DANMAP 2009

DANMAP 2009 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark

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Introduction

This report, DANMAP 2009, describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs. This years report starts with a focus area on ESBL-producing bacteria in pigs, retail meat and human patients. MIC tables and some trend figures are presented in Appendix 1. In addition to the monitoring of antimicrobial resistance and consumption of antimicrobial agents, includes considerable research activities is associated with the DANMAP programme. A few selected research projects are presented as textboxes. Appendix 3 provides a more comprehensive list of DANMAP publications in the international scientific literature.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, was established in 1995 on the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries, as a coordinated national surveillance and research programme for antimicrobial consumption and antimicrobial resistance in bacteria from animals, food and humans. The participants in the programme are Statens Serum Institut, the National Veterinary Institute DTU, the National Food Institute DTU, and the Danish Medicines Agency.

The objectives of DANMAP are:

- To monitor the consumption of antimicrobial agents for food animals and humans
- To monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans
- To study associations between antimicrobial consumption and antimicrobial resistance
- To identify routes of transmission and areas for further research studies

The monitoring of antimicrobial resistance is based on three categories of bacteria: human and animal pathogens, zoonotic bacteria and indicator bacteria. Human and animal pathogens are included because these cause infections and they primarily reflect resistance caused by use of antimicrobial agents in the respective reservoirs. Zoonotic bacteria are included because they can develop resistance in the animal reservoir, which may subsequently compromise treatment effect when causing infection in humans. Indicator bacteria are included due to their ubiquitous nature in animals, foods and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in both reservoirs.

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Statens Serum Institut would like to thank the Danish Medicines Agency for providing data on consumption of antimicrobials in humans, and the clinical microbiology laboratories in the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples.

List of abbreviations and terminology

List of abbreviations

ACD	Defined Animal Course Dose
ADD	Defined Animal Daily Dose
ADDkg	Defined Animal Daily Dose per kg animal
AGP	Antimicrobial Growth Promoter
ATC	Anatomical Therapeutic Chemical
CHR	Central Husbandry Register
CI	Confidence Interval
CNS	Central Nervous System
CPR	Danish Civil Registry
DBD	Defined Daily Doses per 1,000 occupied bed-days
DCM	Department of Clinical Microbiology
DID	Defined Daily Doses per 1,000 inhabitants per day
DDD	Defined Daily Dose
DMA	Danish Medicines Agency
DVFA	Danish Veterinary and Food Administration
EARSS	The European Antimicrobial Resistance Surveillance System
ESBL	Extended spectrum Beta Lactamases
GAS	Group A Streptococcus
GI	Gastrointestinal
GP	General Practitioner
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World organisation for animal health
PMWS	Postweaning multisystemic wasting syndrome
RFCA	Regional Veterinary and Food Control Authorities
SSI	Statens Serum Institut
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
WT	Wild type

List of words

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

Antibacterial agents. Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppresses bacterial growth or reproduction (Source: Dorland's Illustrated Medical Dictionary). Antimycobacterial agents are not included. Only antibacterial agents for systemic use are included (J01 in the ATC system) in the section on human consumption.

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 substancess 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).

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.66 kg.

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

Defined Daily Dose (DDD). This is the assumed average maintenance dose per day in adults. It should be emphasized that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (http://www.whocc.no/atcddd/indexdatabase/). DDD/1,000 inhabitant-days is called DID.

Defined Animal Daily Dose (ADD and ADDkg). This is a national veterinary equevalent to the DDD. This is an assumed average daily dose per animal, defined as the daily maintenance dose for a drug used for its main indication in a specified species. The dose is defined for a „standard animal", i.e. an animal with an estimated average weight within a specified age group. In VetStat, ADDs are calculated for each age group. Otherwise, the general principles for standardisation of dosage for animals are similar to that used by the WHO Collaborating Centre for Drug Statistics and Methodology to calculate Defined Daily Dose (DDD) in humans [Jensen VF *et al*., 2004. Prev. Vet. Med. 64:201-215]. The ADDkg is the ADD per kg animal. Consumption calculated in ADDkg allows summation of consumption across different age groups and animal species.

