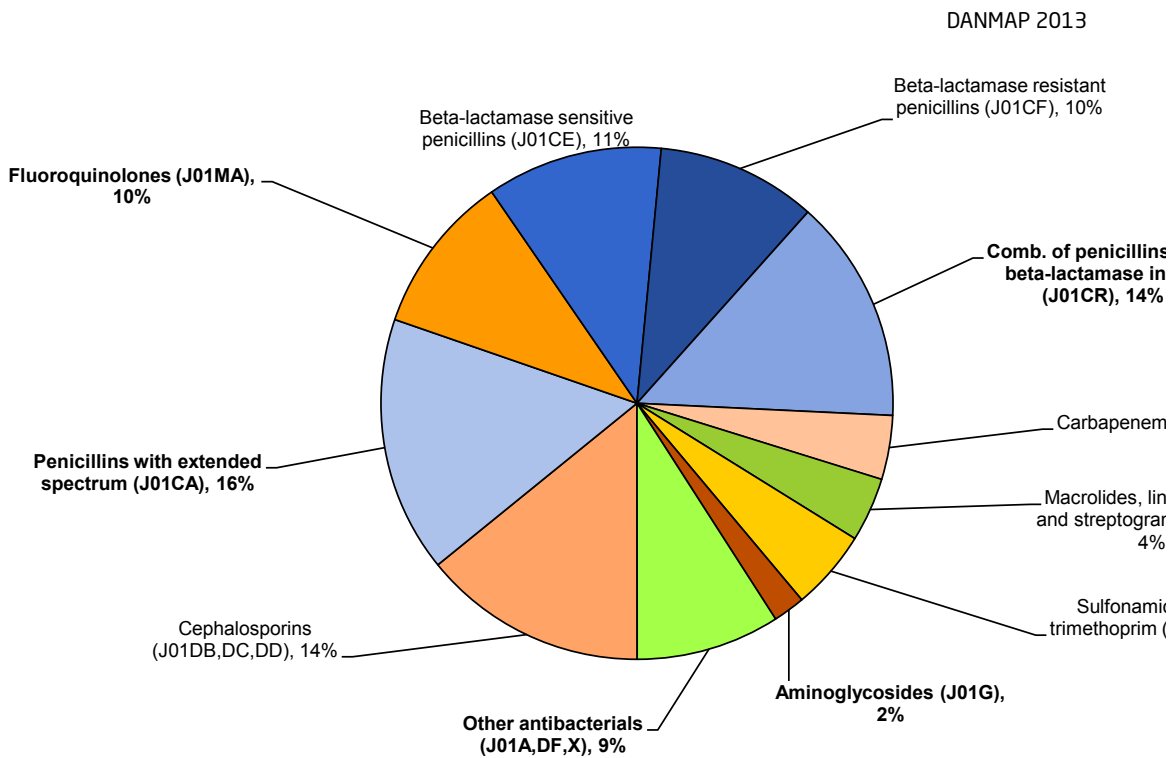


Figure 5.12. Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark



Note: Bold highlights indicate broad-spectrum antimicrobial agents

Figure 5.13. Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), Denmark

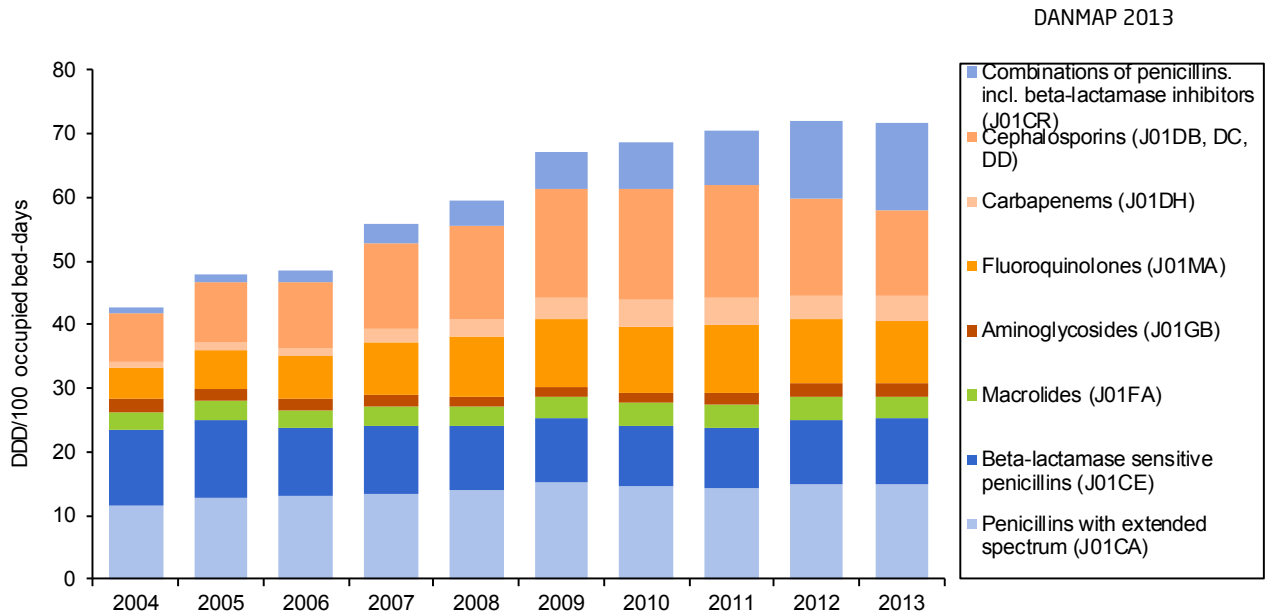
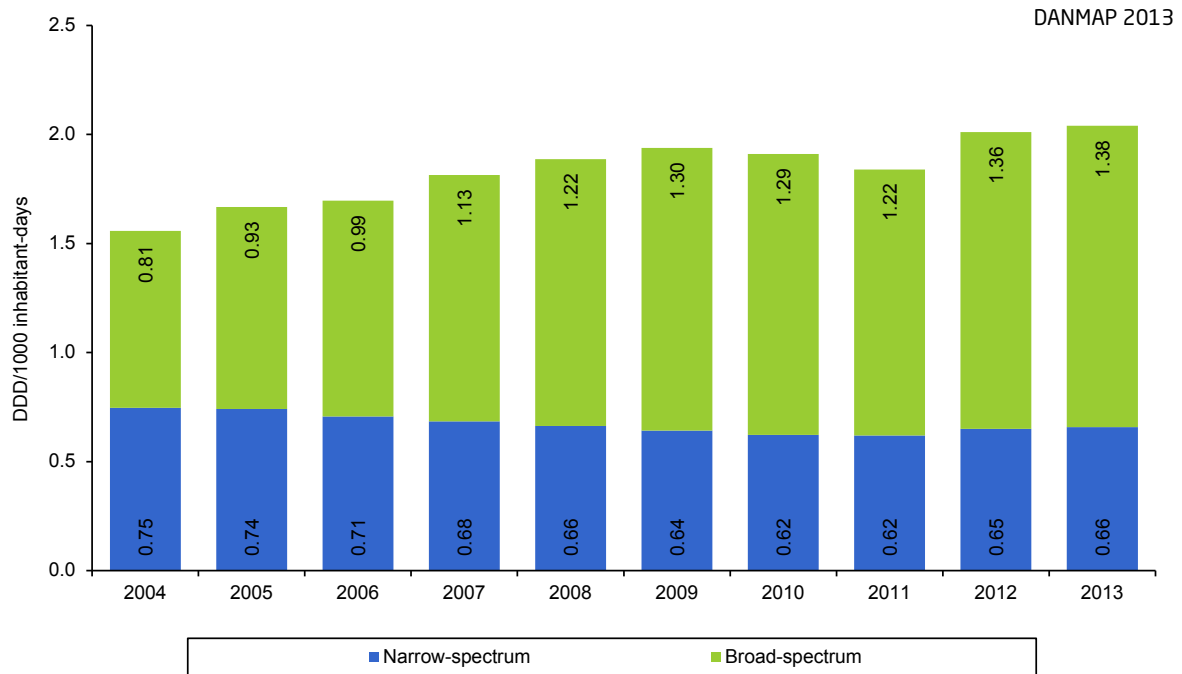


Figure 5.14. Consumption of antimicrobial agents (J01) in hospital care by narrow-spectrum and broad-spectrum agents, Denmark



Note: "Narrow-spectrum" antibiotics includes: beta-lactamase sensitive penicillins, first-generation cephalosporins, beta-lactamase resistant penicillins, monobactams, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran derivatives, and 'other antibiotics'
 "Broad-spectrum" includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, second-generation cephalosporins, third-generation cephalosporins, carbapenems, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones, and polymyxins

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

DANMAP 2013

ATC group ^(a)	Therapeutic group	Year									
		2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
J01AA	Tetracyclines	0.32	0.33	0.39	0.63	0.78	1.04	1.09	1.18	1.58	1.52
J01CA	Penicillins with extended spectrum	11.51	12.90	13.00	13.42	13.96	15.37	14.61	14.41	14.90	15.06
J01CE	Beta-lactamase sensitive penicillins	12.02	12.17	10.67	10.79	9.98	9.90	9.49	9.32	10.13	10.13
J01CF	Beta-lactamase resistant penicillins	6.78	6.71	6.51	6.70	6.81	7.40	7.71	7.30	8.37	9.07
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.84	1.16	1.83	2.95	4.00	5.65	7.13	8.51	12.00	13.64
J01DB	First-generation cephalosporins	0.17	0.15	0.14	0.13	0.18	0.13	0.13	0.13	0.12	0.11
J01DC	Second-generation cephalosporins	6.91	8.39	9.38	12.31	13.32	15.76	16.21	16.14	14.15	12.32
J01DD	Third-generation cephalosporins	0.67	0.83	0.83	1.03	1.25	1.42	1.26	1.39	1.07	1.08
J01DF	Monobactams	0.00	0.00	0.00	0.04	0.07	0.06	0.09	0.19	0.15	0.14
J01DH	Carbapenems	0.85	1.16	1.38	2.13	2.70	3.15	4.02	4.16	3.86	4.02
J01EA	Trimethoprim and derivatives	0.41	0.41	0.42	0.44	0.44	0.44	0.36	0.36	0.38	0.41
J01EB	Short-acting sulfonamides	1.06	0.99	0.75	0.34	0.35	0.35	0.33	0.25	0.20	0.20
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	1.86	2.11	2.12	1.52	1.95	2.28	3.04	4.11	3.33	4.21
J01FA	Macrolides	2.85	2.89	2.83	3.08	3.06	3.42	3.52	3.69	3.56	3.39
J01FF	Lincosamides	0.23	0.24	0.31	0.35	0.41	0.50	0.47	0.53	0.62	0.64
J01GB	Aminoglycosides	2.00	1.95	1.81	1.79	1.64	1.56	1.71	1.91	2.14	2.13
J01MA	Fluoroquinolones	4.93	6.14	6.74	8.16	9.53	10.71	10.44	10.70	10.02	9.77
J01XA	Glycopeptides	0.47	0.52	0.56	0.63	0.68	0.99	1.07	1.24	1.29	1.29
J01XB	Polymyxins	0.06	0.12	0.12	0.05	0.05	0.07	0.10	0.09	0.09	0.16
J01XC	Steroid antibacterials (fusidic acid)	0.22	0.25	0.28	0.28	0.26	0.31	0.34	0.27	0.23	0.22
J01XD	Imidazole derivatives	2.43	2.62	2.78	2.62	3.27	3.84	3.93	4.19	4.16	4.08
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.28	0.29	0.29	0.28	0.29	0.36	0.31	0.33	0.34	0.38
J01XX05	Methenamine	0.10	0.08	0.11	0.09	0.10	0.09	0.08	0.10	0.09	0.09
J01XX08	Linezolid	0.07	0.15	0.20	0.16	0.21	0.22	0.22	0.32	0.32	0.35
J01XX09	Daptomycin	0.00	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02
J01	Antibacterial agents for systemic use (total)	57.04	62.58	63.47	69.94	75.28	85.03	87.72	90.84	93.12	94.41

a) From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system

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

		DANMAP 2013									
ATC group ^(a)	Therapeutic group	Year									
		2004	2005	2006	2007	2008^(b)	2009	2010	2011	2012	2013
J01AA	Tetracyclines	1.45	1.45	1.67	2.59	3.19	3.63	3.55	3.66	5.15	4.97
J01CA	Penicillins with extended spectrum	52.22	56.43	55.13	55.39	57.18	53.76	47.46	44.77	48.60	47.90
J01CE	Beta-lactamase sensitive penicillins	54.53	53.20	45.26	44.55	40.90	34.61	30.83	28.98	33.04	33.13
J01CF	Beta-lactamase resistant penicillins	30.77	29.33	27.60	27.64	27.89	25.86	25.04	22.71	27.30	29.64
J01CR	Comb. of penicillins, incl. beta-lactamase inhibitors	3.82	5.09	7.77	12.17	16.37	19.74	23.15	26.47	39.14	44.60
J01DB	First-generation cephalosporins	0.76	0.67	0.60	0.55	0.72	0.46	0.43	0.41	0.40	0.37
J01DC	Second-generation cephalosporins	31.36	36.70	39.76	50.81	54.55	55.12	52.65	50.19	46.17	40.27
J01DD	Third-generation cephalosporins	3.06	3.62	3.53	4.24	5.10	4.98	4.10	4.33	3.50	3.53
J01DF	Monobactams	0.02	0.02	0.00	0.18	0.27	0.21	0.29	0.60	0.48	0.45
J01DH	Carbapenems	3.85	5.05	5.86	8.78	11.08	11.01	13.07	12.55	12.60	13.14
J01EA	Trimethoprim and derivatives	1.86	1.78	1.78	1.81	1.80	1.56	1.17	1.11	1.23	1.33
J01EB	Short-acting sulfonamides	4.82	4.32	3.18	1.41	1.43	1.21	1.09	0.78	0.63	0.62
J01EE	Comb. of sulfonamides and trimethoprim, incl. derivatives	8.44	9.21	8.98	6.28	7.98	7.96	9.88	12.79	10.87	13.76
J01FA	Macrolides	12.92	12.64	12.01	12.70	12.53	11.97	11.45	11.47	11.61	11.08
J01FF	Lincosamides	1.04	1.05	1.31	1.46	1.69	1.74	1.52	1.63	2.01	2.09
J01GB	Aminoglycosides	9.07	8.55	7.68	7.39	6.71	5.45	5.56	5.95	6.99	6.97
J01MA	Fluoroquinolones	22.38	26.87	28.58	33.66	39.04	37.45	33.92	33.30	32.67	31.96
J01XA	Glycopeptides	2.12	2.28	2.38	2.61	2.77	3.48	3.47	3.87	4.20	4.22
J01XB	Polymyxins	0.27	0.54	0.53	0.22	0.21	0.24	0.32	0.28	0.30	0.54
J01XC	Steroid antibacterials (fusidic acid)	1.01	1.11	1.19	1.17	1.05	1.09	1.12	0.85	0.76	0.71
J01XD	Imidazole derivatives	11.02	11.47	11.81	10.83	13.39	13.43	12.76	13.03	13.55	13.33
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	1.26	1.28	1.24	1.17	1.19	1.27	1.01	1.02	1.12	1.25
J01XX05	Methenamine	0.45	0.36	0.46	0.38	0.43	0.31	0.27	0.32	0.28	0.30
J01XX08	Linezolid	0.34	0.64	0.86	0.68	0.84	0.76	0.72	0.99	1.04	1.14
J01XX09	Daptomycin	0.00	0.00	0.00	0.03	0.06	0.06	0.07	0.05	0.06	0.07
J01	Antibacterial agents for systemic use (total)	258.81	273.67	269.18	288.70	308.39	297.36	284.89	282.53	303.71	308.70

a) From the 2013 edition of the Anatomical Therapeutic Chemical (ATC) classification system

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



6. Resistance in zoonotic bacteria

Zoonoses are 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 2013 [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.

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

Antimicrobial agent	DANMAP 2013	
	Pigs %	Pork Danish %
Tetracycline	47	44
Chloramphenicol	4	3
Florfenicol	3	2
Ampicillin	33	30
Cefotaxime	0	0
Ceftiofur	0	0
Trimethoprim	12	9
Sulfonamide	38	38
Streptomycin	41	41
Gentamicin	2	1
Neomycin	3	0
Apramycin	2	1
Ciprofloxacin	0	0
Nalidixic acid	0	0
Colistin	0	0
Spectinomycin	12	9
Fully sensitive	42	41
Multi-resistant	37	36
Number of isolates	512	148

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 9.3)

In Denmark and the rest of European Union, *S. Enteritidis* and *S. Typhimurium* are the serovars 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 2013 includes isolates from broiler, layer and cattle farms infected during 2013 as well as isolates from Danish and imported broiler meat, turkey meat, beef and pork collected as part of national surveillance and control programmes. From pigs, *Salmonella* isolates recovered from sow herds as well as multiplier and breeding herds as part of the *Salmonella* control programme are included. In addition, caecum samples from healthy pigs were collected at the slaughterhouses and cultured for *Salmonella*. Isolates from all reported human cases are included. Only one isolate per farm, meat sample or human case was included, and data are presented in the report where a sufficient number of isolates were obtained (>15) from a given source. For details on methodology see Chapter 9, Materials and Methods.

In DANMAP 2013, we primarily present resistance among *S. Typhimurium*. During the last ten years, the numbers of poultry flocks and meat infected or contaminated with *S. Enteritidis* has decreased, and therefore resistance in *S. Enteritidis* will not be presented in this report. The occurrence of resistance among *Salmonella* spp. from pigs and Danish pork is presented (Table 6.1).

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. Since 2012, routine analyses of *S. Typhimurium* phage types are no longer performed in Denmark.

MIC distributions for *S. Typhimurium* from pigs, pork and humans, as well as for *Salmonella* spp. from pigs and Danish pork in 2013 are presented in the web annex (Tables A6.1 - A6.5).

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

6.1.1 *Salmonella* spp. in pigs and domestic produced pork

In 2013, *S. Typhimurium* (including the monophasic variants) and *S. Derby* were the most common serotypes isolated from Danish pigs (44% and 43%, respectively) and pork (46% and 41%, respectively). In general, the serotype distribution in the Danish pork has been similar to the distribution among isolates from pigs.

Note that the isolates from pigs in Table 6.1 and 6.2 include isolates from pen faecal samplings from the national control programme as well as isolates from the sampling of healthy pigs

at slaughter. Thus the serotype distributions differ slightly from the ones presented for the slaughter pig herds in Textbox 5.

Among all the *Salmonella* isolates from Danish pigs (n = 512), we observed high levels (range: 33% - 47%) of resistance to ampicillin, streptomycin, sulfonamide, and tetracycline (Table 6.1). Co-resistance of these four antimicrobial agents is often called the ASSuT resistance-profile, also when resistant to additional antimicrobial agents. Resistance to tetracycline increased from 2012 to 2013, whereas resistance to the other tested antimicrobial agents was similar to levels reported in 2012 (Figure 6.1).

The occurrence of resistance in *Salmonella* spp. from Danish pork resembles the occurrences in the isolates from pigs ((Figure 6.1).

The majority of the *S. Derby* isolates were fully sensitive, and the overall occurrences of resistance among *Salmonella* spp. in pigs and Danish pork were lower than observed among the *S. Typhimurium* isolates only. Even though *S. Derby* is very common among pigs, only few human *S. Derby* cases (N=10) were reported in Denmark in 2013 [www.SSI.dk].

Overall, it was estimated that in 2013 multi-resistant *Salmonella* was present in 9% of the tested pigs (24% *Salmonella* positive pigs) and on 0.5% of the tested pig carcasses (1.3% *Salmonella* positive carcasses, Textbox 5 and Table 6.1).

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

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

S. Typhimurium isolated from pigs (n = 148) had very high levels of resistance to ampicillin (61%), streptomycin (66%), sulfonamide (66%), and tetracycline (70%, Table 6.2), and the occurrence of resistance for all four antimicrobial agents increased over the last five years (Figure 6.2). The occurrence of ASSuT resistance as well as multi-resistance in general was comparable with the levels in 2012; however ASSuT resistance among *S. Typhimurium* in pigs has been increasing since 2009, partly due to an increase in the prevalence of the monophasic variants of *S. Typhimurium* often carrying the ASSuT resistance-profile (Figure 6.3).

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.

In 2013, the monophasic *S. Typhimurium* variants constituted 52% of the total number of *S. Typhimurium* isolates from pigs, representing 71% of the multi-resistant isolates. This is comparable to the occurrence in 2012.

The prevalence of *S. Typhimurium* infected slaughter pig herds was 11.6% in 2013 [Annual report on Zoonoses in Denmark 2013], and as 64% of the *S. Typhimurium* isolates were multi-

resistant, the prevalence of pig herds with multi-resistant *S. Typhimurium* amounts to approximately 7% compared to 3% in 2011.

In Danish pork (n = 68), 53% of the isolates were of the monophasic variants and high levels (41%) of ASSuT resistance were observed. As in *S. Typhimurium* isolates from pigs, the occurrence of monophasic variants as well as multi-resistance remained at the same level as in 2012, but has generally increased since 2009, accompanied with a reduced occurrence of isolates fully sensitive to all the antimicrobial agents included in the test panel (Figure 6.3).

For the isolates from pigs and Danish pork, resistance to the other tested antimicrobial agents was similar to levels reported in 2012, except for an increase in resistance to trimethoprim among *S. Typhimurium* in pigs (from 6% to 13%). Levels of resistance were comparable among *S. Typhimurium* isolates from pigs and Danish pork (Table 6.2).

None of the *S. Typhimurium* isolates from Danish pigs and pork were resistant to cephalosporins (ceftiofur, cefotaxime), quinolones (ciprofloxacin, nalidixic acid) or colistin (Table 6.2). Only in 2010, a few DANMAP isolates from pigs were resistant to ciprofloxacin, resulting in a decreasing trend over the last five years (Figure 6.2). Among the ten EU Member States reporting resistance in *S. Typhimurium* from pork in 2012, most Member States reported no resistance to third generation cephalosporins (cefotaxime), whereas quinolone resistance was more common ranging up to 19% of the isolates [EU Summary report on AMR 2012].

It is also important to note that the occurrence of *S. Typhimurium* isolates fully sensitive to all included antimicrobial agents have been decreasing since 2009, this is the case for isolates both from pigs and Danish pork (Figure 6.3). Among the generic *S. Typhimurium* isolates from pigs, 41% of the isolates were fully sensitive isolates, a level comparable to previous years, whereas none of the monophasic isolates from pigs were fully sensitive in 2013.