ESBL. In this 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.* JAC 63:1-4].

Finishers. Pigs from 30 kilogram live weight to time of slaughter at app.100 kilogram live weight.

Heifer. A young female cow before first calving.

Intramammaria. Antimicrobial agents for local application in the mammary gland (udder for the treatment of mastitis.

Intramammary syringe. A one dose applicator for use in the udder.

Layer. A hen raised to produce eggs for consumption.

Minimum Inhibitory Concentration (MIC). This is the lowest concentration of antimicrobial in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

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; does not include horses.

Piglet. The newborn pig is called at piglet from birth till they are permanently separated from the sow at 3–4 weeks of age. The weigh of the piglet at weaning is 7 kilogram.

Poultry. In the DANMAP reports poultry is used when antimicrobial resistance among bacteria from broilers and layers are reported together.

Rearing broilers. Parent flocks producing chickens for broiler production

Sows. Any breeding female pig, that has been served and is on the farm.

Steer. Castrated male cattle.

Weaners. Any pig 7–30 kilogram live weight.

Wild type. The typical form of an organism, strain, gene, or characteristic as it occurs in nature.

Sammendrag

Dette er den fjortende DANMAP rapport. DANMAP 2009 beskriver det årlige forbrug af antibiotika og forekomsten af resistens i forskellige reservoirs. Den kontinuerlige overvågning af antibiotikaresistens og -forbrug gør det muligt at analysere tendenserne i antibiotikaforbrug og -resistens over tid.

Fokusområde: Extended spectrum beta-lactamase producerende bakterier i danske svin, dansk og importeret kød samt patienter (side 19-22)
ESBL-producerende bakterier er resistente overfor bred-spektrede penicilliner, der ofte bruges til behandling, derfor er forekomsten af disse - selv på et lavt niveau - bekymrende. I 2009 blev prævalensen of disse bakterier undersøgt i kød fra svin og patienter.
Svin og kød: Ved slagtning havde 11 % af slagtesvinene ESBL-producerende *Escherichia coli*. I kød-prøverne var prævalensen lav, 0,7 – 3,4 %. I 36 % af det undersøgte importerede fjerkrækød blev der fundet ESBL-producerende *E. coli.* CTX-M-1 (66 %) var det oftest forekommmende gen blandt de ESBL-producerende *E. coli* fra svin, mens CMY-2 (48 %) var det oftest forekommende blandt *E. coli* fra importeret fjerkrækød. CTX-M-15, som ofte findes i *E. coli* fra mennesker, forekom i 2 % af svineisolaterne.
Patienter: Fra 2007 til 2009 steg prævalencen af ESBL-producerende *E. coli* og *Klebsiella pneumonia* signifikant (undtagen for *E. coli* fra blodinfektioner). ESBL-prævalencen hos *K. pneumonie* fra blodinfektioner steg til 14,6 %.

Den parallelle stigning i prævalensen af ESBL-producerende bakterier hos både dyr og mennesker indikerer, at antibiotika selektion finder sted i begge reservoirs og at fødevarebåret spredning af ESBL-producerende *E. coli* kan være årsag til nogle af de humane infektioner.

Antibiotikaforbrug
DANMAP præsenterer antibiotikaforbrug til mennesker og dyr på årsbasis. Lægemiddelstyrelsen har overvåget forbruget af receptordineret medicin på patientniveau siden begyndelsen af 1990erne. Siden 2001 er al anvendelse af receptordineret medicin til dyr registreret på dyreart, aldersgruppe og besætningsniveau i VetStat databasen på Veterinærinstituttet, Danmarks Tekniske Universitet.

Antibiotikaforbrug til dyr (side 25-35)
I 2009 nåede antibiotikaforbruget til dyr i Danmark op på 129,7 ton som følge af en stigning på 10,4 % sammenlignet med 2008. Stigningen skyldtes hovedsagligt et øget forbrug til svin.
Svin. De senere år er sket en stærk stigning i antallet af svin, der eksporteres ved ca. 30 kg legemsvægt, samtidig med et fald i antal slagtesvin, der bliver i DK indtil slagtevægt. Korrigeres for denne produktionsændring, er der i 2009 sket en stigning i antal antibiotika doser per svin produceret på 12.7 %. Antibiotikaforbruget til svin nåede i 2009 op på 4.9 ADD_{kg}/ kg svinekød produceret. Stigningen skete især i forbruget af tetracykliner (12 %), makrolider (16 %) og pleuromutiliner. Disse antibiotika bruges mest til masse-medicinering i foder eller drikkevand. Brugen af bredspektrede cephalosporiner til svin faldt med 25 % i 2009 sammenlignet med 2008.
Kvæg. Antibiotikaforbruget til kvæg nåede i 2009 ca. 15 ton og har været relativt stabilt på omkring 14-15 ton siden 2005. Mælkeproduktionen har været svagt stigende, mens kødproduktionen har været svagt faldende. Beta-laktamase følsomme penicilliner udgjorde 57 % af forbruget til systemisk behandling af køer. Penicilliner udgjorde også størstedelen af antibiotikaforbruget til behandling af yverbetændelse. I 2009 faldt forbruget af bredspektrede cephalosporiner til yverbehandling med 32 % og udgjorde hermed 13 % af yverbehandlingerne. Også brugen af cephalosporiner til systemisk behandling af kvæg faldt med 14,7 % i 2009.
Fjerkræ. I 2009 blev brugt 1070 kg antibiotika til fjerkræ mod 556 kg i 2008. Selv med en fordobling af antibiotikaforbruget i kyllingeproduktionen, er forbruget på 0.15 ADD_{kg} per kg kyllingekød produceret imidlertid fortsat lavt i forhold til andre dyrearter. Dette er også meget lavt i forhold til forbruget i kyllingeproduktionen i ikke-skandinaviske lande. For kalkuner steg antibiotikaforbruget med 165 % og nåede hermed 1.8 ADD_{kg} per kg kød produceret, hvilket er det højeste niveau siden 2002.
I akvakultur faldt forbruget med 4 % til 3300 kg i 2009 som følge af et fald i forbruget i havbrug, der generelt har et højt antibiotikaforbrug per kg kød produceret sammenlignet med andre produktionsdyr.

Antibiotikaforbrug til mennesker (side 41-51)

Primærsektor og hospitaler: Samlet set var det totale forbrug af antibiotika til systemisk brug i mennesker 17,9 DDD pr. 1000 indbyggere pr. dag (DID) i 2009, mod 17,8 DID i 2008. Siden 2000 er forbruget steget med 4,2 DID (31,1 %). Forbruget i primærsektoren udgør ca. 90 % af det totale forbrug.

Primærsektor: I 2009 var det totale antibiotikaforbrug (J01) 15,95 DID sammenlignet med 15,91 DID i 2008. Det lader til, at den kontinuerte stigning i forbruget i primærsektoren er aftaget. Bag denne aftagning ligger dog et faldende forbrug af beta-laktamase følsomme penicilliner (0,18 DID) og makrolider (0,08 DID), der modsvares af en stigning i forbruget af kombinationer af penicilliner/beta-laktamase hæmmere (0,18 DID) samt tetracykliner (0,07 DID). Beta-laktamase følsomme penicilliner (smalspektret) udgør stadig den største gruppe (32 % af det totale forbrug), efterfulgt af penicilliner med udvidet spektrum (21 %) og makrolider (14 %). Det totale antibiotikaforbrug, udtrykt i DID, steg med 31 % i årene 2000-2009.