Relatively few *S. Typhimurium* isolates from imported pork (n = 21) are available (Table 6.2), however higher levels of resistance to tetracycline, chloramphenicol, florfenicol and spectinomycin was observed compared to isolates from Danish pork.

6.1.3 *Salmonella* in humans

In 2013, *Salmonella* continued to be the second most frequent cause of bacterial intestinal infections in Denmark. A total of 1,136 human laboratory-confirmed cases of salmonellosis were reported (20.3 cases per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2013]. The most common serotype was *S. Enteritidis* and a total of 346 confirmed *S. Enteritidis* cases were reported (6.2 cases per 100,000 inhabitants), of which 79% were associated with travel outside Denmark especially in Turkey. This year, only *S. Typhimurium* isolates from humans were systematically susceptibility tested.

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

acquired” if the patients had not travelled within the last week prior to the onset of the disease. Patients were categorised as of ‘unknown origin’ if no telephone interview was conducted and travel information had not been reported to the general practitioners. In 2013, travel information was obtained for 68% of all reported *Salmonella* cases [Annual report on Zoonoses in Denmark 2013].

All human cases associated with a detected outbreak reported in the Annual Report on Zoonoses in Denmark in 2013 were considered ‘outbreak-related’ and all other domestic cases were considered ‘sporadic domestic’ in this report.

6.1.4 *Salmonella* Typhimurium in humans

S. Typhimurium was for the first time since 2008 only the second most common serotype among the human cases (337 cases), and isolates with valid results from susceptibility testing of all antimicrobial agents included in the test panel (n = 319) were included.

Among the reported human *S. Typhimurium* isolates included in DANMAP, 16% of the cases were categorised as travel-associated whereas 33% and 22% most likely had acquired their infection in Denmark as sporadic incidences or as part of detected outbreaks, respectively (Table 6.2). Among the cases where the origin of infection was unknown (29%), the occurrence of resistance falls within the ranges of both domestic cases and travel-associated cases.

There were reported several domestic foodborne outbreaks with *S. Typhimurium*, and compared with 2012 the proportion of outbreak related cases (n = 69) increased from 13% to 22%. In 2013, only 13% of isolates from the outbreak related cases was of the multi-resistant monophasic variants compared with 92% in 2012.

Among the *S. Typhimurium* isolates from domestic as well as travel-associated cases, we observed very high levels of resistance to ampicillin, streptomycin, sulfonamide, and tetracycline. High levels of ASSuT resistance occurred among isolates from sporadic domestic cases (43%, n = 106) as well as and travel-associated cases (47%, n = 51).

Occurrences of the resistance to the other antimicrobial agents were similar to most levels reported in 2012 for the domestic sporadic cases and similar for all antimicrobial agents for the travel-associated cases.

There were no significant changes in levels of resistance from 2012 to 2013 for either domestic sporadic or travel-associated cases. The increasing trend in resistance to ampicillin, streptomycin, sulfonamide and tetracycline, observed previous years discontinued, and for both domestic sporadic cases as well as among the travel associated cases (Figure 6.2). Among the domestic sporadic cases, fluoroquinolone (ciprofloxacin) resistance has decreased over the last five years. In general, the levels of resistance for the domestic cases were similar to those of Danish pork, except for the low occurrence of fluoroquinolone resistance, a resistance not observed among the isolates from Danish pork.

Table 6.2. Resistance (%) among *Salmonella* Typhimurium^(a) from pigs, Danish pork and human cases^(b), Denmark DANMAP 2013

Antimicrobial agent	Pigs	Pork		Human			Unknown origin %
	%	Danish %	Imported %	Domestic sporadic %	Domestic outbreak %	Travel abroad %	
Tetracycline	70	63	95	53	23	57	61
Chloramphenicol	6	6	33	8	0	20	15
Florfenicol	3	4	33	6	0	16	11
Ampicillin	61	59	81	56	30	55	63
Cefotaxime	0	0	0	1	0	4	2
Ceftiofur	0	0	0	1	0	2	2
Trimethoprim	13	9	14	8	7	8	8
Sulfonamide	66	72	81	54	36	59	66
Streptomycin	66	68	81	56	36	61	57
Gentamicin	3	1	0	2	0	6	2
Neomycin	4	0	5	1	0	4	3
Apramycin	3	1	0	1	0	2	1
Ciprofloxacin	0	0	0	3	1	20	8
Nalidixic acid	0	0	0	1	0	10	5
Colistin	0	0	0	7	6	0	5
Spectinomycin	12	12	43	10	4	16	18
Fully sensitive	20	19	0	28	58	27	27
Multi-resistant	64	71	76	54	36	59	63
Number of isolates	148	68	21	106	69	51	93

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel and multi-resistant if resistant to three or more of the ten antimicrobial classes (see Table 9.3)

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

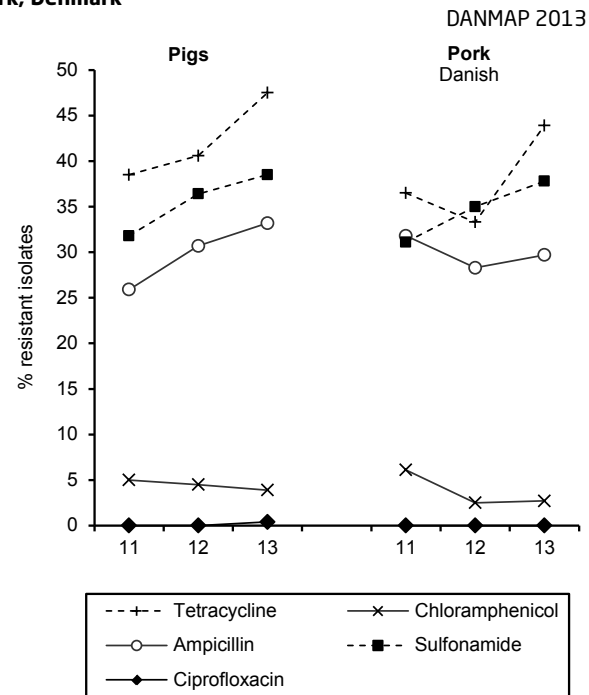
b) An isolate was categorised as ‘domestic sporadic’ if the patient did not travel outside Denmark one week prior to the onset of the disease and was not reported as being part of an outbreak

Since 2009, the proportion of multi-resistant isolates from sporadic domestic cases has increased (no significant change from 2012 to 2013), probably as a consequence of an increasing occurrence of multi-resistant monophasic variants of *S. Typhimurium* (Figure 6.3). In 2013, the monophasic variants represented 44% of all domestic sporadic isolates and 70% of all multi-resistant isolates. Also in line with this, the proportion of fully sensitive *S. Typhimurium* isolates of domestic sporadic origin (28% in 2013) has decreased over the last five years (no significant change from 2012 to 2013).

Among the *S. Typhimurium* from travel-associated cases, resistance to chloramphenicol, ciprofloxacin, florfenicol and nalidixic acid was more frequent than among isolates of domestic sporadic origin, whereas the level of resistance to the other agents included in the panel as well as the proportion of multi-resistant isolates were comparable.

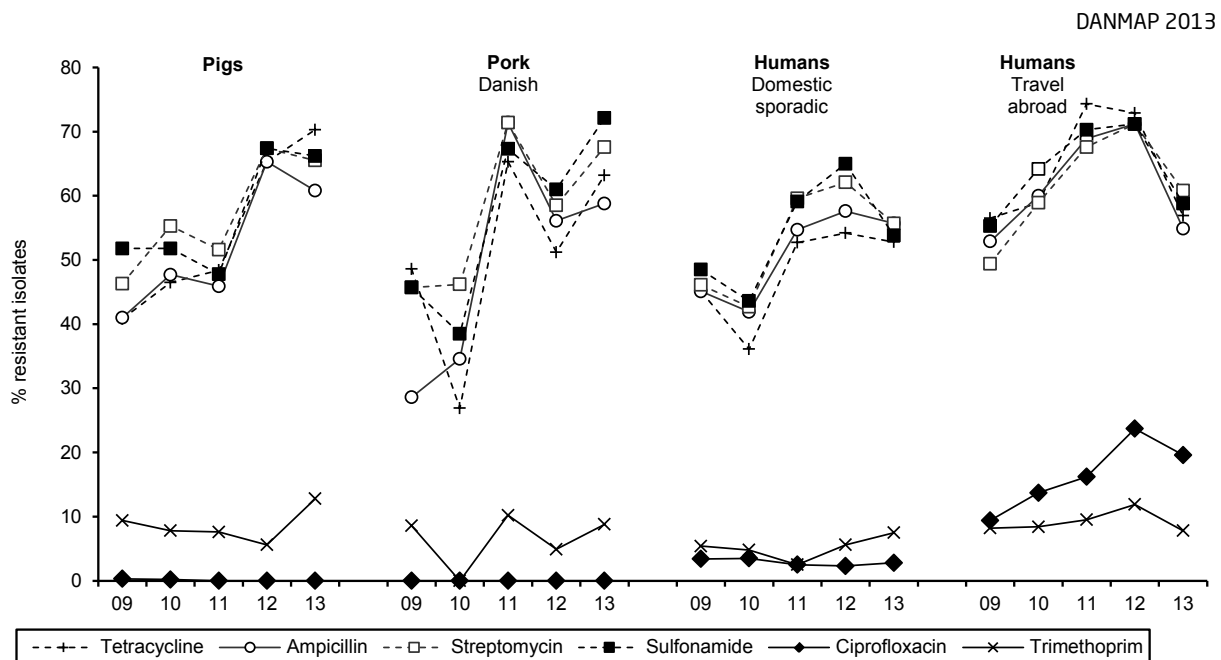
In most of the Member States reporting data on monophasic variants of *S. Typhimurium* from human to the European Food Safety Authority, the number of reported cases was higher in 2012 compared with 2011. Overall, the monophasic variants represented 7.2% of all human *Salmonella* cases in EU in 2012 [EU Summary Report on Zoonoses 2012, EFSA, 2013].

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



Note: The number of isolates varies between years (pigs: n = 340-512, Danish pork: n = 81-148). Before 2011, not all serotypes were susceptibility tested

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)

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

Regarding resistance to antimicrobial agents critical for treatment of human infections, resistance to 3rd generation cephalosporins (ceftiofur) was low and only found in isolates from one travel-associated case, one sporadic domestic case and two cases of unknown origin.

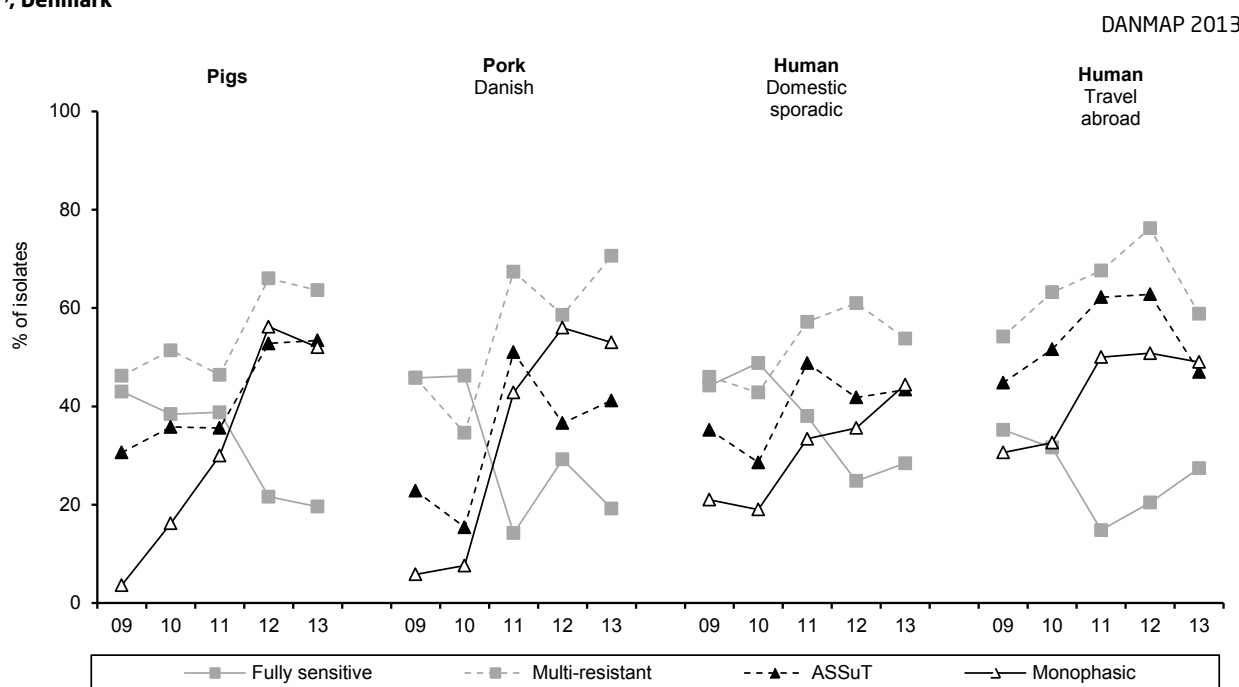
As also observed in previous years, a marked difference in fluoroquinolone (ciprofloxacin) resistance was found between domestically acquired infections (3%) and travel-associated infections (20%). The higher level of ciprofloxacin resistance in

the travel-associated *S. Typhimurium* infections may reflect a higher prescription of fluoroquinolones in production animals in the countries of destination. In Denmark, fluoroquinolones are rarely used in animal husbandry (except for pet animals) since the implementation of legal restrictions in 2002-2003 [DANMAP 2010].

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

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Figure 6.3. Occurrence (%) of multi-resistance^(a,b) and monophasic variants^(c) in *Salmonella Typhimurium* in pigs, pork and human cases^(d), Denmark



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

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

b) 'ASSuT' isolates are resistant to ampicillin, streptomycin, sulfonamide and tetracycline, but can include resistant to other antimicrobial agents such as chloramphenicol

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

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

Surveillance of *Salmonella* and *Campylobacter* in Denmark

Background: In Denmark, all flocks of laying hens, broilers and turkeys, including breeder flocks, are monitored for *Salmonella* according to the EU requirements and the Danish legislation. A *Salmonella* surveillance and control programme is also running in the Danish pig production, as well as a *S. Dublin* monitoring programme in cattle. Since January 2010, a mandatory surveillance of *Campylobacter* in broiler flocks at the farm has also been in place. *Salmonella* and *Campylobacter* in fresh meat are surveyed at the slaughterhouses and at the retail level, and finally, an intensified control of *Salmonella* and *Campylobacter* in fresh meat, based on a case-by-case risk assessment, has been in place for Danish and imported meat ready for retail since 2007. Human salmonellosis and campylobacteriosis are notifiable illnesses in Denmark, and all cases are reported to SSI and recorded in a national database. More information regarding trends and sources of zoonotic infections in humans and animals in 2013 is available in the Annual Report on Zoonoses in Denmark at www.food.dtu.dk.

Salmonella: In layer and broiler flocks, the *Salmonella* prevalence has been low for more than a decade and only 1.1% and 1.0%, respectively, of flocks were positive in 2013. From 2008, all Danish flocks that test positive at the farm are heat treated at slaughter, and as it was in 2011 and 2012, none of the slaughter batches tested at slaughter were *Salmonella* positive in 2013.

The prevalence of *Salmonella* in cattle is low, and in 2013 only 0.3% of the cattle carcasses tested at slaughter were positive. Furthermore, 95.5% of the non-milk producing herds and 92.1% of the milk-producing herds were classified as “probably *S. Dublin* free”. Among pigs sampled prior to slaughter, 24.2% were found *Salmonella* positive, while 1.3% of the pig carcasses tested at slaughter were positive. As in previous years, the most common serotype in pigs and pork in 2013 was *S. Derby* (60% and 39%, respectively) followed by *S. Typhimurium* (30% and 44%, respectively) when including the monophasic variants with antigenic formulas *S. 4,[5],12:i-* (Figure 1).

Only 1,136 cases of human salmonellosis were reported in 2013 with an incidence on 20.3 cases per 100,000 inhabitants. This is the lowest number for several decades and a result of the intensive *Salmonella* control programmes in animal production. In total, six domestic *Salmonella* outbreaks were reported, and five of these were caused by *S. Typhimurium* of which two of the outbreaks were caused by Danish pork.

As in previous years, the *Salmonella* source account estimated that approximately half of the human cases of salmonellosis were acquired during international travel. Similar to previous years, more than 75% of the *S. Enteritidis* cases were acquired abroad whereas the majority of the *S. Typhimurium* cases was acquired in Denmark. The model could not attribute around a quarter of the humane cases to the included food sources, and as previous years Danish pork was estimated to be the most important domestic food source, followed by different types of imported meat (Figure 2).

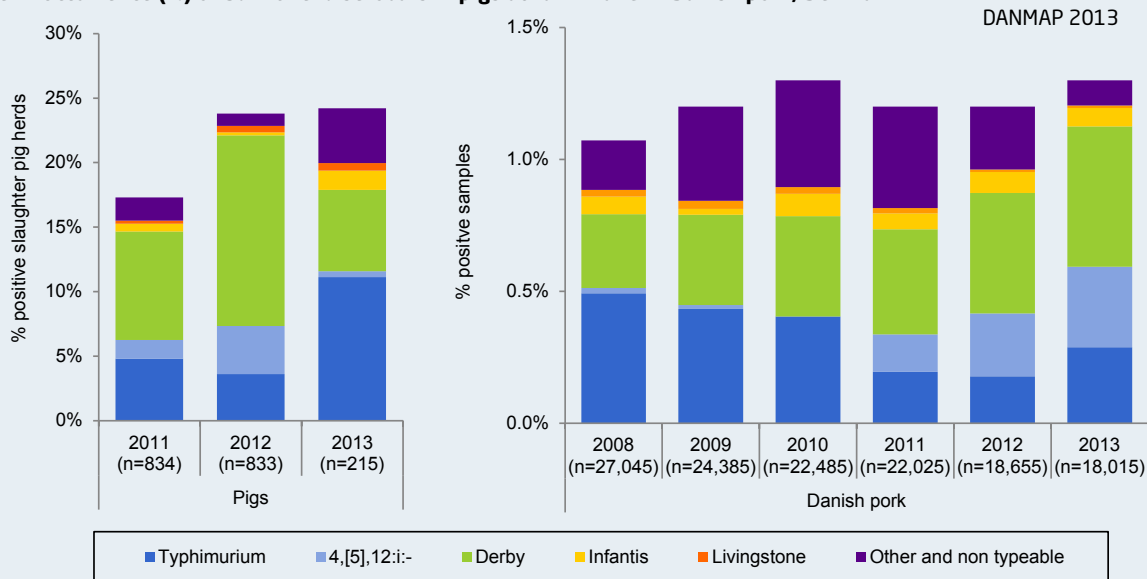
Campylobacter: The proportion of *Campylobacter* positive broiler flocks has decreased since 2010, however in 2013 the decrease leveled out and 13.1% of all broiler flocks tested *Campylobacter* positive. At retail, *Campylobacter* was detected in 17.8% of the chilled broiler meat of Danish origin, compared with 9.7% in 2012 (Figure 3). The most common *Campylobacter* species in broilers are *C. jejuni* (91.7% in 2013). Broilers were also tested at slaughter, where 28.2% of the conventional and 90.3% of the organic-free-range birds were positive. *Campylobacter* is also found in cattle and pigs, where *C. jejuni* is dominant in the cattle and *C. coli* is dominant in pigs.

Campylobacter is the most frequently reported foodborne pathogen in Denmark; however, the number of human campylobacteriosis cases in 2013 (67.1 cases per 100,000 inhabitants) at a level comparable to 2012. Since 2007, approximately one-third of the cases have been related to international travel. Consumption and handling of broiler meat is assumed to be the most important source of human campylobacteriosis (estimated more than 50% of domestic sporadic cases), however other sources such as contaminated water, vegetables and direct contact to farm animals cattle exist.

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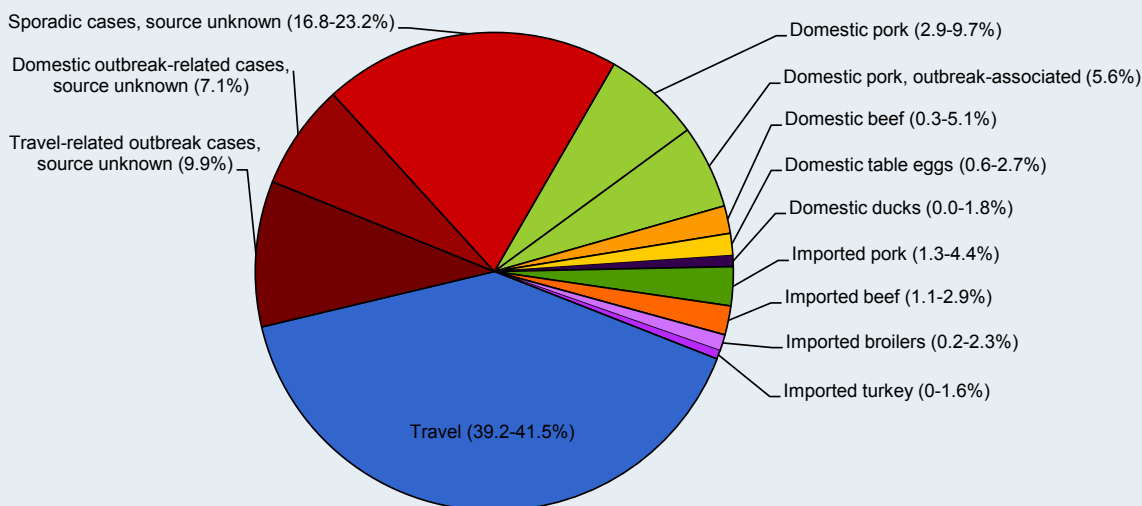
Figure 1. Occurrence (%) of *Salmonella* serovars in pigs at farm^(a) and in Danish pork, Denmark



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

Figure 2. Estimated sources of^(a) 1,136 cases of human salmonellosis, Denmark 2013

DANMAP 2013

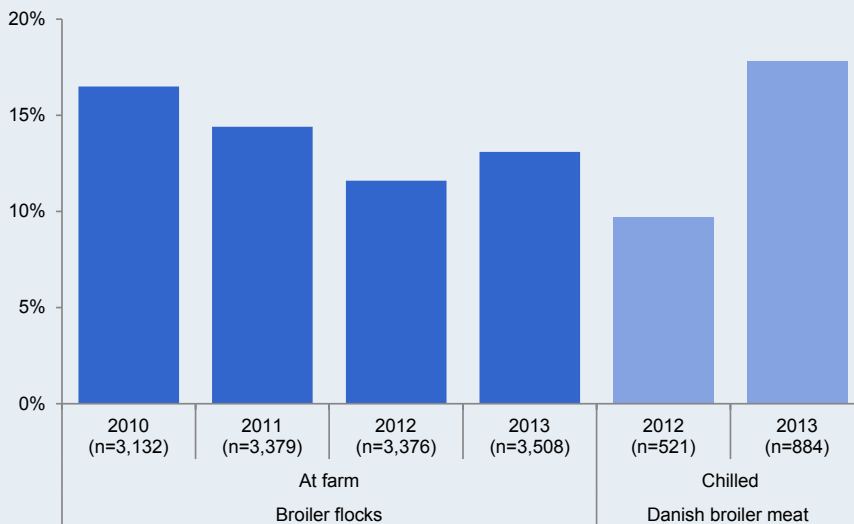


Source: Danish Zoonosis Centre, National Food Institute

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

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

DANMAP 2013



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

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

6.2 *Campylobacter*

Thermotolerant *Campylobacter* are the most commonly reported causes of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union [ECDC, 2014]. 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 2013 includes randomly collected isolates from broilers and cattle at slaughter and from fresh broiler meat sold at wholesale and retail outlets. Isolates from human cases originates from three out of five geographical regions in Denmark. One isolate per farm, meat sample or human case is included, and data are only presented if a sufficient number of isolates were obtained (>15) 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, as well as for *C. coli* from pigs and broiler meat in 2013 are presented in the web annex (Tables A6.6-A6.9). An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the six included antimicrobial classes (see Table 10.3).

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

In 2013, we observed moderate levels of resistance to fluoroquinolones (ciprofloxacin) and tetracycline (26% and 20%, respectively) among *C. jejuni* isolates from broilers (n = 54, Table 6.3) compared with 2012 (15% for both compounds).

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. The consumption of tetracycline increased considerably from 2008 to 2010, decreased from 2011 to 2012, but increased again in 2013. For *C. jejuni* isolates from broilers, the resistance and consumption patterns for tetracycline appear to follow each other (Figure 6.4). We did not observe a similar agreement between the consumption and resistance patterns for fluoroquinolones (ciprofloxacin) in *C. jejuni* broiler isolates. Even though fluoroquinolones have not been used in the broiler production since 2009, the resistance level has increased to a moderate level (26%) during the last five years (Figure 6.4).

In *C. jejuni* isolates from Danish broiler meat (n = 70), we observed levels of resistance to ciprofloxacin (20%) and tetracycline (10%) that were slightly lower than in 2012. The resistance to ciprofloxacin and tetracycline has fluctuated over the last five years, however the ciprofloxacin resistance level observed in 2012 was the highest since 2007.

The levels of antimicrobial resistance were comparable between *C. jejuni* isolated from Danish broilers and Danish broiler meat (Figure 6.4), and macrolide (erythromycin) resistance has remained at a very low level for a decade.

6.2.2 *C. jejuni* from imported meat

In *C. jejuni* isolates from imported broiler meat (n = 30), the levels of resistance to tetracycline (80%) and quinolones (nalidixic acid and ciprofloxacin, 53% and 57%, respectively) remained high (Table 6.3). From 2009 to 2013 there has been an overall decrease in the proportion of fully sensitive *C. jejuni* isolates among isolates from imported broiler meat (from 32% to 13%).

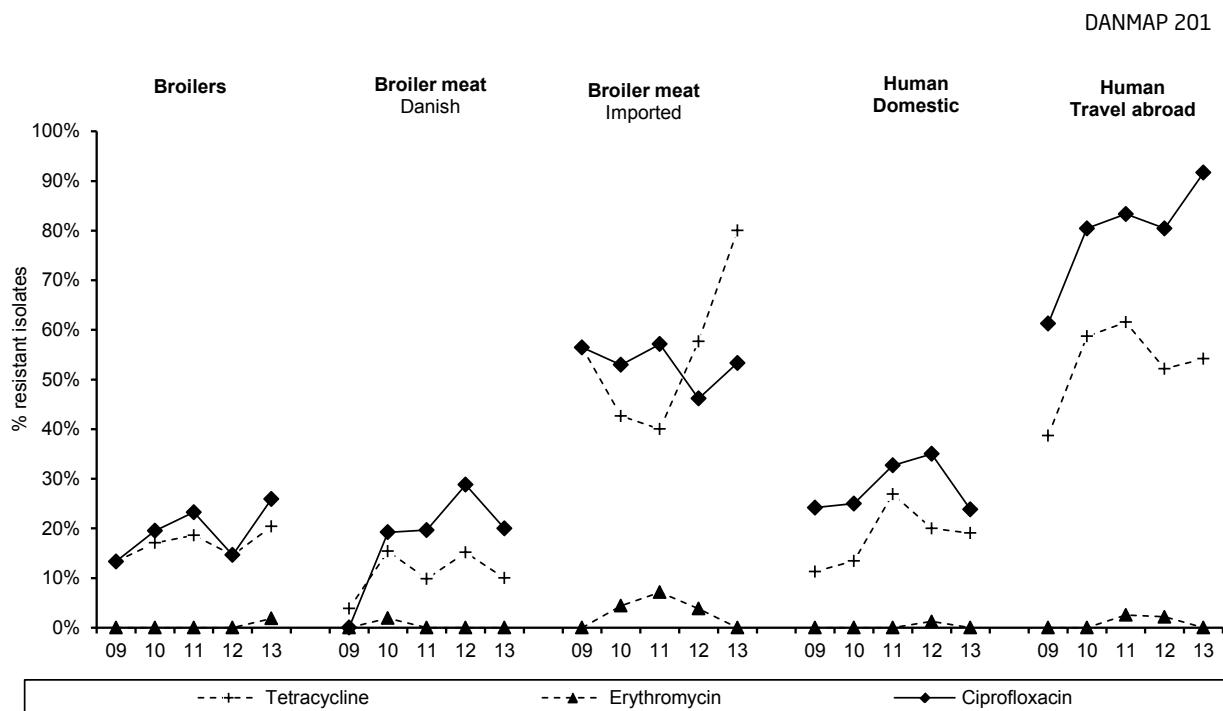
Also, over the past five years, the level of resistance to tetracycline and ciprofloxacin has generally been higher in isolates from imported broiler meat compared with Danish broiler meat. This corresponds with the data reported by EFSA, where Denmark has reported the lowest proportions of resistance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

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

Antimicrobial agent	DANMAP 2013							
	Cattle		Broilers		Broiler meat		Humans	
	Danish %	Danish %	Danish %	Import %	Domestically acquired %	Travel abroad %		
Tetracycline	3	20	10	80	19	54		
Chloramphenicol	0	0	0	0	0	0		
Erythromycin	0	2	0	0	0	0		
Streptomycin	0	4	3	0	0	0		
Gentamicin	0	0	0	0	0	4		
Ciprofloxacin	21	26	20	53	24	92		
Nalidixic acid	22	26	20	57	24	92		
Fully sensitive	76	67	76	13	67	8		
Multi-resistant	0	2	0	0	0	4		
Number of isolates	86	54	70	30	42	24		

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

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

Figure 6.4. Resistance (%) in *Campylobacter jejuni* from broilers, broiler meat and human cases^{a)}, Denmark

Note: The number of isolates varies between years (broilers: n = 41–75, Danish broiler meat: n = 26–70, imported broiler meat: n = 26–70, domestic sporadic 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

6.2.3 *C. jejuni* in cattle

In *C. jejuni* isolates from cattle (n= 86), we observed moderate levels (21%) of resistance to fluoroquinolones (ciprofloxacin) and low levels (3%) of resistance to tetracycline (Table 6.3).

Resistance to fluoroquinolones (ciprofloxacin) among *C. jejuni* from cattle has remained at a moderate level (16%-21%) since 2006 (Figure 6.5). As described in previous DANMAP reports, we observed an increase in the level of fluoroquinolone resistance in 2005 despite low consumption of fluoroquinolones in cattle. In 2012, only one of the fluoroquinolone resistant isolates was also resistant to tetracycline, indicating that co-selection by tetracycline (one of the major drugs for treatment of calves) was not the explanation for the observed levels of fluoroquinolone resistance. It has been discussed [DANMAP 2007] that clonal spread, particularly between farms, could be an explanation for the observed resistance to fluoroquinolones. Initially, fluoroquinolone-resistant *C. jejuni* isolates came from cattle farms in Southern Jutland, but as in recent years, isolates were obtained from farms distributed throughout Jutland and other parts of Denmark.

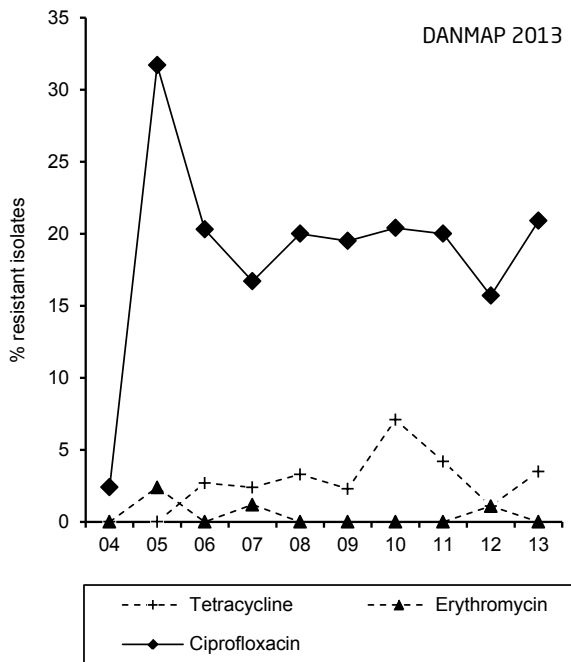
From 2004 to 2010, we observed a general increase in the resistance to tetracycline. However, this trend was discontinued in 2011, and in 2013 the level of tetracycline resistance is comparable to 2006-2009 (3%, Figure 6.4).

6.2.4 *C. jejuni* in humans

In 2013, *Campylobacter* continued to be the most frequent cause of bacterial intestinal infections in Denmark. A total of 3,766 human laboratory confirmed cases of campylobacteriosis were reported (67.1 per 100,000 inhabitants) [Annual report on Zoonoses in Denmark 2013].

A subset (n = 66) of the *C. jejuni* isolates submitted to SSI were selected for susceptibility testing continuously over the year. The isolates were randomly selected from all of the *Campylobacter* isolated from stool samples in the three geographical regions included in the surveillance. Among the tested isolates, 37% were from travel-associated cases and 63% were considered to be domestically acquired. As in previous years, SSI collected information on travel history through phone interviews. 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 6.5. Resistance (%) in *Campylobacter jejuni* from cattle, Denmark



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

Among the domestically acquired infections, 67% were fully sensitive to all the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower (8%) among isolates from travel associated cases (Table 6.3). The level of multi-resistance was low (0%-4%) among both groups, but among the travel-associated cases with resistant *C. jejuni*, all (22/22) were resistant to fluoroquinolones (ciprofloxacin), compared with 71% (10/14) among isolates from the domestically acquired infections.

The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel-associated *C. jejuni* isolates (92% and 54%, respectively) compared to isolates from domestically acquired infections (24% and 19%, respectively, Table 6.3 and Figure 6.4).

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

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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 for several reasons: they are ubiquitous and present as major commensals in both the animal and human reservoirs, they can acquire antimicrobial resistance as response to selective pressures and finally they have the potential for transferring resistance to pathogenic bacteria and between reservoirs and can cause infection in humans.

7.1 Enterococci

For Enterococci, DANMAP 2013 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 were included. We included only one isolate per farm or meat sample, and data are presented in the report where a sufficient number of isolates were obtained (>15) from a given source. For details on methodology, see Chapter 9, Materials and Methods.

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multi-resistant if resistant to three or more of the ten included antimicrobial classes (see Table 9.3).

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

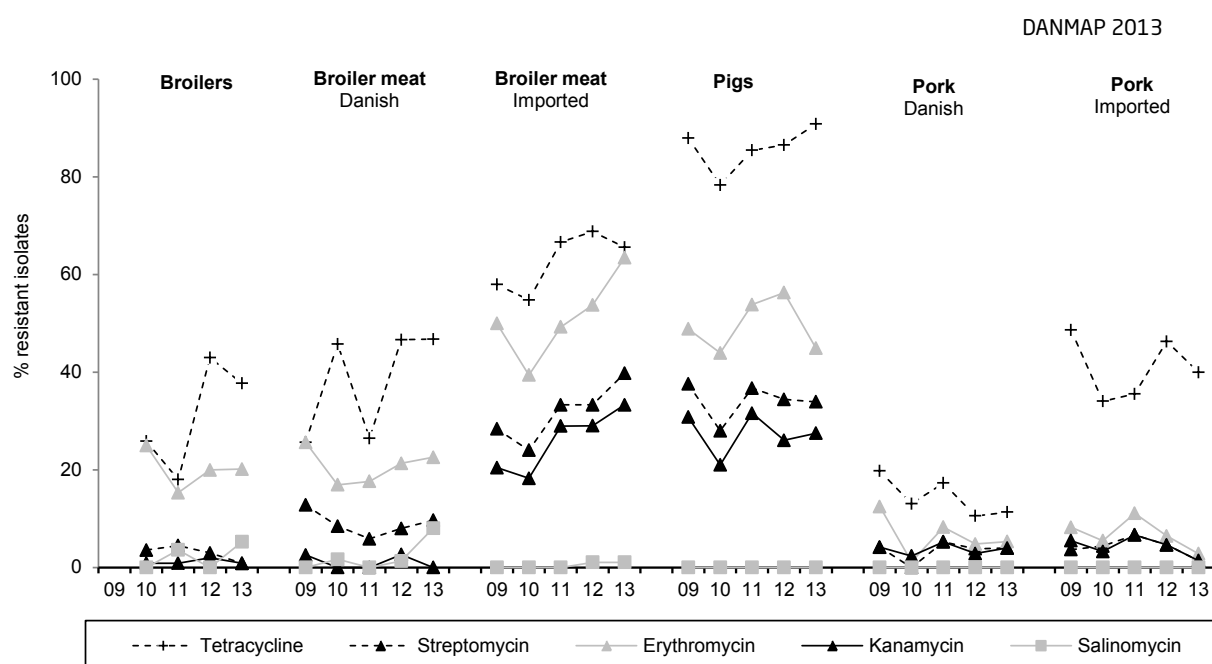
In *E. faecalis* isolates from broilers (n = 114), 38% of isolates were tetracycline resistant followed by erythromycin (20%) and salinomycin (5%). Antimicrobial resistance to salinomycin increased from 0% in 2012 to 5% in 2013, a level comparable to the occurrence in 2011(4%). Only four isolates (4%) were multi-resistant while 52% were susceptible to all tested antimicrobials (Table 7.1), a level comparable to 2012. All four multi-resistant isolates were resistant to tetracycline and erythromycin.

The levels of resistance in *E. faecalis* isolates from broiler meat were comparable to *E. faecalis* isolates from broilers (Figure 7.1) except for streptomycin where significantly higher prevalence (10%) was found in broiler meat. Only three isolates (5%) were multi-resistant and all of these were resistant to tetracycline. Prevalence of multi-resistance has been lowered from 13% in 2009 to 5% in 2013, while resistance to erythromycin has increased from 17% in 2010 to 23% in 2013.

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

Very high occurrence of resistance to tetracycline (91%) and occurrence of resistance to erythromycin (45%), streptomycin (34%) and kanamycin (28%) was found in *E. faecalis* isolates from pigs (n = 109). Resistance to fluoroquinolones

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



Note: The number of isolates varies between years (broilers: n = 100–112, 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)

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

DANMAP 2013

Antimicrobial agent	Broilers	Broiler meat		Beef		Pigs	Pork	
	Danish	Danish	Imported	Danish	Imported	Danish	Danish	Imported
	%	%	%	%	%	%	%	%
Tetracycline	38	47	66	29	25	91	11	40
Tigecycline	0	0	0	0	0	0	0	0
Chloramphenicol	1	2	3	8	2	17	3	1
Ampicillin	0	0	0	0	0	0	0	0
Penicillin	0	0	0	0	0	0	0	0
Erythromycin	20	23	63	12	0	45	5	3
Streptomycin	1	10	40	12	6	34	4	1
Gentamicin	0	0	0	0	0	16	3	1
Kanamycin	1	0	33	8	0	28	4	1
Ciprofloxacin	0	0	6	4	0	1	1	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
Salinomycin	5	8	1	0	0	0	0	0
Fully sensitive	52	42	23	71	75	9	88	59
Multi-resistant	4	5	39	13	2	34	5	2
Number of isolates	114	62	93	24	51	109	150	140

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

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

DANMAP 2013

Antimicrobial agent	Broiler meat		Pork	
	Danish	Imported	Danish	Imported
	%	%	%	%
Tetracycline	9	42	0	19
Tigecycline	0	0	0	0
Chloramphenicol	0	0	0	0
Ampicillin	2	22	0	0
Penicillin	2	22	0	0
Erythromycin	8	53	0	3
Quinupristin/dalfopristin	3	11	0	0
Streptomycin	3	31	0	0
Gentamicin	0	2	0	0
Kanamycin	0	17	0	0
Ciprofloxacin	0	2	0	0
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Linezolid	0	2	0	0
Salinomycin	64	36	5	0
Fully sensitive	26	23	95	77
Multi-resistant	5	34	0	0
Number of isolates	66	64	22	31

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

(ciprofloxacin) was observed in one isolate (1%) from pigs (Table 7.1). The levels of resistance were comparable with 2012. Tetracyclines and macrolides are the most commonly used antimicrobial agents in the Danish pig production, whereas the use of fluoroquinolones cephalosporins in pigs was very low.

All the chloramphenicol (n = 19), kanamycin (n = 30) and gentamicin (n = 17) resistant isolates from pigs were resistant to both tetracycline and erythromycin, while all erythromycin resistant isolates (n = 49) were resistant to tetracycline. Overall, 34% of the isolates were multi-resistant (Table 7.1). Nine percent of the tested isolates were fully sensitive to all the antimicrobial agents tested.

Since 2010 the occurrence of macrolide (erythromycin) resistance among *E. faecalis* isolated from pigs has increased from 44% to 56%, but from 2012 to 2013 a decline to 45% was observed (Figure 7.1). Prevalence of multi-resistant *E. faecalis* (34%) from pigs was comparable to 2012, but lower than in 2011 (40%).

Most of the *E. faecalis* isolates from Danish pork (n = 150) were fully sensitive to all of the antimicrobial agents tested (88%, Table 7.1). The highest prevalence of resistance was seen for tetracycline (11%) followed by erythromycin (5%), and the aminoglycosides streptomycin (4%) and kanamycin (4%). Resistance to fluoroquinolones (ciprofloxacin) was observed in one isolate (1%) from Danish pork. Resistance to tetracycline has been declining from 20% in 2009 to 11% in 2013, a trend that is not observed in the isolates from pigs where tetracycline resistance has been 80%-90% over the last ten years.

7.1.3 *E. faecalis* from domestic produced beef

In *E. faecalis* from beef (n = 24), 29% of the isolates were resistant to tetracycline, 12% resistant to erythromycin and streptomycin and 8% resistant to chloramphenicol and kanamycin (Table 7.1). Levels of antimicrobial resistances were comparable with 2012.

7.1.4 *E. faecalis* from imported meat

Compared with Danish broiler meat, *E. faecalis* from imported broiler meat (n = 93) had higher levels of resistance to tetracycline, erythromycin, kanamycin and streptomycin and were more often multi-resistant (Figure 7.1). Overall, 39% of the *E. faecalis* from imported broiler meat were multi-resistant (all tetracycline resistance) compared with 5% among isolates from Danish broiler meat. Among the multi-resistant isolates from imported broiler meat 72% (26 of 36 isolates) had the same resistance profile (erythromycin, kanamycin, streptomycin and tetracycline) coming from multiple countries in Europe. For salinomycin, occurrence of resistance was higher in Danish broiler meat compared with imported broiler meat (Table 7.1). Compared with Danish pork, *E. faecalis* isolates from imported pork (n = 140) were more frequently resistant to tetracycline, whereas resistance levels to all other antimicrobial agents were comparable. Fifty-nine percent of the *E. faecalis* isolates from imported meat were fully sensitive to all of the antimicrobial agents tested compared to 88% in isolates from Danish pork. All resistant isolates from imported pork were at least resistant to tetracycline. Only 2% were multi-resistant compared with 5% in isolates from Danish pork (Table 7.1).

The levels of resistance in *E. faecalis* isolates in Danish (n = 24) and imported beef (n = 51) were comparable, except for erythromycin and kanamycin where Danish produced beef

were more frequently resistant (Table 7.1). Only resistance to tetracycline (25%), streptomycin (6%) and chloramphenicol (2%) was detected in imported beef. Only one isolate (2%) of the tested isolates from imported beef were multi-resistant, compared to 13% among isolates from Danish beef. Overall 75% of the tested isolates from imported beef were fully susceptible to all tested antimicrobials compared to 71% in Danish produced beef.

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

High and increased occurrence of salinomycin resistance (64%) was observed in *E. faecium* isolates from Danish broiler meat (n = 66, Table 7.2) followed by resistance to tetracycline (9%) and erythromycin (8%). Only three isolates (5%) from the Danish broilers were multi-resistant, and most of the resistant isolates (n = 40) were only resistant to salinomycin (78%). Occurrences of the resistance and multi-resistance were similar to levels reported in 2012. However since 2010, resistance to erythromycin and tetracycline has decreased whereas resistance to salinomycin has increased (from 38% in 2009 to 64% in 2013, Figure 7.2).

In *E. faecium* isolates (n = 22) recovered from Danish pork only one isolate was resistant (to salinomycin only, Table 7.2). Thus, occurrences of resistance were lower than reported in 2012 but no significant differences were detected.

As in 2012, none of the isolates from domestic broiler meat and pork were resistant to fluoroquinolone (ciprofloxacin) or glycopeptides (vancomycin) in 2013. Two isolates from Danish broiler meat and none from pork were resistant to virginiamycin (quinupristin/dalfopristin, Table 7.2).