Hospitaler: Det totale forbrug (J01) udtrykt i DDD pr. 100 sengedage (DBD) steg med 4,8 % (fra 74,56 i 2008 til 78,13 i 2009). Udtrykt som DDD pr. 100 udskrevne patienter steg det med 1,6 % fra 2007-2009 (fra 288,7 i 2007 til 293,3 i 2009). Forskellen afspejler kortere indlæggelser pr. patient men flere indlæggelser på hospitalerne. Med undtagelse af beta-laktamase følsomme penicilliner, aminoglykosider og imidazole derivater steg forbruget af alle betydende antibiotika stofgrupper. Cefalosporiner, hovedsageligt 2. generation, udgjorde 21 % af det totale forbrug i hospitalssektoren. Andre betydningsfulde antibiotikagrupper var penicilliner med udvidet spektrum (18 %), fluorkinoloner (13 %) og beta-laktamase følsomme penicilliner (12 %). Over de sidste 10 år (2000-2009) er det totale forbrug steget med 31,2 DBD (66,4 %).

Antibiotikaforbruget til mennesker var på samme niveau som de to foregående år, men der var en fortsat stigning i forbruget af de bredspektrede antibiotika (så som cefalosporiner, fluorkinoloner).
Den modsatte situation gjorde sig gældende i landbruget, hvor brugen af cefalosporiner faldt og brugen af fluorkinoloner var meget lav. Derimod var der en stigning i det totale veterinære forbrug – specielt til svin – blandt andet i forbruget af makrolider.

Resistens i zoonotiske bakterier (side 58-64)

Fra 2008 til 2009 blev der ikke observeret signifikante ændringer i antibiotikaresistens blandt ***Salmonella* Typhimurium** isolater fra danske svin. Blandt *S.* Typhimurium fra svinekød var resistensforekomsten højere i importeret svinekød sammenlignet med dansk. Der var adskillige udbrud af *S*. Typhimurium blandt mennesker i Danmark i 2009, de to største bestod af hhv. 212 og 83 tilfælde. Resistensforekomsten i humane *S*. Typhimurium tilfælde erhvervet i Danmark var generelt lavere end forekomsten i både dansk og importeret svinekød, hvilket delvist kan forklares af udbruddene. Der blev observeret en signifikant højere forekomst af nalidixan syre- og ciprofloxacin resistens i rejseassocierede tilfælde, sammenlignet med hjemmeerhvervede tilfælde.
***Salmonella* Enteritidis** er relativt sjælden i dansk fjerkræproduktion og blev i 2009 kun isoleret fra importeret kyllingekød. Blandt de testede isolater var specielt resistensen mod nalidixan syre og ciprofloxacin steget, og 49 % af isolaterne var resistente overfor nalidixan syre og ciprofloxacin. Resistensen overfor ampicillin, ciprofloxacin og nalidixan syre var signifikant højere i rejseassocierede tilfælde sammenlignet med tilfælde erhvervet i Danmark.

Fra 2008 til 2009 sås ingen signifikante ændringer i resistensforekomsten blandt *C. jejuni* fra danske kyllinger, *C. jejuni* fra kvæg eller blandt ***Campylobacter coli*** fra svin. Ciprofloxacin og nalidixan syre resistensen i *C. jejuni* fra danske kyllinger faldt signifikant i 2009 og nåede 0 %. Importeret kyllingekød indeholdt *C. jejuni* med signifikant højere resistens overfor ciprofloxacin (56 %) og nalidixan syre (56 %) og tetracyklin (52 %) sammenlignet med dansk kyllingekød. Resistensforekomsten for ciprofloxacin, nalidixansyre og tetracyklin var signifikant højere blandt rejserelaterede *C. jejuni* isolater sammenlignet med *C. jejuni* isolater fra infektioner erhvervet i Danmark.

Resistensovervågningen af de zoonotiske bakterier viser, at selvom det er fornuftigt at overvåge og begrænse resistensudviklingen i Danmark, er resistens-niveauet i dansk kød bedre end i kødet fra mange andre lande indenfor og udenfor Europa, som vi importerer fra.