7.1.5 *E. faecium* from imported meat

As in 2012, resistance to tetracycline, ampicillin, penicillin, erythromycin, quinupristin/dalfopristin, streptomycin and kanamycin, as well as multi-resistance was significantly more frequent in *E. faecium* from imported broiler meat (n = 64) compared with isolates from Danish broiler meat (n = 66). Resistance to salinomycin was higher in Danish broiler meat compared with imported (Table 7.2). Overall, 34% of the *E. faecium* isolates from imported broiler meat were multi-resistant, and among the resistant isolates (n = 41), 30% of the isolates were resistant to both erythromycin and tetracycline. In imported pork, 77% of the *E. faecium* isolates (n = 31) were fully sensitive to the tested antimicrobial agents (Table 7.2), and only resistance to tetracycline (19%) and erythromycin (3%) were detected. Occurrences of resistance were similar between Danish and imported pork, except for a higher level of resistance to tetracycline in imported pork.

7.1.6 One Health perspective

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

The level of resistant *E. faecalis* is generally higher in pigs than in broilers, indicating that pig production is a potential greater reservoir for resistance genes. Tetracycline is predominantly used against *E. coli* infections in pigs and since resistance

among *E. coli* isolates from pigs is still low (35%, Table 7.3) tetracycline can still be used. However, a side effect of this usage has given resistance levels of 91% in *E. faecalis* and most likely in other bacterial species isolated from pigs (62% resistance in *E. faecium* from pigs in 2012). A very high and increasing level of resistance to tetracycline (80%-90%) has occurred over the last years.

Apart from tetracycline, significantly higher resistance to chloramphenicol, erythromycin, streptomycin, gentamicin and kanamycin was found among *E. faecalis* isolated from pigs when compared to broilers; reflecting the higher usage of antimicrobials in pigs. All these antimicrobial agents are used for human treatment (chloramphenicol for eye infections only). Higher occurrence of salinomycin resistance was found in isolates from broilers when compared to pig isolates (5% vs 0%). Salinomycin is not used to treat human infections, so salinomycin resistance in itself does not pose a public health problem. However, continuously growing prevalence and co-resistance with other antimicrobial agents can be of importance, and in 2013, 4 out of 6 salinomycin-resistant isolates were also resistant to other antimicrobial agents, especially tetracycline.

No vancomycin resistant enterococci were detected in Danish produced meat in 2013 and only very few vancomycin resistant DANMAP isolates have been reported from pigs during the last decade. An increased occurrence of vancomycin resistant *E. faecium* infections has been observed in Danish hospitals (Textbox 8), however, it does not seem likely that, these infections are related to Danish meat or pigs. The clones causing the hospital infections are all resistant to ampicillin, in contrast to the vancomycin resistant *E. faecium* previous isolates from pigs.

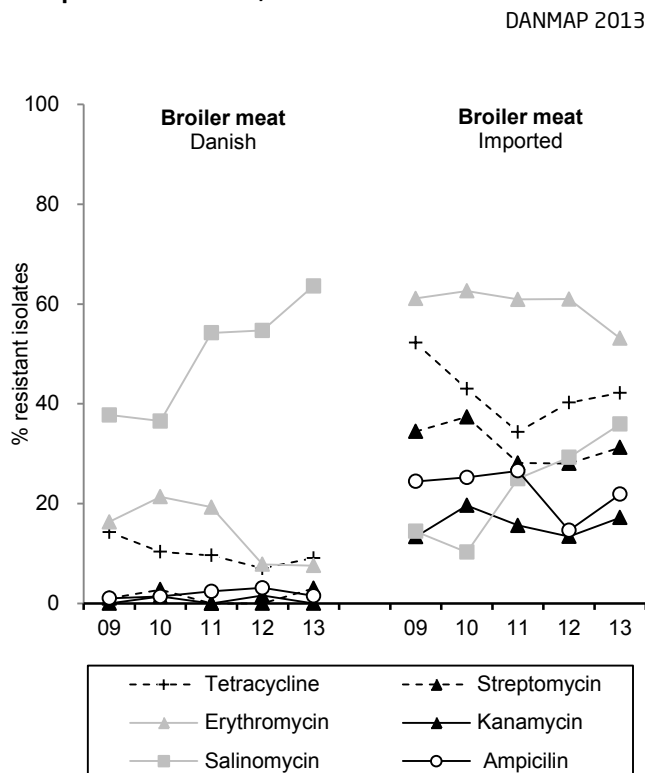
As in previous years, the occurrence of antimicrobial resistance in *E. faecalis* from Danish pork is much lower than from Danish pigs. This is not observed among *E. faecalis* from broiler meat where equal levels of resistance are observed except for streptomycin. These results may indicate that enterococcal populations in the live animal and on pork constitute different sub populations. Pork cuts for sampling are collected from wholesale and retail outlets. Possibly, enterococci on the product may reflect the processing environment, rather than direct contamination of the meat during slaughter and dressing. In contrast, cutting of broilers is done in slaughter plants, which may explain why the enterococcal populations from live broilers and from broiler meat do not appear too dissimilar.

In isolates from imported broiler meat, especially the prevalence of fluoroquinolone resistance is noticeable and could be of importance for human treatment but also the presence of multi-resistant *E. faecalis* (erythromycin, kanamycin, streptomycin and tetracycline) among poultry meat isolates from multiple countries raise concern. Imported broiler meat contains resistant *Enterococcus* isolates more often than Danish broiler meat, especially tetracycline, erythromycin, streptomycin and kanamycin (also ampicillin and penicillin in *E. faecium*).

The tendency that imported meat contains higher prevalence of resistance is also seen for pork (tetracycline), but not for beef. There is a significantly higher occurrence of resistance to erythromycin and kanamycin in Danish beef compared with imported beef.

Lars Bogø Jensen, Lars Stehr Larsen og Helle Korsgaard

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)

7.2 Indicator *Escherichia coli*

For indicator *E. coli*, DANMAP 2013 includes randomly collected isolates from healthy pigs, broilers and cattle at slaughter and from fresh broiler meat, beef and pork sold at wholesale and retail outlets. We included only one isolate per farm or meat sample, and present only data where a sufficient number of isolates were obtained (>15). For details on methodology see Chapter 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). Data for each of the figures are also presented in the web annex. Carbapenems are not included in the test panel, but *E. coli* isolated after selective enrichment with 3rd generation cephalosporins are examined for carbapenemase and ESBL resistance genes (see Textbox 6).

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

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

E. coli isolates from broilers (n = 125) were most often resistant to sulfonamide and ampicillin (both 26%, Table 7.3). Resistance was comparable to the levels observed in 2012, except for an increase in trimethoprim resistance. Over the last five years resistance to ampicillin and sulfonamide has increased whereas resistance to fluoroquinolones (ciprofloxacin) has declined (Figure 7.3). In 2013, resistance to tetracycline increased, apparently reflecting the increased use of this antibiotic in poultry production.

Ampicillin resistance conveys cross-resistance to amoxicillin which has been the most frequently used antimicrobial agent in the broiler production for at least a decade whereas sulfonamides have only been used in the last 2-3 years. Historical use of sulfonamide and co-selection by ampicillin may partly explain the occurrence of sulfonamide resistance, however in 2013 sulfonamide use in poultry increased markedly.

We found 3% of the *E. coli* broiler isolates resistant to nalidixic acid and 6% to ciprofloxacin. During the period 2003-2007, fluoroquinolone consumption in poultry was significantly higher than for the other production animal species in Denmark, because antimicrobial agents approved for poultry were limited to amoxicillin and fluoroquinolones. However, since 2008, fluoroquinolone usage in the broilers has been very low. One isolate was resistant to colistin but with a MIC <16, which is seen occasionally.

Among *E. coli* isolates (n = 116) from domestic broiler meat, we found the highest levels of resistance for ampicillin (24%) and sulfonamide (18%). Resistance was comparable to the levels observed in 2012 and the occurrence of ciprofloxacin and nalidixic acid resistance remained at a low level (5%).

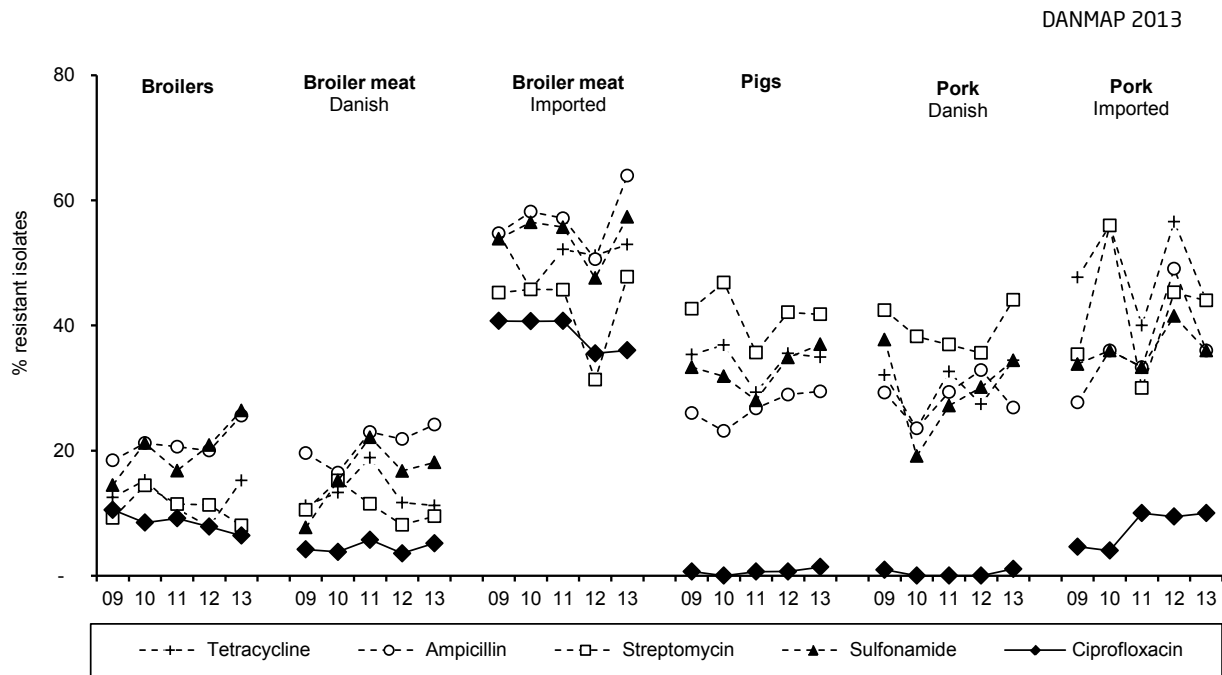
The levels of resistance in *E. coli* from Danish broiler meat were similar to what was found among isolates from Danish broilers (Table 7.3).

Among *E. coli* isolates from Danish broilers and broiler meat, it is noteworthy that the level of multi-resistance has more than doubled within the past five years; reaching 16% - 18% in 2013, while the level of fully sensitive strain has remained relatively stable. The increase in multi-resistance is mainly driven by an

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

Antimicrobial agent	DANMAP 2013									
	Broilers		Broiler meat		Cattle	Beef		Pigs	Pork	
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	
Tetracycline	15	11	53	12	4	11	35	34	44	
Chloramphenicol	0	0	14	2	4	6	5	6	4	
Florfenicol	0	0	5	2	4	6	1	0	0	
Ampicillin	26	24	64	4	4	9	29	27	36	
Cefotaxime	1	2	9	0	0	3	0	1	0	
Ceftiofur	2	2	9	0	0	0	0	1	0	
Trimethoprim	19	12	40	1	0	6	28	25	34	
Sulfonamide	26	18	57	8	4	9	37	34	36	
Streptomycin	8	9	48	8	4	9	42	44	44	
Gentamicin	0	0	2	0	0	3	2	0	2	
Neomycin	1	0	7	0	0	0	2	4	4	
Apramycin	0	0	1	0	0	0	1	0	0	
Ciprofloxacin	6	5	36	0	0	3	1	1	10	
Nalidixic acid	3	5	35	0	0	3	1	1	10	
Colistin	1	0	1	0	0	0	1	0	0	
Spectinomycin	2	2	34	1	0	3	19	19	20	
Fully sensitive	57	62	15	88	96	86	42	46	38	
Multi-resistant	18	16	67	8	4	9	35	31	38	
Number of isolates	125	116	136	103	24	35	146	93	50	

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

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

Note: The number of isolates varies between years (broilers: n = 115-152, Danish broiler meat: n = 116-197, imported broiler meat: n = 136-221, pigs: n = 146-160, Danish pork: n = 68-106 and imported pork: n = 30-65)

increase in occurrence of the ASSuTr phenotype (Figure 7.4) and the increased use of tetracycline in poultry may be a driver in this trend.

Resistance to cephalosporins (ceftiofur and cefotaxime) was observed in 1%-2% of the *E. coli* isolates from Danish broiler meat. Based on a more sensitive selective enrichment method, a high level of cephalosporinase producing *E. coli* (ESC) was also observed in broiler meat (see Textbox 6).

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

In cattle, we found similar levels of resistance in *E. coli* isolates (n = 103) as in 2012. The highest levels of resistance were found for tetracycline (12%), sulfonamide (8%) and streptomycin (8%, Table 7.3). The level of multi-resistance has remained at the same level, fluctuating between 2% and 8% during the past five years.

As in cattle, the levels of resistance in *E. coli* isolates (n = 24) from Danish beef were very low, and at the same level as in 2012.

None of the *E. coli* isolates from cattle or Danish beef were resistant to cephalosporins (ceftiofur and cefotaxime) or to quinolones (ciprofloxacin and nalidixic acid). The use of fluoroquinolones in cattle has been negligible since 2003, and the use of 3rd and 4th generation cephalosporins has been gradually decreased since 2008 (Figure 4.5).

7.2.3 Indicator *E. coli* from pigs and pork

Resistance to ampicillin (29%), streptomycin (42%), sulfonamide (37%) and tetracycline (35%) was common in *E. coli* isolates from pigs (n = 146, Table 7.3). Resistance was comparable to the levels observed in 2012, and the trend analysis over the period 2009-2013 shows an increasing occurrence of resistance to ampicillin (Figure 7.3).

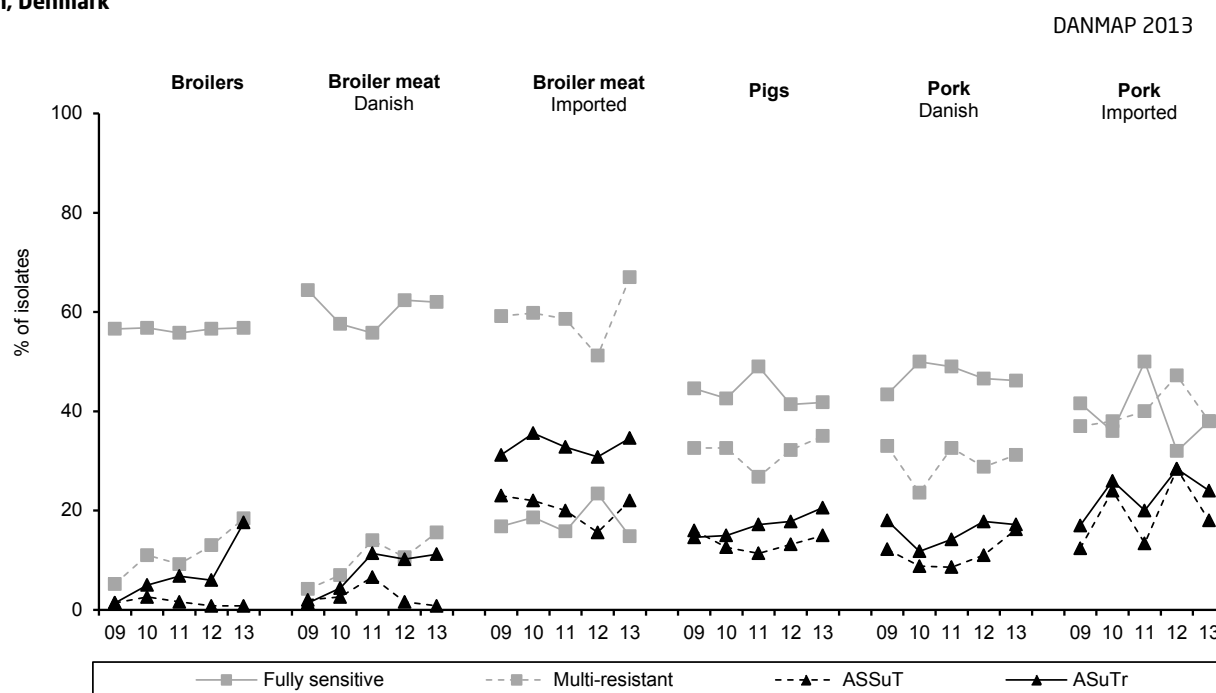
Overall, we found 35% of the isolates from pigs were multi-resistant (Table 7.3). The level of multi-resistance in *E. coli* from Danish pigs has been comparable during 2008-2013. This coincides with an increasing use of antibiotics, among them tetracyclines, in Danish pig production in recent years.

Among the *E. coli* isolates (n = 146) from Danish pork the level of resistance to streptomycin (44%), tetracycline (34%), and sulfonamide (34%), ampicillin (27%) as well as multi-resistance was similar to 2012 (Figure 7.3 and Table 7.3).

The level of fluoroquinolone (ciprofloxacin) resistance remained very low at one percent. This reflects the low consumption of fluoroquinolones in pigs since 2002-2003 (web annex, Table A4.1), when the use in production animals was restricted by legislation. Also, occurrence of cephalosporin (ceftiofur and cefotaxime) resistance remained very low; we observed only one such resistant isolate from Danish pork. The use of cephalosporins in pigs has been close to zero since July 2010, when a voluntary ban was adopted by the industry.

Surveillance based on selective methods has shown a decrease in the number of cephalosporin resistant (ESC) in samples from pigs at slaughter in 2011-2013 compared with 2009-2010 (see Textbox 6).

Figure 7.4. Occurrence (%) of multi-resistant^(a b c) and fully sensitive *Escherichia coli* from animals and meat of Danish and imported origin, Denmark



Note: The number of isolates varies between years (broilers: n = 115–152, Danish broiler meat: n = 116–197, imported broiler meat: n = 136–221, pigs: n = 146–160, Danish pork: n = 68–106, imported pork: n = 30–65)

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

b) 'ASSuT' isolates are resistant to ampicillin, streptomycin, sulfonamide and tetracycline, but can include resistant to other antimicrobial agents such as chloramphenicol

c) 'ASuTr' isolates are resistant to ampicillin, sulfonamide and trimethoprim, but can include resistant to other antimicrobial agents such as chloramphenicol

7.2.4 Indicator *E. coli* from imported meat

The level of multi-resistance (67%) in imported broiler meat was considerably higher compared to Danish broiler meat (16%, Table 7.3), and also compared to other Danish meat types included in the DANMAP programme.

The highest occurrences of resistance in *E. coli* isolates (n = 136) from imported broiler meat were found for ampicillin (64%), sulfonamide (57%), tetracycline (53%) and streptomycin (48%). One isolate was resistant to colistin with a MIC <16. For almost all antimicrobial agents in the test panel (except for gentamycin, apramycin and colistin) the level of resistance was significantly higher for imported broiler meat than in broiler meat of domestic origin (Figure 7.3, Table 7.3). The exceptions were gentamycin, apramycin og colistin, where only one isolate among the isolates from imported broiler meat was resistant and none from the Danish broiler Meat.

In imported beef, 86% of the *E. coli* isolates (n = 35) were fully sensitive and three isolates (9%) multi-resistant (Table 7.3). The level of fully sensitive and multi-resistant isolates in imported beef has not changed during the past 5 years, and is similar to what we find in Danish beef. One isolate was resistant to quinolones (ciprofloxacin and nalidixic acid) and one to 3rd generation cephalosporins (cefotaxime).

The highest occurrence of resistance in *E. coli* isolates (n = 50) from imported pork was for tetracycline and streptomycin (44%), ampicillin and sulfonamide (36%), and trimethoprim (34%), Table 7.3). Overall, 38% of the isolates were multi-resistant, a decline from 47% in 2012 (Figure 7.4). Resistance to fluoroquinolones (ciprofloxacin) was found in five isolates whereas cephalosporin (ceftiofur cefotaxime) resistance was not observed. Compared with Danish pork, resistance to quinolones (ciprofloxacin and nalidixic acid) was significantly higher in imported pork (Figure 7.4).

7.2.5 One Health Perspective

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

Transfer of genes coding for resistance to antimicrobial agents that are critically important in human medicine such as third generation cephalosporins is especially worrisome, as is transmission through food of *E. coli* resistant to fluoroquinolones. Resistance to fluoroquinolones is generally low in Danish animals, but higher in imported meats, in particular broiler meat (Figure 7.4). This also applies to cephalosporin resistance. 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.

Multi-resistance is of importance, because high levels of resistance decrease the number of good 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 imported broiler meat, where we also observed high levels (>30%) of multi-resistance (Figure 7.3 and 7.4).

With co-resistance to critically important antimicrobial agents, the risk of maintenance and spread of the critical 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.

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Occurrence of Extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in meat and slaughter pigs, but no sign of carbapenemase producing *E. coli*

Background: Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide in both humans and production animals. Lately, several studies have found the same ESBL genes, plasmids and clones of *E. coli* isolates originating from animals and involved in human infections, suggesting a zoonotic link.

The occurrence of Enterobacteriaceae resistant to carbapenems is a growing threat in human medicine as carbapenems are the last line antimicrobial agents for treatment of infections caused by multidrug resistant Gram-negative bacteria; this has been pointed out by both EFSA and ECDC. The presence of carbapenemase producing bacteria in food-producing animals is not known, but recently carbapenemase producing *E. coli* has been detected in livestock pigs in Germany, and carbapenemase producing *Salmonella enterica* subsp. *enterica* has been detected in both livestock pigs and poultry in Germany [Fischer *et al.* 2012. J. Antimicrob Chemother. 67:1793-5]. The presence and possible spread of carbapenemase producing bacteria in production animals is thus considered extremely important for the assessment of potential zoonotic risks.

Carbapenems are not used in the Danish animal production. Both ESBL and carbapenem resistance can be driven by use of cephalosporins. In July 2010, the use of cephalosporins in the Danish pig production was discontinued, but it is still used for systemic and intramammary treatment in cattle. Cephalosporins have not been used in the Danish broiler production for at least a decade, but was used outside of Denmark in the production of grandparent animals of the Danish broilers before 2012.

The aim of this study was to investigate the occurrence of ESBL/carbapenemase-producing *E. coli* in pigs at slaughter and in meat at retail (see Chapter 10. for definition of ESBL).

Materials and methods: During January through December 2013, faecal samples were taken from pigs at slaughter (n = 381). One animal represented one herd and no herds were sampled more than once in the same month. In the same period, broiler meat (Danish: n = 146, imported: n = 172), beef (Danish: n = 70, imported: n = 102) and pork (Danish: n = 238, imported: n = 225) samples were collected randomly in retail stores and outlets in all regions of Denmark. *E. coli* was isolated from 1 g of faecal sample or 5 g of meat after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL-resistance was revealed by use of whole genome sequencing (WGS). The reads were assembled de novo prior to prediction of genes. The web-server ResFinder (www.genomicepidemiology.org) was used to identify acquired ESBL and carbapenemase genes in the WGS data. For isolates where no genes were detected, the sequences were investigated for up-regulation of chromosomal *ampC* by use of CLCbio Genomic Workbench v6.

Results: For pigs at slaughter, 5.8% (22/381) contained ESBL-producing *E. coli*, which was significantly lower than in 2009 (11%) and in 2010 (12%). Among the ESBL producing *E. coli* isolates from pigs the most commonly detected gene was, as in previous years, CTX-M-1 (59%) followed by CMY-2 (18%), *ampC* upregulation (14%) and CTX-M-14 (9%) (Figure 1). From meat samples, the highest prevalence of ceftriaxone resistant *E. coli* was found among imported broiler meat (52%, 89/172) and were at the same level as in 2010 to 2012 (between 50 % to 62%). The prevalence (25%, 36/146) of ceftriaxone resistant *E. coli* in Danish broiler meat was significantly lower than in 2012 and a decreasing trend since 2011, where the occurrence of ceftriaxone resistant *E. coli* (44%) was the highest observed. The occurrence of ceftriaxone resistant *E. coli* was significantly higher in imported broiler meat when compared with Danish broiler meat.

Among ESBL producing *E. coli* isolates the most commonly detected gene in Danish broiler meat was, as found in previous years, CMY-2 (83%), whereas CTX-M-1 (37%) and CMY-2(42%) were almost equally present in imported broiler meat. Eight isolates of the ESBL-producing isolates from broiler meat contained two ESBL genes. In the other meat types the prevalence was generally low (0-1.4%, Figure 2). None of the isolates from meat and slaughter pigs contained any known carbapenemase genes.

Discussion: The usage of cephalosporins was still low (approximately 3 kg in total, Table 4.1) compared with usage of other antimicrobial agents, and the occurrence of cephalosporinase producing *E. coli* in slaughter pigs was significantly lower than than before the voluntary ban of cephalosporin usage in the Danish pig production was effectuated.

The occurrence of cephalosporinase producing *E. coli* in Danish produced broiler meat was significantly lower in 2012 than in 2011, which could be explained by the voluntary discontinuation of the use of 3rd generation cephalosporins in the top of the breeding pyramid (in the country producing the grandparents for the Danish broilers) leading to a reduction in imported parentflocks, but other explanations may also exist.

Since none of the tested ESBL-producing *E. coli* isolates contained known carbapenemase genes, meat or food-producing animals in Denmark are most likely not a source to carbapenemase producing bacteria causing human infections in Denmark; however monitoring of carbapenemase producing bacteria in animals and meat is still important as the situation may change over time.

Conclusion: The voluntary ban of cephalosporins in the pig production seems to retain the ESBL producing *E. coli* in pigs at slaughter at a low level. For the first time a significant reduction in the occurrence of ESBL producing *E. coli* in Danish broiler meat was observed, which could be due to less introduction of ESBL producing *E. coli* from imported parent animals, but this was not investigated in this study and also other factors may influence the occurrence.

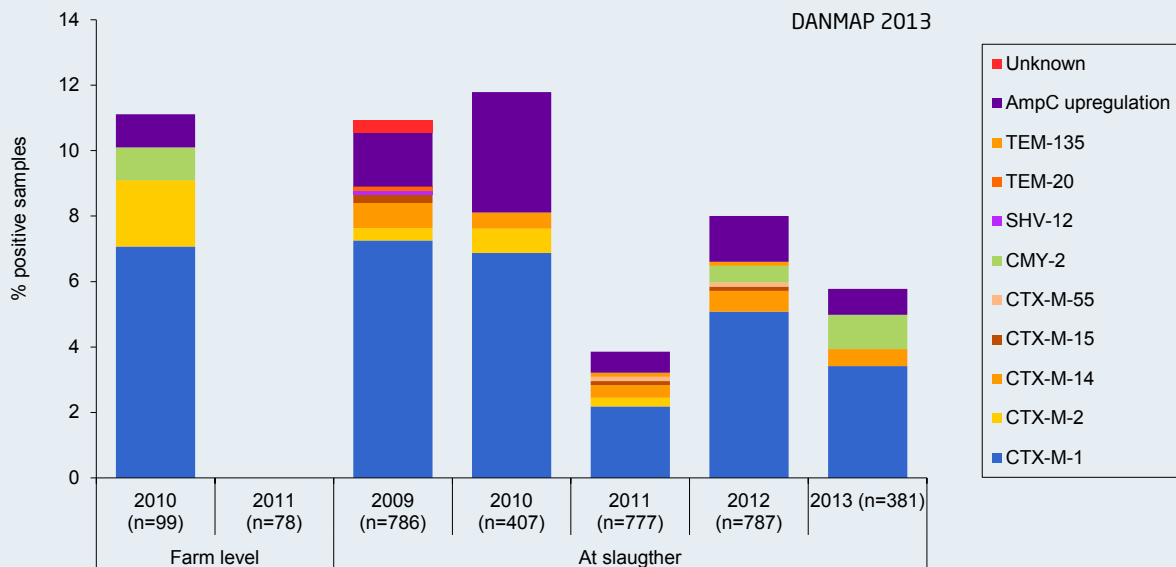
ESBL genes found in ESBL-producing *E. coli* from human infections are still present in pork and pigs, and broiler meat from both Danish and imported origin. A possible zoonotic link will be studied further in the coming years.

Currently, and based on the data presented here, carbapenemase producing *E. coli* from meat or food-producing animals are considered a low, if any, risk to human health.

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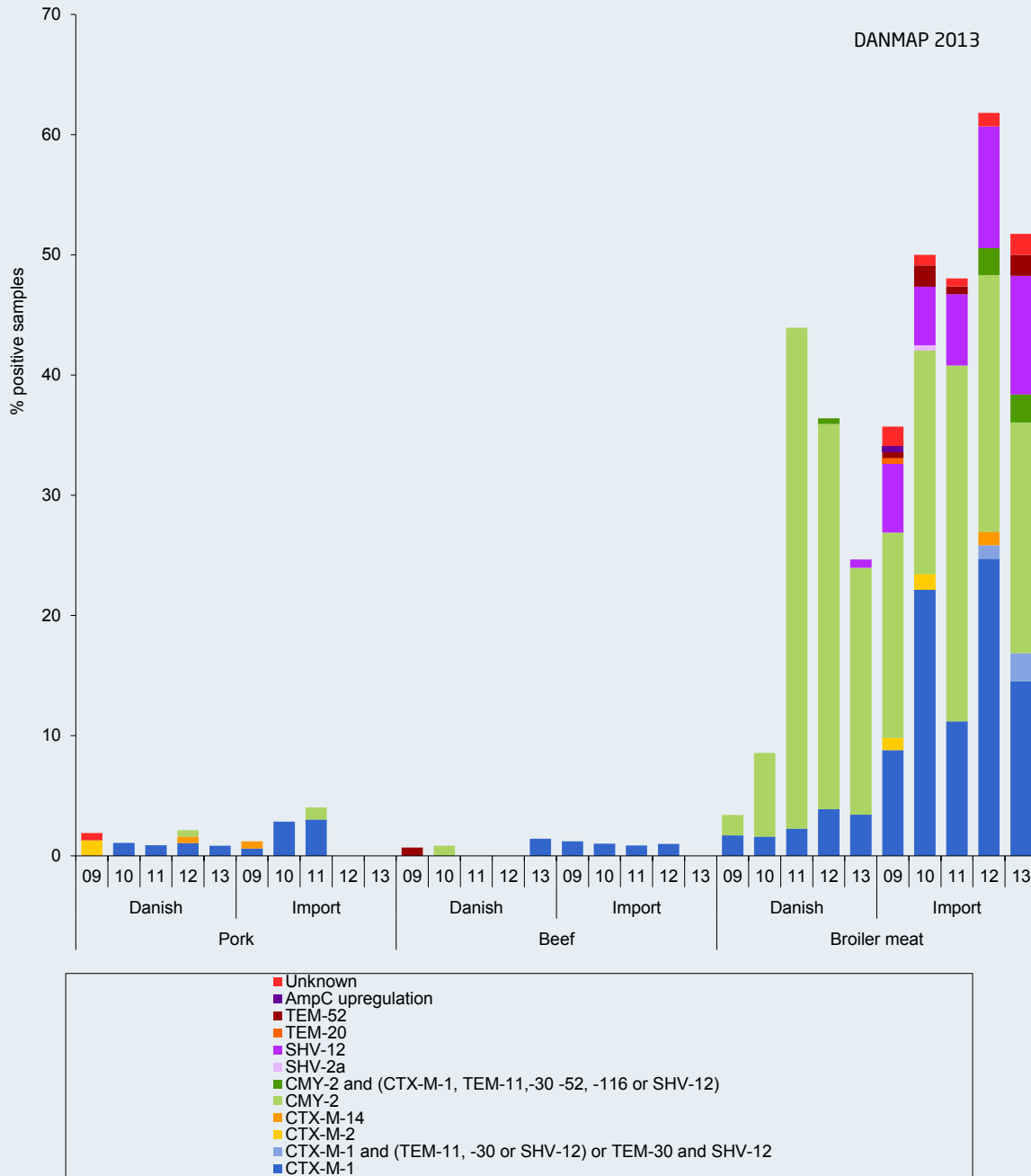
For further information: Yvonne Agersø (yvoo@food.dtu.dk)

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



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

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



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



8. Resistance in human clinical bacteria

8.1 *Escherichia coli*

Escherichia coli is part of the normal intestinal flora of both humans and animals but also cause infections. In humans, *E. coli* is the most frequent cause of bacteraemia and community- and hospital-acquired urinary tract infections. For *E. coli*, DANMAP 2013 includes data from 10 out of 11 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. Nine of the 10 DCM working with primary healthcare isolates contributed data on antimicrobial resistance in urine isolates of *E. coli* from primary healthcare (Table 8.1).

Blood isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 4,000 *E. coli* isolates from blood (Table 8.1 and Figure 8.1).

In 2013, resistance to 2nd generation cephalosporins (cefuroxime) was 9% and resistance to 3rd generation cephalosporins 8%. Both levels are similar to the levels reported in 2012. The level of 3rd generation cephalosporin resistance in Denmark was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by other European countries in 2012 [EARS-Net 2012].

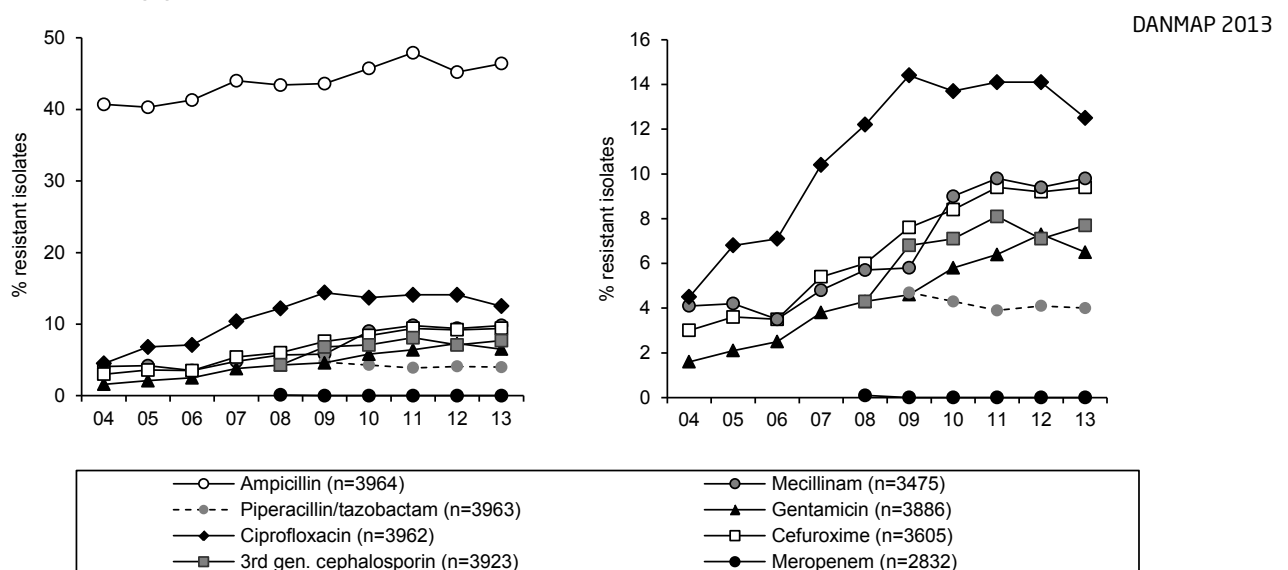
Table 8.1. Resistance (%) in *Escherichia coli* isolates from humans, Denmark

Substance	DANMAP 2013		
	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary healthcare %
Ampicillin	46	42	40
Mecillinam	10	8	6
Piperacillin/tazobactam	4		
Sulfonamide		33	33
Gentamicin	7	5	
Ciprofloxacin	12 #	12	10
Cefuroxime	9	6	
3rd generation cephalosporins ^{a)}	8	6	4
Meropenem	<1	<1	<1
Max. number of isolates tested	3967	39820	43770

#) A number sign indicates a significant decrease from 2012 to 2013

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

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



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2013

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

The occurrence of ciprofloxacin resistance decreased from 14% in 2012 to 12% in 2013. This level was the same as reported by many other countries in Europe [EARS-Net 2012].

Aminoglycoside (gentamicin) resistance was 7%, which is at the same level as reported in 2012. This level was the same as reported by many other countries in Europe [EARS-Net 2012].

Mecillinam resistance was 10% and ampicillin resistance was 46% in 2013, which are at the same levels as in 2012.

Resistance data on piperacillin/tazobactam was reported for the first time in the present DANMAP report. The DCMs were asked to report resistance data for five years (2009-2013) (Table 8.1 and Figure 8.1). In all five years, the resistance was at the same level around 4%.

In the 10-year period from 2004 to 2013, resistance in *E. coli* blood isolates has increased steadily: resistance to 2nd generation cephalosporins from 3% to 9%; ciprofloxacin resistance from 5% to 12%; aminoglycoside (gentamicin) resistance from 2% to 7%. These increases parallel the increased antimicrobial consumption, which has been seen in the same period (Table 5.4).

Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 40,000 *E. coli* isolates from hospital patients with a urinary tract infection (Table 8.1 and Figure 8.2).

The occurrence of resistance to 2nd generation cephalosporin (cefuroxime) (6%) and 3rd generation cephalosporin (6%) were at the same level as in 2012.

In 2013, carbapenem (meropenem) resistance was observed in five *E. coli* urine isolates from hospitalised patients.

The occurrence of ciprofloxacin resistance was at the same level as in 2012, but a steady increase has been seen in ciprofloxacin resistance from 3% in 2004 to 12% in 2013 (Figure 8.2).

Aminoglycoside (gentamicin) resistance was 5% and sulfonamide resistance was 33%, which are at the same levels as in 2012.

Urine isolates from primary healthcare

DANMAP received data on the antimicrobial susceptibility of approximately 44,000 *E. coli* isolates from urinary tract infection in patients from primary healthcare (Table 8.1 and Figure 8.3).

The occurrence of resistance to 3rd generation cephalosporin was 4% and this was the same level as reported in 2012.

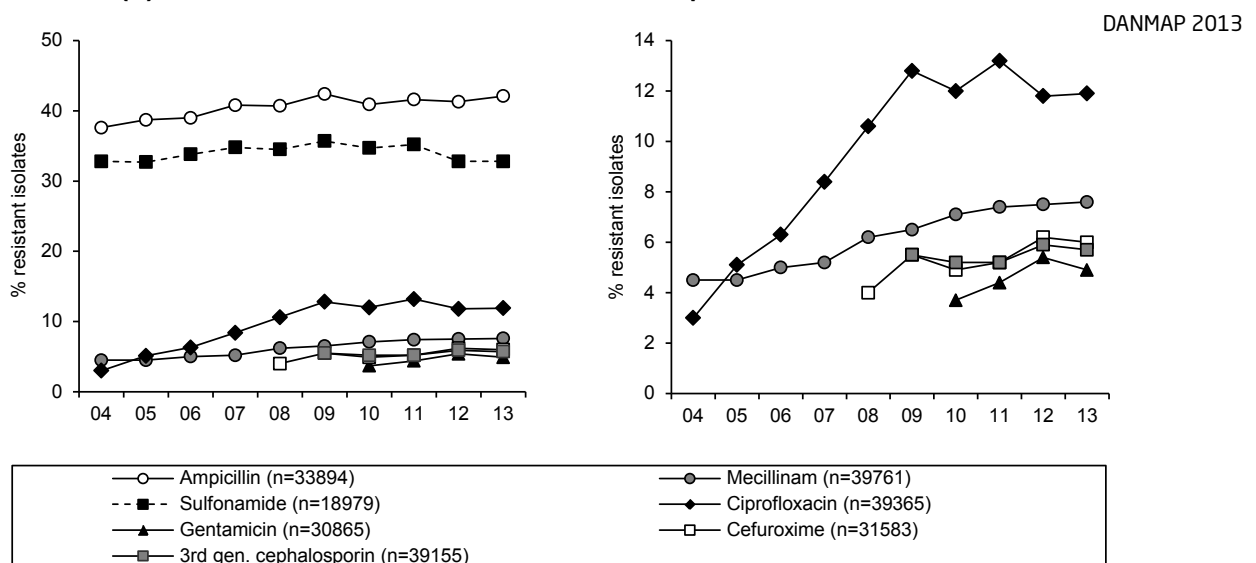
Since 2003, the level of ciprofloxacin resistance has increased steadily from 3% in 2004 to 10% in 2013 (Figure 8.3), which parallels the increasing trend observed in the consumption of fluoroquinolones during the last decade (Table 5.2).

The occurrence of ampicillin resistance was 40% and sulfonamide resistance was 33%, both levels were similar to levels reported in 2012.

In 2013, carbapenem (meropenem) resistance was observed in five *E. coli* urine isolates from primary healthcare.

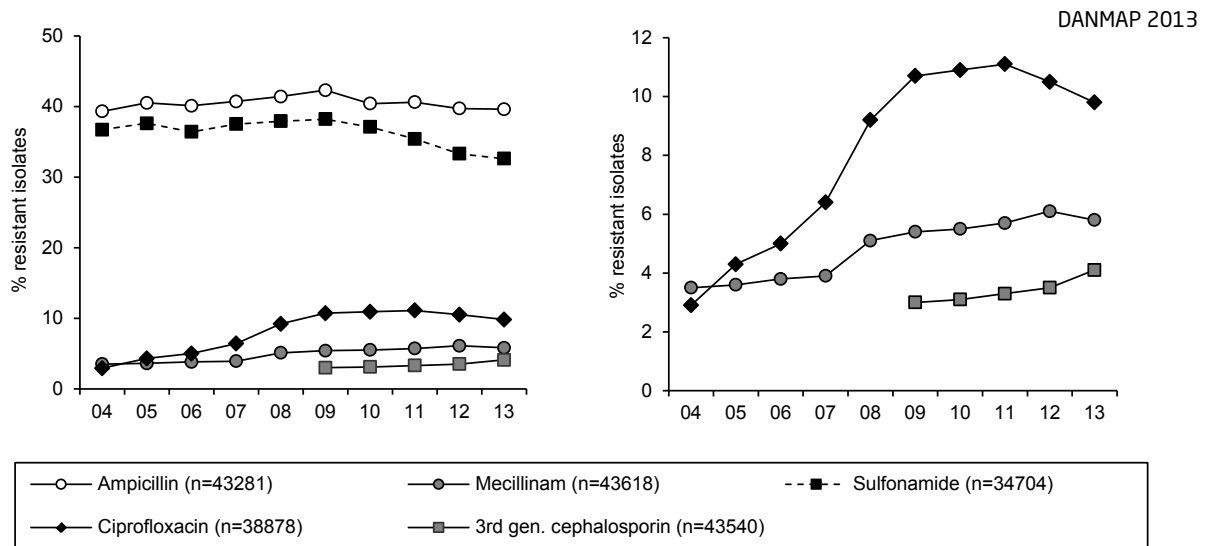
Line Skjøt-Rasmussen, Stefan S. Olsen
and Anette M. Hammerum

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



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2013

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



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2013

8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the normal intestinal flora in humans but also cause infections such as urinary tract-, respiratory tract-, wound- and bloodstream infections. Many of these infections are hospital acquired and can be life threatening, especially if the strains are resistant to antimicrobial agents. *K. pneumoniae* is intrinsically resistant to aminopenicillins (e.g. ampicillin). Therefore, infections caused by *K. pneumoniae* are treated with broad spectrum antimicrobial agents such as ciprofloxacin, gentamicin, cephalosporins and carbapenems. For *K. pneumoniae*, DANMAP 2013 includes data from 10 out of 11 DCM, representing 95% of the Danish population. Nine of the 10 DCM working with primary healthcare isolates contributed data on antimicrobial resistance in urine isolates of *K. pneumoniae* from primary healthcare (Table 8.2).

Blood isolates from hospital patients

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

In general, the level of antimicrobial resistance in 2013 was similar to 2012 (Figure 8.4).

Resistance to 2nd generation cephalosporins (cefuroxime) was 12% and resistance to 3rd generation cephalosporins was 9%. In 2013, 3rd generation cephalosporin resistance was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by other European countries in 2012 [EARS-Net 2012].

In 2013, two carbapenem (meropenem) resistant *K. pneumoniae* blood isolates were detected.

Ciprofloxacin resistance (9%), and resistance to aminoglycoside (gentamicin) (4%) were similar to the level reported in 2012.

The level of resistance to ciprofloxacin was above the level reported from the other Nordic countries and the same as reported to EARS-Net by other European countries in 2012 [EARS-Net 2012]. Whereas, the level of resistance to aminoglycosides was the same as in the other Nordic countries reporting to EARS-Net [EARS-Net 2012].

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

Substance	DANMAP 2013		
	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary healthcare %
Mecillinam	8	10 #	10
Piperacillin/tazobactam	6		
Sulfonamide		20 #	22 #
Gentamicin	4	4 #	
Ciprofloxacin	9	8	7
Cefuroxime	12	9 #	
3rd generation cephalosporins ^{a)}	9	7 #	6
Meropenem	<1	<1	<1
Max. number of isolates tested	879	5919	3660

#) A number sign indicates a significant decrease from 2012 to 2013

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

Urine isolates from hospital patients

DANMAP received data on the antimicrobial susceptibility of approximately 5,900 *K. pneumoniae* isolates from hospital patients with a urinary tract infection (Table 8.2 and Figure 8.5).