Slagtesvin og kød (svine-, okse- og kyllingekød) blev undersøgt for forekomst af MRSA. Tretten % af svinene ved slagtning var positive for MRSA og af disse var 93 % CC398. I dansk kød blev der fundet MRSA i 4,6 %, 1,4 % og 0 % af henholdsvis svine-, okse- og kyllingekød. I importeret kød var forekomsten 7,5 %, 0 % og 18 % i henholdsvis svine- okse- og kyllingekød. Humane data tyder indtil videre ikke på, at kød udgør en væsentlig smittekilde.

Resistens i indikator bakterier (side 67-71)

Indikatorbakterier er inkluderet i overvågningsprogrammet for at give information om de generelle resistensniveauer i sunde og raske produktionsdyr, idet resistens fra ikke sygdomsvoldende bakterier kan overføres til andre reservoirer.
Fra 2008 til 2009 var der en signifikant stigning i ampicillinresistensen i både ***Enterococcus faecium*** og ***Enterococcus faecalis*** fra svin, antageligt som resultat af et øget penicillinforbrug. Forekomsten i svinekød var dog signifikant lavere for nogle antibiotika end forekomsten i de levende dyr.
I enterokokker fra kyllinger var der stigninger i bestemte typer resistensfænotyper fra 2008 til 2009. Sammenlignet med kyllinger var resistensforekomsten signifikant lavere i kyllingekød for salinomycin, ampicillin og avilamycin blandt *E. faecium* samt tetracyklin blandt *E. faecalis*. Sammenlignet med importeret kyllingekød var resistensforekomsten signifikant lavere i dansk produceret kylllingekød for en række antibiotika.

I ***E. coli*** fra svin fandt vi stigende resistensforekomster overfor en række antibiotika. Nogle resistenstendenser var tilsyneladende relateret til tendenserne i forbruget af de pågældende antibiotika. Tilsvarende stigninger kunne ses for nogle antibiotika i *E. coli* fra svinekød.
I modsætning til hvad vi så for de zoonotiske bakterier, var der ingen signifikant forskel på resistensforekomsten i *E. coli* fra dansk og importeret svinekød.
I *E. coli* fra kyllinger sås for første gang et tilfælde af resistens mod cefalosporinet ceftiofur. I *E. coli* fra importeret kyllingekød var resistensniveauet signifikant højere end i dansk produceret kyllingekød.
Blandt *E. coli* isolater fra kvæg og fra dansk og importeret oksekød var resistensen lav.

Resistensforekomsten var generelt lavere i bakterier fra det danske kyllingekød sammenlignet med det importerede kyllingekød. I de seneste år har resistensforekomsten været stigende i indikatorbakterier fra dansk svinekød og er ikke længere lavere end i det importerede kød.
Dog er forekomsten af ciprofloxacin resistens lav i *E. coli* fra dansk svinekød.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker (side 76-85)

Rapporteringen af antibiotikaresistens i bakterier fra diagnostiske prøver fra mennesker er baseret på frivillig indsendelse af data fra DANRES gruppen, som dækker de klinisk mikrobiologiske afdelinger i Danmark. De eneste undtagelser omfatter methicillin resistente *Staphylococcus aureus* og invasive *Streptococcus pneumoniae*, som er anmeldepligtige. Data vedr. disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt ***E. coli* isolater fra blod** steg resistensforekomsten signifikant for fluorkinoloner (ciprofloxacin 16 %) og cefalosporiner (3. generations cefalosporiner 7 %) fra 2008 til 2009 og nåede resistensniveau som i andre europæiske lande. Stigningen i resistens ses samtidig med stigningen i forbruget af disse antibiotika observeret i de senere år. Ingen *E. coli* isolater fra blodinfektioner var carbapenem resistente.

Blandt ***E. coli* isolater fra urin**, både fra primærsektoren og fra hospitalerne, steg resistensforekomsten i 2009 signifikant for ampicillin, ciprofloxacin, nalidixan syre og cefuroxim. Resistens for 3. generations cefalosporiner nåede 6 % i isolater fra både hospitaler og primærsektor.