Resistance to mecillinam (10%), sulfonamide (20%), gentamicin (4%), 2nd generation cephalosporins (cefuroxime) (9%) and to 3rd generation cephalosporins (7%) decreased from 2012 to 2013. Ciprofloxacin resistance was at the same level as reported in 2012.

In 2013, carbapenem (meropenem) resistance was observed in eight *K. pneumoniae* urine isolates from hospitalised patients.

Urine isolates from primary healthcare

DANMAP received data on the antimicrobial susceptibility of approximately 3,600 *K. pneumoniae* isolates from urinary tract infection in patients from primary healthcare (Table 8.2 and Figure 8.6).

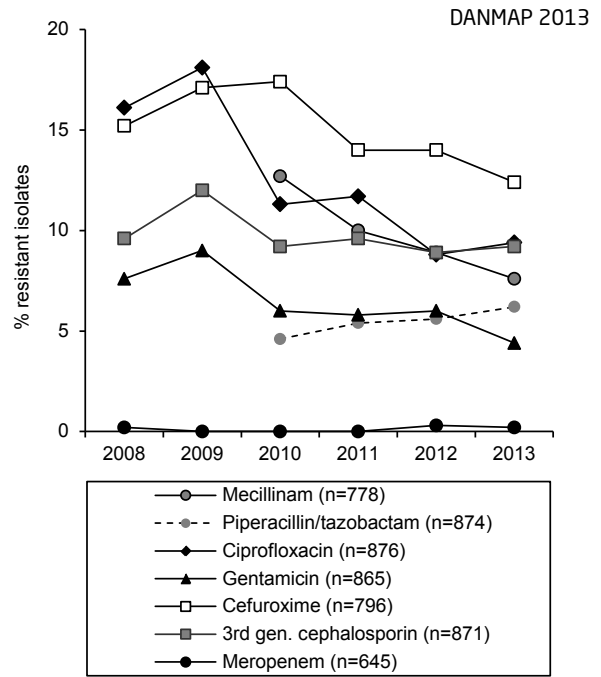
In 2013, resistance to 3rd generation cephalosporins was 6%, which is similar to the level reported in 2012.

In 2013, three carbapenem (meropenem) resistant *K. pneumoniae* urine isolates from patients in primary healthcare were found.

Resistance to ciprofloxacin was 7% and resistance to mecillinam was 10%, which were similar to the levels reported in 2012. Sulfonamide resistance decreased from 26% in 2012 to 22% in 2013.

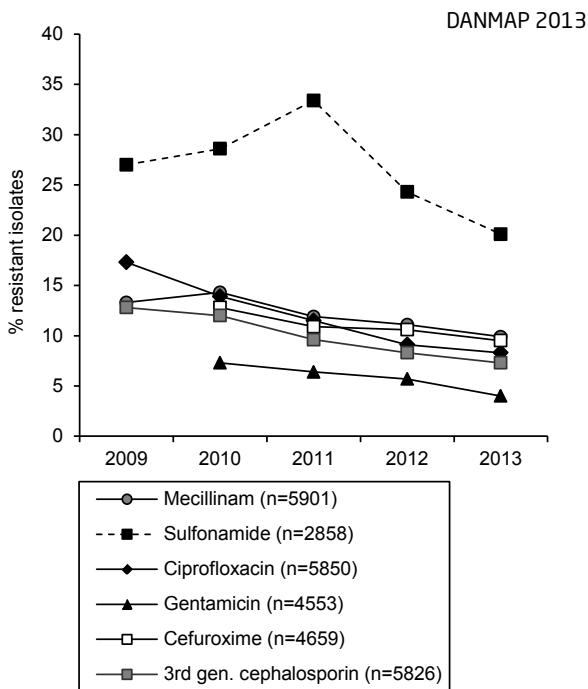
Line Skjøt-Rasmussen, Stefan S. Olsen and Anette M. Hammerum

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



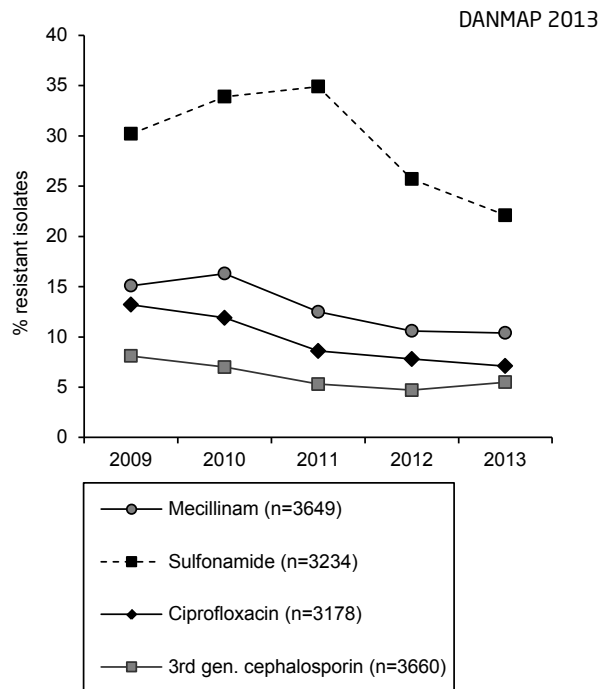
Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2013

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 2013

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



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2013

Carbapenemase producing bacteria in Denmark

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant bacteria such as *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). Furthermore, Imipenemase (IMP) can be detected in *P. aeruginosa* isolates.

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

During 2013, 35 carbapenemase producing bacteria were detected. 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. In many of the cases, the carbapenemase producing isolates were related to travelling, but spread between patients in Denmark was also reported for both *Enterobacteriaceae* and *A. baumannii* in 2013.

Enterobacteriaceae: In 2013, 18 CPE were detected compared to 19 CPE during 2008–2012 (Figure 1). Fifteen of the 18 isolates harboured NDM genes (*K. pneumoniae* (n = 4), *Citrobacter freundii* (n = 4), *E. coli* (n = 2), *Providencia stuartii* (n = 2) and *Proteus mirabilis* (n = 1)), OXA-48 was detected in 3 isolates (*K. pneumoniae* (n = 2), *E. coli* (n = 1)), and KPC (*K. pneumoniae* (n = 1)) and VIM (*Klebsiella oxytoca* (n = 1)) in one isolate each.

During 2013, the first spread of CPE in Denmark was confirmed by comparing epidemiological information with molecular data. The first spread of CPE was between two patients, who had been admitted to the same ward in a hospital in the North Denmark Region in 2012. None of these patients had a prior history of travel noted in their hospital records. In 2013, it was shown by molecular typing that the patients shared the same VIM-4 producing *E. coli*. This was the first detected spread of CPE in Denmark. The origin of VIM-4 producing *E. coli* was unknown.

In 2013, NDM-1 producing *C. freundii* isolates were detected from four patients at the same hospital ward in the North Denmark Region. Spread of NDM-1 *C. freundii* was shown by molecular typing and comparison of epidemiological data. None of the four patients had been travelling recently and the origin of the NDM-1 producing *C. freundii* was unknown. Besides the NDM-1 producing *C. freundii*, two of the four patients had NDM-1 producing *K. pneumoniae*.

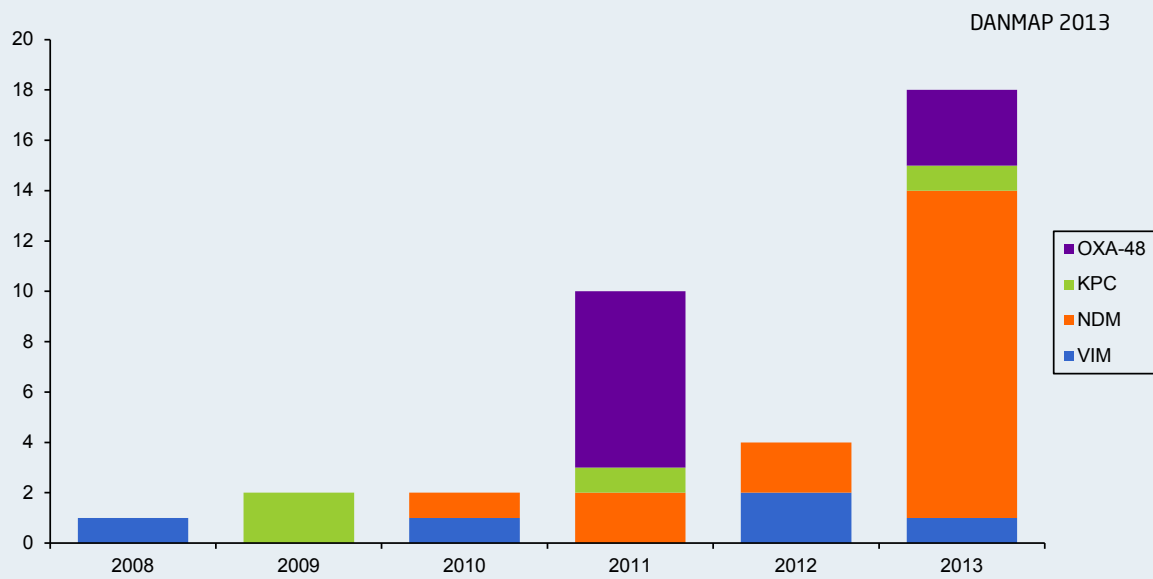
A. baumannii: During 2013, seven OXA-23 producing *A. baumannii* isolates were detected. Spread of OXA-23 producing *A. baumannii* was detected two times from one patient to another patient. Furthermore, three OXA-40-like producing *A. baumannii* were detected, two of these being part of the same transmission chain. Furthermore, two NDM-1 producing *A. baumannii* isolates were detected.

P. aeruginosa: In 2013, three VIM producing *P. aeruginosa* isolates were detected. Furthermore, an NDM and VIM producing *P. aeruginosa* was detected. For the first time, an IMP producing *P. aeruginosa* isolate was detected in Denmark. Both patients had been hospitalized abroad prior to detection of these isolates.

Conclusion: The occurrence of carbapenemase-producing bacteria in Denmark is increasing. Most isolates were multi-resistant, which makes infections caused by these bacteria extremely difficult to treat with antimicrobial agents. The increasing trend of carbapenemase producing bacteria is therefore worrying. 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 infection control difficult.

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

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

8.3 *Pseudomonas aeruginosa*

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

P. aeruginosa blood isolates obtained from hospitalised patients

For *P. aeruginosa*, DANMAP 2013 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 approximately 414 *P. aeruginosa* isolates from blood. Resistance to all the tested antimicrobial agents was not significantly different from the level in 2012, but an increasing trend has been observed for gentamicin during 2007–2013 (Figure 8.7). The occurrence of resistance to fluoroquinolones, carbapenems, ceftazidime and piperacillin/tazobactam was at the same level or lower than most of the other countries reporting to EARS-Net in 2012 [EARS-Net 2012].

Meropenem resistance was observed for 3% (n = 12) of the *P. aeruginosa* isolates in 2013. A Danish study of *P. aeruginosa* carbapenem non-susceptible isolates from 2011 showed that carbapenemases were present in a minority of the isolates (7%) [Textbox 11, DANMAP 2012]. As in previous years, presumable carbapenemase producing *P. aeruginosa* isolates were sent on a voluntary basis from the DCM to SSI for national surveillance on carbapenemase producing bacteria, including not only isolates from bloodstream infections but also from other origins. During 2013, three VIM producing *P. aeruginosa* isolates and an NDM and VIM producing isolate were identified. For the first time an IMP producing isolate was detected in Denmark [Textbox 7].

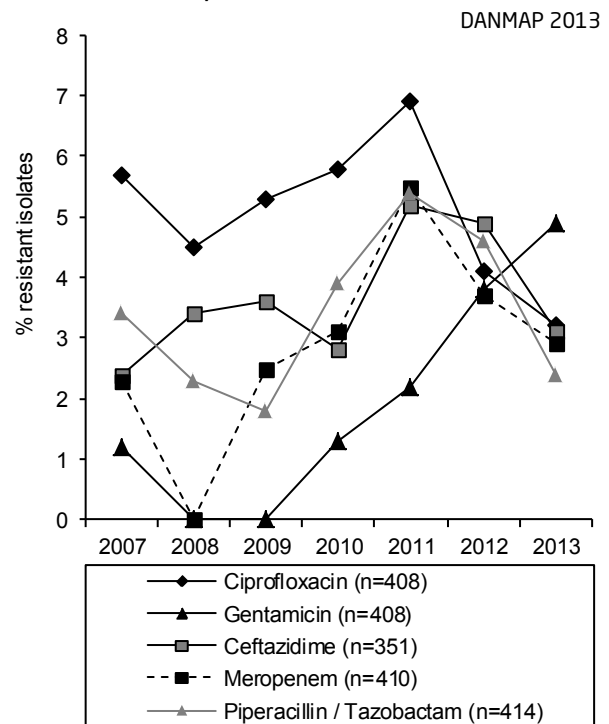
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8.4 Streptococci

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

In this report, data on resistance in invasive streptococcal isolates (only from blood or cerebrospinal fluid) were obtained from the Neisseria and Streptococcus Reference laboratory covering all DCM in Denmark. Infections with streptococci are primarily treated with penicillins and macrolides. All invasive non-duplicate *Streptococcus pneumoniae* and group A, B, C and G streptococci were susceptibility tested against erythromycin and penicillin.

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 2013

Streptococcus pneumoniae

S. pneumoniae is a leading cause of bacterial pneumonia, otitis media, bacteraemia and meningitis. In 2013, susceptibility testing was performed on 824 non-duplicate *S. pneumoniae* isolates from invasive infections (blood and spinal fluid) (Figure 8.8).

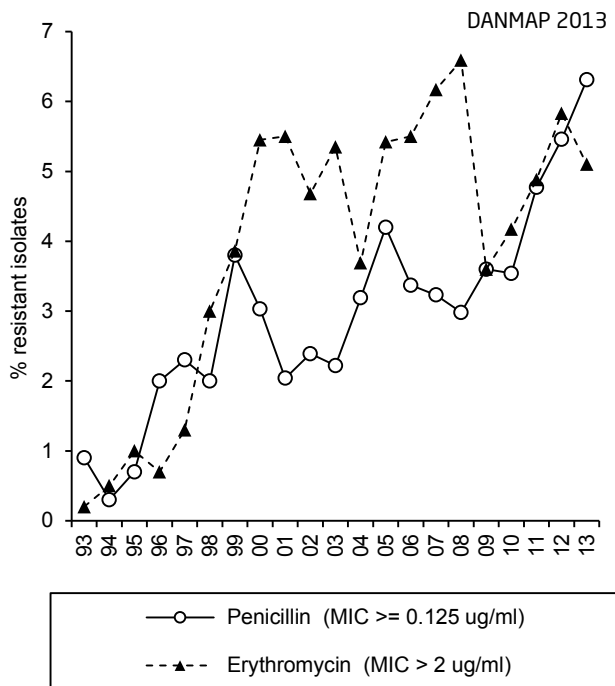
A total of 42 (5.1%) *S. pneumoniae* invasive isolates from blood and cerebrospinal fluid were non-susceptible (resistant and intermediary resistant) to erythromycin in 2013 compared to 5.4% in 2012 (Figure 8.8). The 42 isolates belonged to 12 different serotypes and the most commonly found erythromycin non-susceptible serotypes were type 15A (n = 19), 19A (n = 7) and 24F (n = 4).

Regarding penicillin, 52 (6.3%) invasive isolates from blood and cerebrospinal fluid were non-susceptible (resistant and intermediary resistant) in 2013 compared to 5.1% in 2012 (Figure 8.8). The 52 isolates belonged to 12 different serotypes and the most commonly found penicillin non-susceptible serotypes were type 15A (n = 20), 23B (n = 10), 19A (n = 8) and 24F (n = 4). None of the isolates were resistant to penicillin according to EUCAST.

Four serotypes were particularly non-susceptible to either penicillin or erythromycin or both, namely 15A (20 of 32 received isolates), 23B (10 of 16 received isolates), 19A (8 of 29 received isolates) and 24F (4 of 22 received isolates).

The levels of erythromycin (macrolide) and penicillin non-susceptibility in Denmark were similar to the levels reported in 2012 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 2012 [EARS-Net 2012].

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



Group A streptococci

In 2013, susceptibility testing was performed on 167 non-duplicate group A streptococci (GAS, *Streptococcus pyogenes*) isolates from invasive infections (blood and spinal fluid). Erythromycin resistance was detected in five isolates (3.0%) as compared to one isolate in 2012 (0.6% of 167). No resistance to penicillin was detected.

Group B, C and G streptococci

In 2013, susceptibility testing was performed on 129 non-duplicate group B streptococci (GBS, *Streptococcus agalactiae*) isolates from invasive infections (blood and spinal fluid). Erythromycin resistance was detected in 22 isolates (17.1%) as compared to 17 in 2012 (12.7% of 134). No resistance to penicillin was detected.

In 2013, susceptibility testing was performed on 66 non-duplicate group C streptococci (GCS, *Streptococcus equisimilis* and *S. zooepidemicus*) isolates from invasive infections (blood and spinal fluid). Erythromycin resistance was detected in 3 isolates (4.5%) as compared to 4 in 2012 (5.5% of 73). No resistance to penicillin was found.

In 2013, susceptibility testing was performed on 154 non-duplicate group G streptococci isolates from invasive infections (blood and spinal fluid). Erythromycin resistance was detected in 16 isolates (10.4%) as compared to 17 in 2012 (10.6% of 160). No resistance to penicillin was found.

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8.5 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but also cause infections. Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteraemia and bacterial endocarditis. *E. faecalis* and *E. faecium* can cause life-threatening infections in humans, especially in the hospital environment. The naturally high level of antimicrobial resistance found in *E. faecalis* and *E. faecium* makes infections difficult to treat. Antimicrobial therapy for serious enterococcal infections requires the use of synergistic combinations of a cell-wall-active agent such as penicillin (ampicillin) and an aminoglycoside (gentamicin) or a glycopeptide (vancomycin).

For *E. faecalis* and *E. faecium*, data from 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 657 *E. faecium* isolates and 579 *E. faecalis* isolates from blood.

As in previous years, most of the *E. faecium* isolates from bloodstream infections were ampicillin resistant. In 2013, 93% of the *E. faecium* isolates were resistant to ampicillin. Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as a risk factor for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has been observed in hospitals in Denmark during the 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.

Only one of the DCMs (Aalborg) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates, 27% 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 2012 [EARS-Net 2012].

In 2013, vancomycin resistance was detected in 3.4% of the *E. faecium* isolates ($n = 22$) and 0.2% ($n = 1$) 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. The level of vancomycin resistant *E. faecium* was above the level reported to EARS-Net 2012 by the other Nordic countries [EARS-Net 2012].

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Increased occurrence of vancomycin resistant enterococci in Danish hospitals

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 vancomycin resistant enterococci for species identification, genotyping (*vanA*, *vanB*, *vanC*) and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (Figure 1).

In 2010 and 2011, an increase in the number of *vanA E. faecium* was detected at hospital wards in the Central Denmark Region and screening of faecal samples was initiated [DANMAP 2010 and DANMAP 2011].

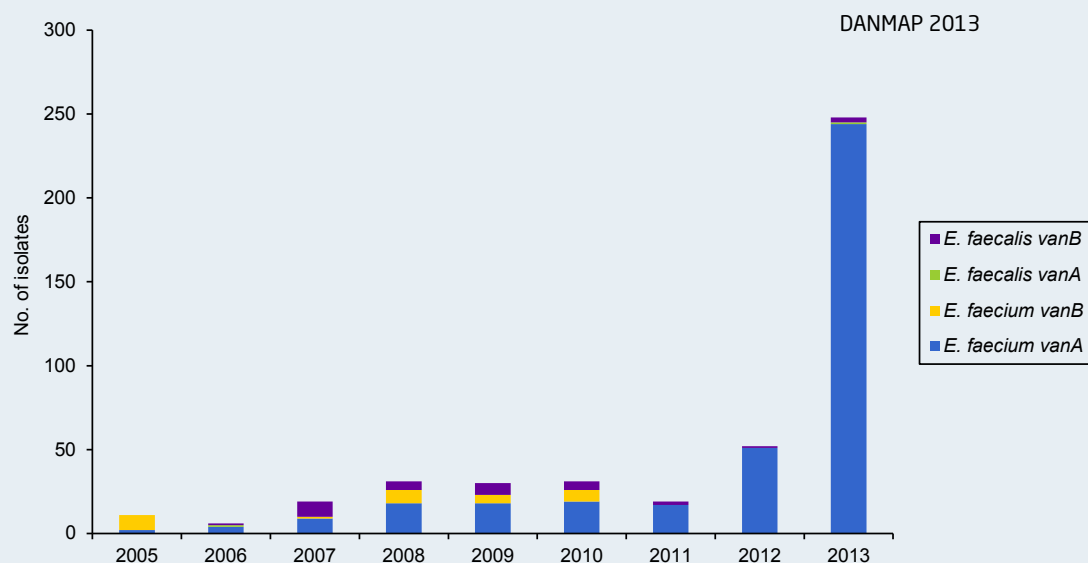
In 2012, 54 VRE isolates from clinical infections (UTI, wounds and bloodstream infections) were received at SSI (only one isolate per patient was included) (Figure 1). In 2013, the number increased further to 248 clinical isolates. In addition, 168 faecal screening isolates were received. Most of the patients with VRE were 60 years of age or older. Nearly all VRE isolates from 2012 and 2013 were *vanA E. faecium* isolates (Figure 1). 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. Pulsed-field gel electrophoresis typing showed spread of several *vanA E. faecium* types both inside hospitals and between hospitals.

Conclusion: The increasing number and spread of VRE in Denmark is worrying, since only limited number of antimicrobial agents are left for treatment of infections. 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. National infection control guidelines are needed to contain the current increase and spread of VRE. The guidelines 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 and van genes, Denmark



***Neisseria gonorrhoeae* 2013**

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. Most of the received isolates are from urethra or cervix, while specimens from rectum and pharynx are only rarely obtained by clinicians. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

At the NSR laboratory ceftriaxone and ciprofloxacin 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, as modified by ECDC. Both fully and intermediary resistant isolates were categorized as resistant. Penicillinase production was tested using the nitrocephin technique.

As part of NSRs participation in ECDCs surveillance of sexually transmitted infections since 2009, approximately 60 gonococcus isolates are investigated twice per year for susceptibility to an expanded panel of antimicrobial agents. In addition to ceftriaxone and ciprofloxacin, this panel includes azithromycin, cefixime, spectinomycin, and gentamicin.

Results and discussion: The ciprofloxacin resistance rate increased steadily from 30% in 2003 reaching a peak of 75% in 2009, followed by a decrease to 56% in 2013 (Figure 1). The percentage of strains producing penicillinase fluctuated between 24% in 2003 and 11% in 2013.

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have been reported from several countries during recent years. No cases from Denmark have ever been reported. 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. Thus, there is no evidence of emerging ceftriaxone resistance in Denmark.

In 2013, azithromycin resistance including intermediary resistance (MIC > 0.25 mg/L) in gonococci was 45%, corresponding to the rate in 2009 (Table 1). It is worth noting that 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 9% in 2013, i.e. unchanged compared to 2012. Cefixime is an oral cephalosporin which has never been used in Denmark. During 2009–2013 none of the isolates were resistant to spectinomycin (MIC > 64 mg/L); however, this parenteral drug is not marketed in Denmark and is not readily accessible in most countries.

Gentamicin has been used successfully for the treatment of gonorrhoea in several countries in Africa but no randomized clinical trials evaluating its efficacy have been published. Gentamicin breakpoints for gonococci have not been determined. In 2011 through 2013, 98% of the examined strains from Denmark had gentamicin MIC ≤ 4 mg/L.

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

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Figure 1. Ciprofloxacin resistance and penicillinase production in gonococci, Denmark, 2003-2013

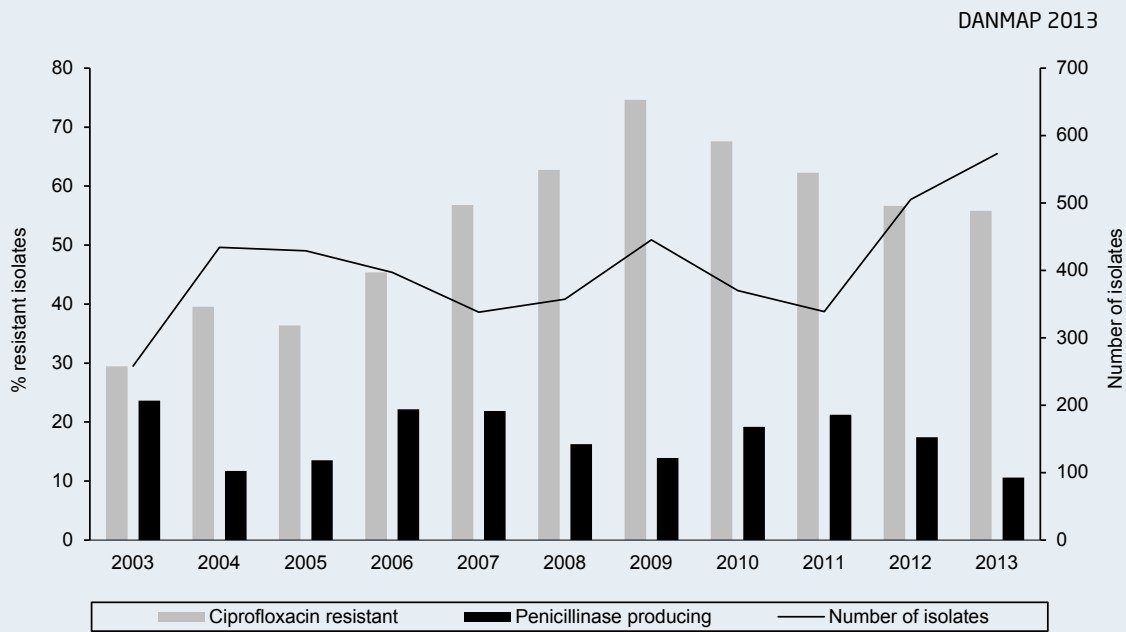


Figure 2. Distribution of ceftriaxone MIC values in gonococci, Denmark, 2003-2013

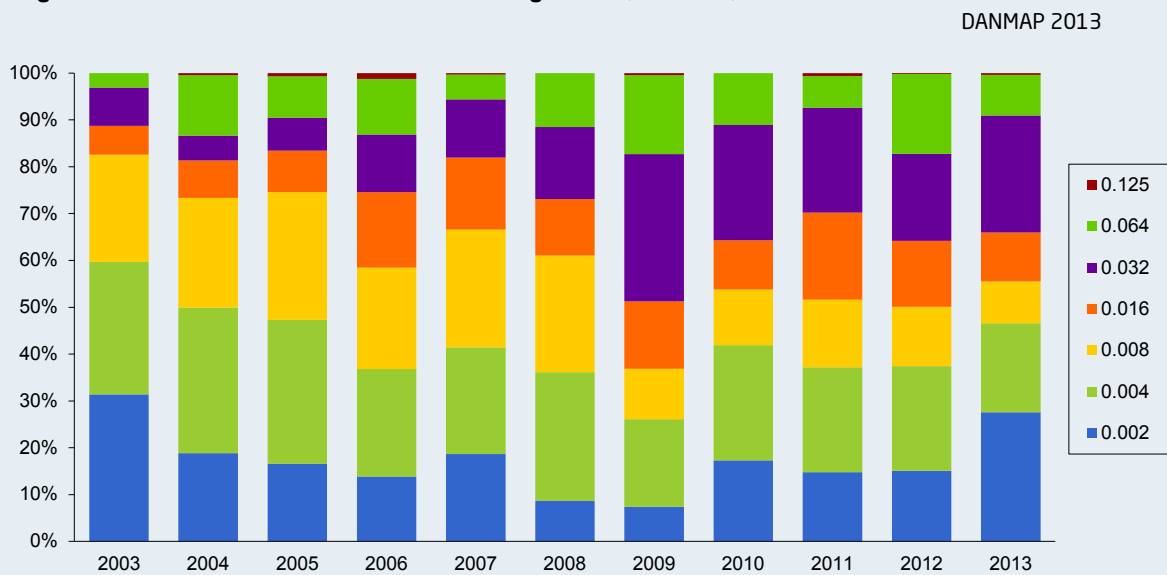


Table 1. Resistance rates (%) against azithromycin, cefixime and spectinomycin in gonococci, Denmark, 2009-2013

Antimicrobial agent	DANMAP 2013				
	2009	2010	2011	2012	2013
Azithromycin	46	23	12	12	45
Cefixime	15	18	21	11	9
Spectinomycin	0	0	0	0	0
Number of isolates	119	111	126	114	111

8.6 *Staphylococcus aureus*

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

In Denmark, a voluntary surveillance programme of all *S. aureus* bacteraemia cases was established in 1957. Methicillin-resistant *S. aureus* (MRSA) has been both laboratory and clinical notifiable since November 2006. At SSI, all isolates are typed by *spa* typing and epidemiological information registered. Based on this information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO) and import (IMP). MRSA belonging to clonal complex 398 (CC398) has in recent years attracted special attention as this type has been closely connected to livestock animals, especially pigs, and increasingly affects people in direct contact with pigs.

The CC398 cases have in the past years constituted an increasing part of the CA cases and due to these increasing numbers, cases belonging to CC398 are analysed as a separate group as both epidemiology and exposition are different.

Surveillance of bacteraemia

In 2013, 1,769 *S. aureus* bacteraemia cases, corresponding to 32.9 per 100,000 inhabitants, were reported from the Departments of Clinical Microbiology (DCM) in Denmark. This is somewhat higher than in the previous five years (c. 1,500 annual cases) and may likely reflect underreporting in previous years rather than a true increase. Thirty (1.7%) of the cases were caused by MRSA. This is at the same level as in previous years, and very low compared to most other countries reporting to EARS-Net [EARS-Net 2012].

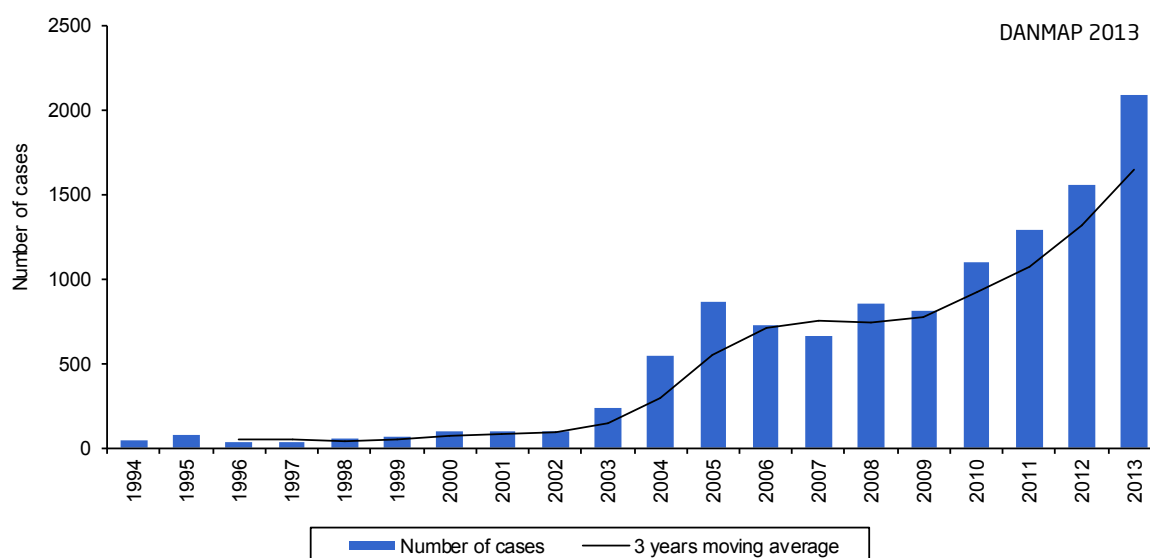
Antimicrobial resistance in *S. aureus* bacteraemia isolates from 2008–2013 is presented in Table 8.3. The highest frequency of resistance to other than penicillin was observed for fusidic acid (15%), erythromycin (7%), clindamycin (6%) and norfloxacin (5%). Susceptibility to all tested antimicrobial agents was at the same level as in 2012, but both fusidic acid and norfloxacin resistance have increased steadily since 2008 (Table 8.3). Resistance to at least 1, 2 or 3 other antimicrobial agents in addition to penicillin was demonstrated in 22%, 8% and 4% of the cases, respectively.

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

Antimicrobial agent	2008	2009	2010	2011	2012	DANMAP 2013
	%	%	%	%	%	%
Methicillin	1.3	1.6	1.4	1.4	1.2	1.7
Penicillin	77	77	75	77	74	76
Erythromycin	5	7	5	7	6	7
Clindamycin	4	6	4	6	6	6
Tetracycline	3	2	3	2	2	3
Fusidic acid	9	9	13	13	14	15
Rifampicin	<1	<1	<1	<1	<1	0
Norfloxacin	2	2	3	4	4	5
Kanamycin	1	1	1	<1	1	2
Linezolid	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	<1	1	1
Number of isolates	1344	1480	1418	1525	1528	1769

Note: nt = not tested.

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



Surveillance of methicillin-resistant *S. aureus*

In 2013, 2,094 new MRSA cases were detected (37.4 per 100,000 inhabitants). This is the highest number of cases observed in over 25 years (Figure 8.9). A case was a person found positive for the first time with a specific MRSA strain regardless of whether the patient was infected or colonised only.

In 2013, the number of MRSA increased by 31% compared to 2012. The number of new cases was more than three times as high as in 2007. In 2013, 24 persons were found with their second case of MRSA (i.e. MRSA of a new subtype). At the time of diagnosis, 45% (n = 940) of cases had infection which is lower than in 2012 (57%). CC398 cases increased substantially in 2013 and constituted 31% of new MRSA cases in 2013 (Table 8.4). The increase was for a large part due to inclusion of contact to pigs as a risk factor in December 2012, requiring screening for MRSA when admitted to hospitals. This also explains the lower fraction of infections seen for CC398 compared to other CC groups. See also Textbox 10.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.4. Most of the cases (83%) were acquired in Denmark. The epidemiological classification of MRSA infections 2007–2013 is shown in Figure 8.10. Despite the increasing total number of cases, both the number of hospital-acquired infections and the number of healthcare-associated with community onset (HACO) infections were at a stable very low level. The number of CA infections continued the increasing trend in 2013 and was by far the largest group (n = 404) (Figure 8.10). The proportion of bloodstream infections with MRSA was 1.7% in 2013 (see surveillance of *S. aureus* bacteraemia).

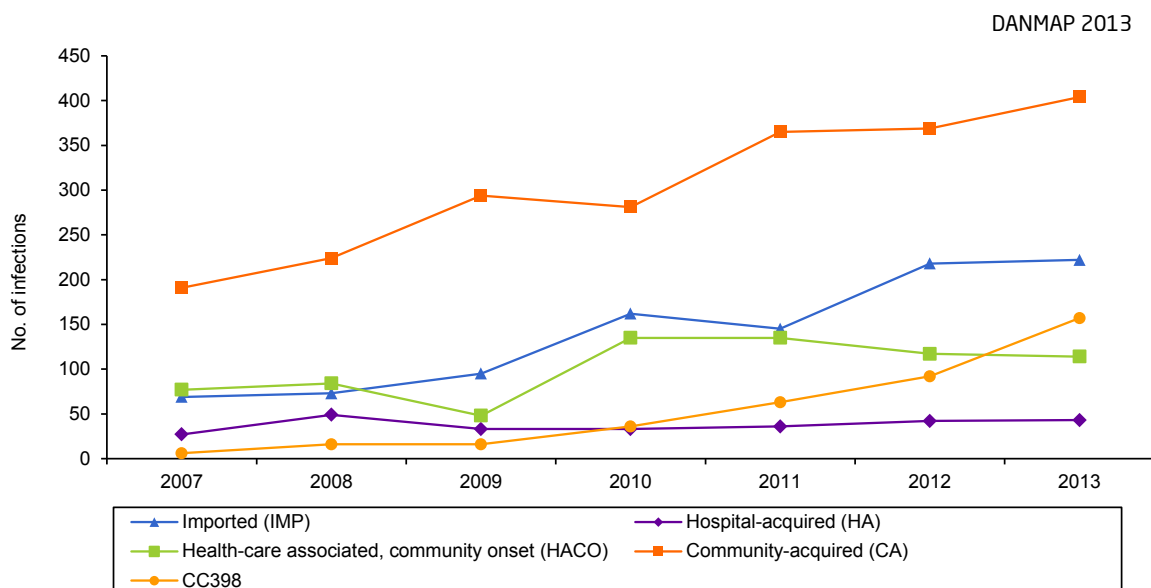
Table 8.4. Epidemiological classification of new MRSA cases, Denmark

Epidemiologic classification	Exposure	2012		2013	
		No. of cases (% of total)	No. (%) of cases with infections	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		324 (21)	218 (67)	366 (17)	222 (61)
Hospital-acquired (HA)		67 (4)	42 (63)	52 (2)	27 (52)
Healthcare associated, community onset (HACO)	with healthcare risk	178 (11)		158 (8)	
	with known exposure	60	24 (40)	39	17 (44)
	without known exposure	118	88 (75)	119	96 (81)
Healthcare worker		29 (2)	5 (17)	40 (2)	17 (43)
Community-acquired (CA)	without healthcare risk	726 (47)		834 (40)	
	with known exposure	378	69 (18)	408	75 (18)
	without known exposure	348	300 (86)	426	329 (77)
CC398		232 (15)	92 (40)	643 (31)	157 (24)

Note: Numbers shown in bold are totals

Unresolved cases in 2013 (notifications not received): 1

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



Molecular typing of the MRSA strains

In total, *spa* typing revealed 270 different strain types of which 194 types were associated with clinical infections. The number of isolates belonging to the 10 dominating *spa* types isolated in 2013 is shown in Table 8.5. They constituted 61% of the total number of MRSA isolates. Ten *spa* types constituted 56% of the 940 clinical infections with MRSA. Most prevalent *spa* types causing clinical infections at time of presentation were t034 (n = 126), t002 (n = 90), t008 (n = 72), t019 (n = 67), t127 (n = 43), t024 (n = 28), t044 (n = 28), t304 (n = 26), t437 (n = 25) and t032 (n = 21). The PVL encoding gene *lukF-PV* was demonstrated in 36% of the infections and in 14% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 97), t019 (n = 90), t002 (n = 59) and t044 (n = 45).

Resistance among MRSA isolates

The resistance patterns varied considerably between *spa* types (Table 8.6). In 2013, 100% of CC398 *spa* type t034 isolates were resistant to tetracycline and 94% of t034 were resistant to clindamycin. In contrast, the majority of t019, a primarily community-acquired *spa* type, and t304 were susceptible to all tested antimicrobial agents except for beta-lactams. It is noteworthy that 47% (304 of 643) of CC398 isolates had the unusual phenotype of being susceptible to erythromycin while resistant to clindamycin. Whole genome sequencing of 25 CC398 isolates showed that this was due to presence of the *lnu(B)* gene – a gene previously reported in MRSA CC398 strains from Spain, Portugal and USA. Whether *lnu(B)* is responsible for this phenotype in general needs to be investigated. Resistance to at least 1, 2 or 3 other antimicrobial agents in addition to β -lactam antibiotics (cefoxitin/penicillin) was demonstrated in 73%, 62% and 43% of the cases, respectively.

Andreas Petersen, Robert L. Skov and
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Table 8.5. The ten most prevalent *spa* types demonstrated in MRSA cases, Denmark

DANMAP 2013			
<i>spa</i> type	CC group ^(a)	No. of cases	No. causing infections (%)
t034	CC398	527	126 (24)
t002	CC5	161	90 (56)
t008	CC8	113	72 (64)
t019	CC30	92	67 (73)
t127	CC1	87	43 (49)
t304	CC8	76	26 (34)
t011	CC398	73	19 (26)
t032	CC22	49	21 (43)
t223	CC22	48	16 (33)
t044	CC80	47	28 (60)

a) CC = Clonal complex

Table 8.6. Resistance (%) in the six most prevalent *spa* types demonstrated in MRSA cases compared with all MRSA cases, Denmark 2013

DANMAP 2013							
<i>spa</i> type	t034	t002	t008	t019	t127	t304	All cases
Clonal complex	CC398	CC5	CC8	CC30	CC1	CC8	
	%	%	%	%	%	%	%
Erythromycin	44	33	54	0	61	1	36
Clindamycin	94	26	12	0	57	1	44
Tetracycline	100	8	10	0	59	1	45
Fusidic acid	3	27	6	0	26	4	13
Rifampicin	0	0	0	0	1	0	1
Norfloxacine	28	19	51	5	11	3	24
Kanamycin	7	17	50	2	68	3	23
Linezolid	<1	<1	0	0	0	0	<1
Mupirocin	0	0	1	2	0	0	<1
Trimethoprim-sulfamethoxazole	1	0	0	0	2	0	3
Number of isolates	527	161	113	92	87	76	2094

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) among humans in 2013

Background: Since 2003, livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has emerged worldwide. LA-MRSA primarily belong to clonal complex 398 (CC398) and is especially associated with pigs. In humans MRSA CC398 has especially been found in persons with contact to pigs.

A new type of MRSA carrying a different resistance determinant *mecC* has been found in humans, cattle and sheep in Denmark and internationally but do not appear to have the same close relationship to animals as CC398 [García-Álvarez *et al.* 2011. *Lancet Infect Dis.* 11: 595–603].

Livestock associated MRSA in humans: The number of persons positive for MRSA CC398 steeply increased to 643 cases in 2013 (42 in 2009, 111 in 2010, 164 in 2011 and 232 in 2012). A significant part of the increase was associated with inclusion of contact to pigs as a risk factor requiring screening for MRSA when admitted to hospitals in December 2012 [<http://sundhedsstyrelsen.dk/publ/Publ2012/11nov/MRSAvej2udg.pdf>]. The most frequent *spa* type related to CC398 was type t034 (n = 527). The majority of CC398 cases (n = 562, 87%) were in persons with documented close contact to pigs or being a household member to a person with direct contact. This resembles the proportion from previous years, which means that the actual number of CC398 cases without documented contact to livestock increased concordantly from the previous years. There were however, no signs of significant spread of CC398 to urban areas.

One hundred and fifty-seven CC398 cases (24%) presented with infections. In 2013, four bacteraemia cases were MRSA CC398. Two of the patients died within 30 days. In both cases, the patients had severe comorbidity. The patients did not have any direct contact to pigs.

MRSA isolates carrying the new *mecA* homologue *mecC*, were demonstrated in 41 cases (2%) in 2013 (9 in 2009, 21 in 2010, 37 in 2011, and 24 in 2012). Twenty-eight of the cases (68%) had infections, including one bacteraemia at the time of diagnosis. Only one possible livestock contact was registered for the 41 *mecC* cases.

In 2013, no animal surveillance of LA-MRSA was performed, but sampling at farm level is ongoing and expected to be finalized in 2014.

Conclusions: In 2013, a steep increase in CC398 cases was detected and CC398 became the most common CC group among human MRSA cases. An increasing number of CC398 cases had no documented contact to pigs. The number of MRSA isolates carrying the *mecC* gene seems to have stabilized.

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9. Materials and methods

9.1 General information

For the DANMAP 2013 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

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

Unless stated specifically, all differences and temporal trends noted in the text are statistically significant ($p < 0.05$) using either Chi-square tests or linear logistic regression (see Section 10.6.4).

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 (two in 2011) were permitted - on certain conditions (identical conditions as for pharmacies) - to sell prescribed veterinary medical products for animals. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration costs related to sale of large quantities to the veterinarians.

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

In 2013, the animal owners and veterinarians purchased the antimicrobial agents almost equally between the pharmacies and the veterinary drug trading companies, while only 2% was purchased from the feed mills. Sales from feed mills additionally comprised zinc chloride for the pig production on veterinary prescription, and non-prescription sales of coccidiostatic agents for domestic fowl (*Gallus gallus*).

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

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

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" - a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www.whocc.no]. See further description of the ADD system in the DANMAP 2009 report [www.danmap.org].

Methods

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

The animal daily doses (ADDs) used in previous DANMAP reports are an integrated part of the VetStat database and have been described elsewhere [DANMAP 2009; Jensen *et al.*, *Prev Vet Med.* vol: 64, 201-215, 2004]. In principle, the ADD should be identical for all products within medicinal groups and defined by the active compound, route of administration and the formulation. However, in VetStat, the ADDs are defined by the dosage level that was part of the product registration, and over time doses for products within the same medicinal group sometimes have changed. The greatest variation occurs when the ADD for some products have been defined solely based on the approved dosage. For example, the approved dosage of a product registered in 2011 may differ from a similar product registered in 2001.