Blandt ***Klebsiella pneumoniae* isolater fra blod** var resistensforekomsten for 3. generations cefalosporiner (12 %) (rapporteret som ceftazidim, ceftriaxon, cefpodoxim eller cefotaxim) og gentamicin (9 %) over niveauet for de andre nordiske lande i 2008. Resistensforekomsten for ciprofloxacin var 18 %; forekomsten var signifikant højere i den østlige del af Danmark (23 %) end i den vestlige del (9 %). Ingen *K. pneumoniae* isolater fra blod var carbapenem resistente. Forekomsten af multiresistente isolater (3. generations cefalosporiner, kinoloner og gentamicin) steg fra 1 % i 2006 til 8 % i 2009. Der kunne også observeres en 24 % stigning i antallet af *K. pneumoniae* blodisolater siden 2006.

I denne DANMAP rapport blev der for første gang medtaget data om resistens i ***Klebsiella pneumoniae* urinisolater** fra hospitaler og primærsektor. Forekomsten af ciprofloxacin resistens var 17 % i isolater fra hospitaler og 13 % i isolater fra primærsektor, resistens for 3. generations cefalosporiner (rapporteret som ceftazidim, cefpodoxim eller cefotaxim) var 13 % i isolater fra hospitaler og 8 % i isolater fra primærsektor. Der blev observeret

carbapenem (meropenem) resistente isolater, men da forekomsten af antibiotikaresistens i *K. pneumoniae* ikke er anmeldepligtig, kunne en frekvens for carbapenem resistens ikke beregnes.

Resistensforekomsten i *Pseudomonas aeruginosa* isolater fra blod var lav for alle de testede antibiotika.

I 2009 var penicillin og erythromycin resistensforekomsten stadig lav blandt ***Streptococcus pneumoniae*** og gruppe **A, B, C** og **G streptokokker**. I *S. pneumoniae* isolater faldt makrolid resistensen (4%) signifikant i 2009.

Forekomsten af ampicillin resistens var høj (87%) blandt ***Enterococcus faecium*** isolater fra blod. Forekomsten af vancomycin resistens var 1.6 % i *E. faecium* og mindre end 1 % i ***E. faecalis*** blodisolater. Høj niveau gentamicin resistens (HLGR) i alle enterokokker fra blodinfektioner blev kun testet på én afdeling for klinisk mikrobiologi. Her var 34 % af de testede *E. faecalis* isolater HLGR og 56 % af de testede *E. faecium* isolater HLGR. Behandling med fluorkinoloner, cefalosporiner eller carbapenemer er beskrevet som risikofaktorer for udvikling af en *E. faecium* infektion. I de senere år er der netop observeret en stigning i forbruget af disse antibiotika på hospitaler i Danmark, og dette kan muligvis forklare det stigende antal *E. faecium* infektioner.

Der blev indrapporteret 1466 tilfælde af ***Staphylococcus aureus*** bakteriæmi i 2009 svarende til en incidens på 26,6 per 100.000 indbyggere. Antallet af methicillin-resistente *S. aureus* (MRSA) bakteriæmier var 23 (1,6 %). Frekvensen er meget lav sammenlignet med landene i det øvrige Europa.

Frekvensen af resistens mod øvrige antibiotika lå på samme niveau som de foregående år.

I 2009 var antallet af nye tilfælde af **MRSA** på samme niveau som i 2008. Nitten procent af tilfældene var erhvervet i udlandet, 7% på hospitaler, 10 % af tilfældene blev fundet hos personer med hospitals-/plejehjemskontakt, mens 61% tilfælde var samfundserhvervede. Tres procent havde en infektion på diagnosetidspunktet. Tendensen set siden 2006, at hovedparten af tilfældene erhverves i samfundet, er således fortsat, mens antallet af hospitalserhvervede tilfælde er uændret. Der blev fundet 39 nye tilfælde af MRSA CC398, som har relation til svin. Dette udgør fortsat en relativt lille del af det samlede antal, og der er ingen tegn til, at der ses spredning til befolkningen i almindelighed. Bekæmpelsen af MRSA på hospitalerne er således effektiv, mens forekomst af samfundserhvervet MRSA udgør en stadig større udfordring.

Forekomsten af multiresistente bakterier fra blod- og urinvejsinfektioner steg, specielt var der en øget forekomst af ESBL-producerende *E. coli* og *K. pneumoniae.* Dette kan til dels forklares ved det stigende forbrug af cefalosporiner og flurokinoloner. Det stigende forbrug af disse antibiotika kan også være en forklaring på den stigende forekomst af ampicillin resistente Enterokok blodinfektioner. Resistentsforekomsten var stadig lav hos *P. auroginosa* and streptokokker. Antallet af hospitals erhvervede MRSA var uændret, mens stigningen i MRSA infektioner generelt skyldes en spredning i samfundet uden for hospitalerne.

Summary

This report is the 14th DANMAP report. DANMAP 2009 describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs. The continuous monitoring of antimicrobial resistance and consumption makes it possible to analyse the trends in antimicrobial consumption and resistance over time.

Focus Area: Extended spectrum beta-lactamase (ESBL) producing bacteria in Danish pigs, Danish and imported retail meat and human patients (main report pp 19-22)

Bacteria that produce extended spectrum beta-lactamase (ESBL) are resistant to the wide-spectrum penicillins normally used for treatment and their occurrence – even at low levels - is therefore a matter of concern. In 2009, we examined the prevalence of these bacteria in meat and pigs as well as among human patients.

Pigs and meat: Eleven percent of pigs carried ESBL producing *Escherichia coli* at slaughter. In samples from meat, the prevalence was low, 0.7 – 3.4%; however, in 36% of the imported broiler meat, ESBL producing *E. coli* were detected. The most commonly detected gene among ESBL positive isolates from pigs was CTX-M-1 (66%) and among isolates from imported broiler meat CMY-2 (48%) was most commonly found. CTX-M-15 a gene often found among human isolates was found among 2% of the isolates from pigs.

Humans: The prevalences of ESBL-producing *E. coli* and *Klebsiella pneumonia* from blood and urine infections increased significantly (except for *E. coli* from blood) from 2007 to 2009. ESBL resistance in *K. pneumoniae* from bloodstream infections reached 14.6%.

The parallel increase in prevalence of ESBL-producing bacteria in both humans and animals indicate that antimicrobial selection takes place in both reservoirs, and food derived spread of ESBL-producing *E. coli* may be the origin in at least part of the human cases.

Antimicrobial consumption

DANMAP presents the use of antimicrobial agents in humans and animals. In humans, the use of prescription medicines has been monitored by the Danish Medicines Agency at the level of the individual patient since the early 1990s. In animals, data on all medicines prescribed by veterinarians for use in animals have been registered at farm and species level by the VetStat programme at the Veterinary Institute (Technical University of Denmark) since 2001.

Antimicrobial consumption in animals (main report pp 25-35)

In 2009, the total consumption of antimicrobial agents in animals amounted to 129.7 tonnes. This was a 10.4% increase compared with 2008. The increase could mainly be attributed to consumption in pigs.

An increasing number of pigs were exported at 30 kg live weight. When we adjust the statistic for this, we find that antimicrobial consumption in pigs increased by 12.7% from 2008 to 2009. The consumption in pigs reached 4.9 ADD_{kg}/kg pork produced.

The increase could largely be attributed to tetracyclines (12%), macrolides (16%) and pleuromutilins; these antimicrobial agents are commonly used for mass medication in feed or drinking water in pig herds with disease problems. The use of wide-spectrum cephalosporins in pigs decreased by 25% compared to 2008.