In DANMAP 2012, we therefore introduced two new metrics to follow trends in antimicrobial consumption to ensure robustness of the analyses over time. The new metrics, DADD and DAPD, are defined below in the highlighted boxes.

DADD - Defined animal daily dose

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

The DADDs used in DANMAP 2013 are presented in the web annex.

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

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

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

Denominator

Trends in veterinary consumption, both within and across species, are presented in DADD per 1,000 animals per day – DAPD. The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live body-weight and the average life-span of the species.

DAPD - DADD per 1,000 animals per day

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

DAPD is a statistical measure, 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 (30% of pigs produced in 2013, 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 bodyweight, 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.

Broiler and layer production (*Gallus gallus*).

The live biomass is estimated based on number of broilers produced (Table 3.1), and an average live weight at slaughter of

1.97 kg [Statistics Denmark, 2013] after an estimated average life span of 30 days. The mean live biomass per broiler is assumed to be half of the weight at slaughter. In addition, the biomass of the parental animals (rearing and breeding) for the broiler production was estimated for 2011 based on number of hens per year (2,9 mill) annual rotations, length of the empty periods, percentage of cocks, and average weight of the cocks and hens in rearing and in breeding [DANHATCH, 2013; S. Kabell, Danish Agriculture and Food Council, personal communication]. For the other years, the biomass of rearing and breeders was assumed to be proportionate to the broiler production as in 2011.

In the layer production chain, the biomass of the parent flocks is estimated separately for each production type, based on number of eggs produced, eggs per hen, and average production length [Danish Poultry Producers, Statistics Denmark]. We have assumed an average weight of 2.3 kg per hen, based on slaughter weight [Statistics Denmark, 2013].

Turkey production. The live biomass is estimated based on the number of turkeys produced (Table 3.1) and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Pig production. The estimation was based on number of pigs produced, including exports at different ages [Statistics Denmark; Danish Agriculture and Food Council], productivity data for each year [Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and lifespan 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, 2014] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Fur animals. The live biomass of mink is estimated from production data [Statistics Denmark, 2013; Copenhagen Fur, 2013] and the average weight at pelting was 2.45 kg [Kopenhagen Fur, 2013]. The progeny live for approximately 7 months. The biomass for the breeding animals (female) was estimated based on census data and an assumed average live weight of 2 kg.

Pet animals. Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The 2011 population is based on census data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been

relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture. The estimation is based on data from the Danish AgriFish Agency (NaturErhvervstyrelsen) on produced amounts in each subtype of production, and information on the typical lifespan and entrance and exit body weights, and were calculated in cooperation with Danish Aquaculture [NH Henriksen, Danish Aquaculture]. Data from 2012 were not available at the time of publication.

9.2.3 Data on antimicrobial consumption in humans

Data on consumption of antibacterial agents in humans were obtained from Statens Serum Institut (SSI), National Register of Medicinal Products Statistics. SSI has the legal responsibility for monitoring the consumption of all human medicinal products. This is performed by monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies, to SSI. Data from the primary healthcare sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

Certain categories of hospitals were excluded when the consumption was measured by occupied bed-days and admissions. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

In Denmark, all antimicrobial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code, which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDDs), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and place (pharmacy, hospital pharmacy, institution) of the transaction, and information regarding reimbursement of cost, if applicable. The data are transferred monthly to SSI in an electronic format.

From DANMAP 2012 and onwards, the consumption of certain infusion substances, such as cephalosporins, carbapenems and trimethoprim, has been directly reported by the hospital pharmacies to SSI. In previous DANMAP reports, the consumption of these substances was corrected by direct data collection from all Danish hospital pharmacies; however, since DANMAP 2012 all data were delivered only by SSI.

The present report includes data on the consumption of antibacterial agents for systemic use, or group J01, of the 2013 update of the ATC classification, in primary healthcare and in hospitals. As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary healthcare is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitant-days). Consumption in primary healthcare 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 healthcare, 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 animals are collected from healthy production animals randomly selected at slaughter. From pigs, isolates of *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis*, and thermophilic *Campylobacter* spp. were collected. From cattle, isolates of *E. coli* and thermophilic *Campylobacter* spp. were collected, and from broilers isolates of *E. coli*, thermophilic *Campylobacter* spp., *E. faecalis* and *E. faecium* were collected. In addition, isolates of *E. coli* O149, were collected from diagnostic submissions.

Campylobacter spp., indicator *E. coli* and enterococci. Samples from healthy pigs, cattle and broilers were collected for the DANMAP programme at slaughter by meat inspection staff or abattoir personnel and sent for examination at DTU National Food Institute. For broilers, cloacal swabs were collected weekly from May through October and the sampling programme represented 86% of all broiler farms in Denmark. A Danish broiler farm is typically comprised of more than one unit each generating several flocks per year, but even though a farm was sampled more than once through the sampling period, only one isolate per farm of each bacterial species was included.

For pigs and cattle, the slaughter plants included in the DANMAP programme accounted for 99% and 95% of the total number of animals slaughtered in Denmark during 2013, respectively. The number of pigs and cattle samples from each slaughter plant was proportional to the annual number of animals slaughtered at the plant. Samples were collected once a month from January through November as caecum samples from pigs and rectum samples from cattle. Only one isolate per farm of each bacterial species was included.

Accordingly, the bacterial isolates from the Danish production animals may be regarded as representing a stratified random sample of the respective populations, and the observed prevalence of resistant isolates provides an estimate of the true occurrence in the population.

An overview of the number of samples analysed, isolates obtained and MIC determinations performed for pigs, cattle

and broilers is presented in Table 9.1. The isolation rates of *C. jejuni* from pigs and *C. coli* from cattle and broilers were low and therefore MIC-analyses were not performed.

Salmonella. National Food Institute DTU is the national reference laboratory for *Salmonella* in animals and food and receives all isolates for typing. Only one isolate per serotype per farm was selected for the DANMAP report, except for isolates from broilers, where one isolate per flock was included. Isolates of *S. Typhimurium* include the monophasic variants with antigenic formulas *S.* 4,5,12:i:- and *S.* 4,12:i:-.

The *Salmonella* isolates from pigs originated both from the random sampling of healthy animals at slaughter for DANMAP and from the national *Salmonella* surveillance programme where the results of a serological surveillance at the slaughterhouses and in all breeding herds appoint risk herds to be further examined by microbiological analysis of pen-faecal samples.

No *Salmonella* data from layers, broilers and cattle are presented in DANMAP 2013 due to the low findings of serotype *S.* Enteritidis and *S. Typhimurium*. *Salmonella* isolates from diagnostic submissions were not included in DANMAP 2013.

Further details on the sampling procedures and the findings of the Danish *Salmonella* surveillance programs are presented in Textbox 5, and in the Annual Report on Zoonoses in Denmark, 2013 [www.food.dtu.dk].

Isolates from diagnostic submissions were specifically collected for the DANMAP programme at the Laboratory of Swine Diseases, the Danish Agriculture and Food Council, Kjellerup (*E. coli* O149 from diarrheic pigs). Only one isolate per farm was included.

9.3.2 Meat

Campylobacter, indicator E. coli and enterococci. The meat isolates originated from meat samples collected at wholesale and retail outlets in all regions of Denmark. All samples were collected by the Danish Veterinary and Food Administration's (DVFA) Food Control Offices and were collected during the routine inspection by the authorities or on specific request from the for the DANMAP programme. The sampling included both Danish and imported meat. Only one isolate per bacterial species per meat sample was selected for DANMAP.

Salmonella. The *Salmonella* isolates from Danish pork originated from the national *Salmonella* surveillance programme (swab samples from pork and beef carcasses taken at the slaughterhouse after cooling). *Salmonella* isolates from imported poultry meat and other imported fresh meats derived from a case-by-case risk assessment control programme (DFVA), are not presented due to the low number of isolates (<15). Further details on findings are presented in Textbox 5. Only one isolate per positive swab sample or batch of meat was included for DANMAP. Isolates of *S. Typhimurium* include the monophasic variants, antigenic formula *S.* 4,5,12:i:- and *S.* 4,12:i:-.

9.3.3 Humans

S. Typhimurium, S. Enteritidis and C. jejuni. Antimicrobial susceptibility was performed on human faecal isolates submitted

to Statens Serum Institut (SSI). *Campylobacter* isolates were submitted from Departments of Clinical Microbiology (DCM) covering three geographical regions: Northern Jutland, Funen and Roskilde/Køge. Information on travel history was obtained for the patients. *Salmonella* isolates were submitted from all DCM in Denmark. Exact figures of the proportion tested and the sampling strategy for the different species can be found in Sections 6.1 and 6.2.

***Staphylococcus aureus*.** All blood isolates were referred to the Staphylococcus reference laboratory at SSI on a voluntary basis. In November 2006, methicillin-resistant *S. aureus* (MRSA) became a notifiable disease in Denmark and it became mandatory to submit all MRSA isolates to the reference laboratory.

Invasive *Streptococcus pneumoniae*, *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all 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*, invasive *E. faecium* and invasive *E. faecalis*.** Data were provided on all isolates recorded from either blood samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecium* and *E. faecalis*) or urine samples (*E. coli* and *K. pneumoniae*) submitted for susceptibility testing to the DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev/Hillerød, Region Zealand, Odense, Esbjerg, Vejle, Herning/Viborg, Aarhus and Aalborg. No samples were collected from healthy humans.

9.4 Isolation and identification of bacteria

9.4.1 Animals

***Campylobacter*.** The samples from broilers was inoculated directly onto mCCD agar (Oxoid, Denmark) and incubated in microaerophilic atmosphere for 2-3 days at 41.5°C. For samples from cattle, selective enrichment in Preston broth at a ratio of 1:10 incubated in microaerophilic atmosphere for 24 h at 41.5°C was performed followed by inoculation of 10 µl of the enrichment broth to mCCD agar. *Campylobacter*-like colonies were verified by microscopy and species identification of *C. jejuni* and *C. coli* was performed by a real-time PCR assay [Mayr *et al.* 2010, J Food Prot. 73(2):241-50]. All isolates of *C. jejuni* and *C. coli* were stored at -80°C.

Indicator *E. coli*. The material was inoculated directly onto Drigalski agar (SSI Diagnostica, Denmark) and incubated o/n at 37°C. Yellow colonies were inoculated onto BBL CHROM agar Orientation Medium (Becton Dickinson, Germany) and red colonies were collected as *E. coli* after o/n incubation at 37°C. All isolates were stored at -80°C.

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* and *E. faecium* morphology were sub-cultivated on blood agar. *E. faecalis* and

E. faecium were identified by motility- and arginine dihydrolase tests and the ability to ferment mannitol, sorbitol, arabinose and raffinose. All isolates of *E. faecium* and *E. faecalis* were stored at -80°C.

9.4.2 Meat

Salmonella was isolated by the regional laboratories at the DVFA according to the open reference methods issued by the NMKL (NMKL No. 187, 2007 or NMKL No. 71, 1999), the ISO 6579:2002 or alternative methods validated against the reference method according to ISO 16140:2001. Sero-typing was performed at the National Food Institute, DTU.

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. Isolation and identification was performed by the laboratories at the DVFA in Ringsted, Denmark. All isolates of *C. jejuni*, *C. coli* and *C. lari* were sent to the National Food Institute, DTU for MIC-testing and storage at -80°C.

Indicator *E. coli* was isolated by the regional laboratories at the DVFA by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulphate-broth, which was incubated o/n at 44°C, subsequently streaked onto violet red bile agar and incubated for 24 h at 44°C. Presumptive *E. coli* was identified using TBX agar (β-glucuronidase activity) incubated o/n at 44°C. *E. coli* isolates were sent to DTU National Food Institute for MIC-testing and storage at -80°C.

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

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*.** Sequencing of the *S. aureus* specific *spa* gene was performed for species confirmation and typing. *Spa*-negative isolates were confirmed as *S. aureus* by MALDI-TOF. The *spa*-typing [Harmsen *et al.* 2003. J Clin Microbiol. 41: 5442–5448] and additional typing by multi locus sequence typing (MLST) [Enright *et al.* 2000. J Clin Microbiol. 38: 1008–1015] were annotated using eBURST v.3 software (www.mlst.net). Based on the *spa* and MLST typing, each isolate was assigned to a clonal complex (CC). For MRSA isolates, presence of the *mecA* or *mecC* methicillin resistance genes was confirmed by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611–614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395–400]. For all isolates, presence of *lukF-PV* gene (PVL) was demonstrated by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611–614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395–400].

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*, *Staphylococcus aureus* and the veterinary pathogens was performed as microbroth dilution MIC with Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, UK). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

The quality control strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560 were used.

Isolates from animals and meat were tested at DTU National Food Institute, and the *Salmonella*, *Campylobacter* and *Staphylococcus aureus* isolates of human origin were tested at SSI. MIC-testing at DTU National Food Institute is accredited by DANAK (the national body for accreditation).

One isolate per bacterial species per farm, per meat sample or per patient was tested for antimicrobial susceptibility. For *Salmonella* isolates from poultry, one isolate per serotype per flock was tested. For isolates in excess numbers (e.g. isolates from healthy animals), a random selection was appointed to MIC.

Table 10.2 presents the interpretation of MIC-values used for any combination of bacteria and antimicrobial agent. Since 2007, data were interpreted by EUCAST epidemiological cut-off values (ECOFFs) with a few exceptions described in Table 10.2. The corresponding clinical breakpoints validated by EUCAST are presented both in Table 10.2 and in the MIC-distribution tables to visualize the impact of the use of ECOFFs contra clinical breakpoints. In general, if ECOFFs were re-validated and changed by EUCAST during the past year, all data presented from previous years were interpreted using the changed ECOFFs. 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 number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals. Multi-resistance was defined as resistance to three or more of the antimicrobial classes listed in Table 10.3. Isolates were considered fully sensitive if susceptible to all the antimicrobial agents included in the test.

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. As per September 2012, all DCM except Rigshospitalet used breakpoints defined by EUCAST.

Data on antimicrobial resistance from private hospitals and clinics and from psychiatric hospitals were excluded. All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

9.6 Data handling

9.6.1 Animal

The results from the analysis of all animal samples – positive as well as negative findings – and of the bacteria isolated and the susceptibility testing were stored in an Oracle Database 9i Enterprise Edition® at DTU National Food Institute. The susceptibility data were stored as continuous values as well as categorised as susceptible or resistant as defined by the relevant ECOFF. Each isolate was identified by the bacterial species, the subtype as applicable and by the date of sampling and species of animal. Information on the farm of origin was also recorded. All handling and evaluation of results were carried out using SAS® Software, SAS Enterprise Guide 4.3.

9.6.2 Meat

Results from the analysis of food samples were reported via the database administrated by the DVFA, except for the data on *Salmonella*, which were reported to and extracted from the laboratory database at the National Food Institute, DTU. For each bacterial isolate, information was available on food type, bacterial species, date and place of sampling, date of examination, country of slaughter, and an identification number, which makes it possible to obtain further information about the isolate from the relevant authorities. Furthermore, information about the country of origin was recorded whenever possible.

9.6.3 Human

Salmonella and Campylobacter. Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

Staphylococcus aureus. For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of whether it was colonisation or infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonization/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonization), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 12 months as either healthcare associated with community onset (HACO) or community-acquired (CA). Healthcare associated risk factors included prior hospitalizations or stay in long-term care facilities within 12 months prior to MRSA isolation and being a healthcare worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398 were treated separately as both epidemiology and exposition 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 placed on a SQL server at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

E. coli, K. pneumoniae, invasive P. aeruginosa, invasive E. faecium and invasive E. faecalis. 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 and 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.

9.6.4 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 4.3 or StatCalc in EpiInfo™ v. 6. Difference in pair-wise comparisons were tested using Chi-square, or Fisher's Exact Test when the number of samples is low (<25). When appropriate, significance of temporal trends is tested using linear logistic regression using Proc LOGISTIC procedure in SAS (Likelihood ratio test).

In the text, commented differences imply statistically significant differences where $p < 0.05$.

When comparing proportions between years, the EUCAST epidemiological cut-off values for 2013 were also used for interpretation of previous years MICs.

Jeppe Boel and Line Skjøt-Rasmussen

Table 9.1. Number of DANMAP samples, number of isolates and MIC-tests from healthy production animals at slaughter, Denmark

		DANMAP 2013		
		<i>E. coli</i>	<i>E. faecalis</i>	<i>C. jejuni</i>
Pigs ^(a)	No. of samples analysed (1 per farm)	260	709	0
	No. of isolates obtained	258	134	0
	No. of isolates MIC-tested/reported	146	109	0
Cattle	No. of samples analysed (1 per farm)	166	0	166
	No. of isolates obtained	150	0	95
	No. of isolates MIC-tested/reported	103	0	86
Broilers	No. of samples analysed (no. of flocks)	165	165	165
	No. of farms represented	150	134	61
	No. of isolates MIC-tested/reported	125	114	54

Note: Data in this table should not be used for reporting of prevalences of the bacterial species

a) From 2011, the DANMAP samples from pigs were also part of the surveillance programme for *Salmonella*

Table 9.2. Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values (blue fields) and the corresponding EUCAST clinical breakpoints (white fields)

Antimicrobial agent	<i>Salmonella</i>		<i>E. coli</i>		<i>E. faecium</i>		<i>E. faecalis</i>		<i>C. jejuni</i>		<i>C. coli</i>	
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8*	>8*	>8*	>8*	>4*	>8*	>4*	>8*				
Apramycin	>16		>16									
Cefotaxime	>0.5*	>2*	>0.25*	>2*								
Cefoxitin												
Ceftiofur	>2*		>1*									
Chloramphenicol	>16*	>8*	>16*	>8*	>32*		>32*		>16*		>16*	
Ciprofloxacin	>0.06*	>1*	>0.06*	>1*	>16 ^(b)		>8 ^(b)		>0.5*	>0.5*	>0.5*	>0.5*
Colistin	>2*/>8 ^(c)	>2*	>2*	>2*								
Erythromycin					>4*		>4*		>4*	>4*	>8*	>8*
Florfenicol	>16*		>16*									
Gentamicin	>2*	>4*	>2*	>4*	>32*		>32*		>2*		>2*	
Kanamycin					>1,024		>1,024					
Linezolid					>4*	>4*	>4*	>4*				
Nalidixic acid	>16*		>16*						>16*		>16*	
Neomycin	>4*		>8*									
Penicillin					>16*		>16*					
Quinupristin/ dalfopristin					>4 ^(a)	>4*						
Salinomycin					>4		>4					
Spectinomycin	>64		>64*									
Streptomycin	>16*		>16*		>128*		>512*		>4*		>4*	
Sulfonamide	>256		>64*									
Teicoplanin					>2*	>2*	>2*	>2*				
Tetracycline	>8*		>8*		>4*		>4*		>1*	>2*	>2*	>2*
Tiamulin												
Tigecycline					>0.25*	>0.5*	>0.25*	>0.5*				
Trimethoprim	>2*	>4*	>2*	>4*								
Vancomycin					>4*	>4*	>4*	>4*				

* EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints. Changes in ECOFF values since DANMAP 2011 are highlighted by orange.

a) The EUCAST ECOFF (>1) was not applied for quinopristin/dalfopristin (tradename synercid) according to investigations presented in DANMAP 2006.

b) The EUCAST ECOFF (>4) was not applied for ciprofloxacin. The aim was to look for high level ciprofloxacin-resistance as described by Werner et al, 2010 (Int J Antimicrob Agents;35:119-125). DANMAP 2006.

c) The EUCAST ECOFF (>2) for colistin was applied for *S. Typhimurium* and other serotypes, except for *S. Enteritidis* and *S. Dublin* where ECOFF >8 was applied as recommended by Agerstøe et al, 2011 (DANMAP 2011, Textbox 6).

Table 9. 3. Definitions of antimicrobial classes for calculation of multi-resistance (MR) in zoonotic and indicator bacteria, DANMAP
DANMAP 2013

Antimicrobial classes	<i>Salmonella</i> and <i>E. coli</i> ^(a)	<i>Campylobacter</i> ^(b)	<i>Enterococcus</i> ^(a)
Tetracyclines	Tetracycline	Tetracycline	Tetracycline
Phenicoles	Chloramphenicol and/or florfenicol	Chloramphenicol	Chloramphenicol
Penicillins	Ampicillin		Ampicillin and/or penicillin
Cephalosporins	Ceftiofur and/or cefotaxime		
Sulfonamides	Sulphonamides		
Trimethoprim	Trimethoprim		
Aminoglycosides I	Gentamicin	Gentamicin	Gentamicin and/or kanamycin and/or streptomycin
Aminoglycosides II	Streptomycin	Streptomycin	
Quinolones	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin and/or nalidixic acid	Ciprofloxacin and/or nalidixic acid
Polymycins	Colistin		
Macrolides		Erythromycin	Erythromycin
Glycopeptids			Vancomycin and/or teicoplanin
Ionophores			Salinomycin
Oxazolidinones			Linezolid
Glycylcyclines			Tigercycline

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the panel for the selected bacterial species

a) An isolates is considered multi-resistant if resistant to three or more of the ten antimicrobial classes

b) An isolates is considered multi-resistant if resistant to three or more of the six antimicrobial classes



List of abbreviations

DADD	Defined animal daily dose
DAPD	Defined animal daily dose per 1,000 animals per day
AGP	Antimicrobial growth promoter
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
CC	Clonal complex
CHR	Central Husbandry Register
CI	Confidence interval
CNS	Central nervous system
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1000 inhabitant days)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	Extended spectrum beta-lactamase
GI	Gastrointestinal
GP	General practitioner
HLGR	High-level gentamicin resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
RFCA	Regional Veterinary and Food Control Authorities
SSI	Statens Serum Institut
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin resistant enterococci
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification. International classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) (www.whocc.no/atcddd/indexdatabase/). The ATC classification for veterinary medicinal products, 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 1.97 kg.

Central Husbandry Register (CHR). This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined animal daily dose (DADD). DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage

in the Summaries of Product Characteristics (SPC). In contrast to the ADD previously used in DANMAP, the DADD has not been defined for each antimicrobial agent, administration route and animal species but at product level. In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which has been used since DANMAP 2003. For more details, see Chapter 9, Materials and Methods. The DADDs used in DANMAP 2013 are presented in the web annex.

DADD per 1,000 animals per day (DAPD). Trends in veterinary consumption, both within and across species, are presented in DAPD, allowing for comparison between sectors and adjusting for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight and lifetime. This may also be referred to as the 'standard-animals-at-risk' and takes into account species differences in body-mass and lifespan. 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 9, 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 healthcare, 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 9.3, 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 until 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 2013