The consumption of antimicrobial agents in cattle, increased to 15 tonnes, but has been relatively stable at around 14-15 tonnes since 2005. Narrow spectrum penicillins comprised 57% of the systemic treatment in cows. Penicillins were also the most frequently used agents for intramammary use. In 2009, the use of wide-spectrum cephalosporins for intramammary use decreased by 32%, and comprised 13% of the intramammary consumption in 2009. Also, the consumtpion of cephalosporins for systemic use in cattle decreased by 14.7%.

The consumption of antimicrobial agents in poultry increased from 556 kg in 2008 to 1070 kg in 2009. However, even with a doubling of, the consumption of antimicrobial agents in Danish broilers, the consumption remains at a very low level, equivalent to 0.15 ADD_{kg} perkg meat produced.

In turkeys, the consumption increased by 165%, reaching 1.8 ADD_{kg} per kg meat produced, the highest level since 2002.

In aquaculture, the antimicrobial consumption decreased by 4% to 3300 kg in 2009 compared with 2008, due to a reduction in consumption in salt water fish.

Antimicrobial consumption in humans (main report pp 41-51)

Primary health care and hospitals: The total consumption of antibacterial agents for systemic use in humans amounted to 17.9 DDDs per 1000 inhabitants per day (DID) compared with 17.8 DID in 2008. Since 2000, consumption has increased by 4.2 DID (31.1%). Consumption in primary health care comprises app. 90% of the total consumption.

Primary health care: In 2009, the total antibacterial consumption (J01) amounted to 15.95 DID compared with 15.91 DID in 2008. It seems that the continuing increase in consumption in primary healthcare has levelled off. However, the levelling in total consumption was mainly due to a reduction in beta-lactamase sensitive penicillins (0.18 DID) and macrolides (0.08 DID) counterbalanced by an increase in combinations of penicillins/beta-lactamase inhibitors (0.18 DID) and tetracyclines (0.07 DID). Beta-lactamase sensitive penicillins (narrow spectrum) still represented the largest group of antibacterial agents consumed (32% of the total consumption) followed by penicillins with extended spectrum (21%) and macrolides (14%). Total consumption expressed in DID increased by 31% during 2000–2009.

Hospitals: The total consumption (J01) expressed in DDDs per 100 occupied bed-days (DBD) increased by 4.8% (from 74.56 in 2008 to 78.13 in 2009). When expressed as the number of DDDs per 100 discharged patients it increased by 1.6% during 2007–2009 (from 288.7 in 2007 to 293.3 in 2009). The difference reflects shorter duration of hospitalisation per patient, but more admissions to hospitals. The consumption of all the major antibacterial groups (>1.0 DBD) increased with the exception of beta-lactamase sensitive penicillins, aminoglycosides and imidazole derivates. Cephalosporins, mainly 2nd generation, accounted for 21% of the total consumption in the hospital sector. Penicillins with extended spectrum (18%), fluoroquinolones (13%) and beta-lactamase sensitive penicillins (12%) were other major contributing antibacterial groups. Over the last decade (2000–2009), total consumption has increased by 31.2 DBD (66.4%).

Overall, the antimicrobial consumption in humans has been at a steady level the past two years, but the use of broad spectrum antimicrobial agents (e.g. cephalosporins, fluoroquinolones) has increased. The opposite situation is seen in the veterinary sector, where the consumption of the most critically important antimicrobial agents is decreasing (cephalosporins) or the use is very low (fluoroquinolones). Concurrently, a steep increase in overall antimicrobial consumption – particularly in pigs – occurred in 2009, including increasing consumption of macrolides (critically important).

Resistance in zoonotic bacteria (main report pp 58-64)

Among ***Salmonella*** **Typhimurium** isolates from Danish pigs, no significant change in occurrence of antimicrobial resistance was observed from 2008 to 2009, although significant increasing trends throughout the decade continued for sulfonamide, ampicillin and streptomycin. In *S*. Typhimurium from pork the occurrence of resistance to four antimicrobial agents was significantly higher in imported pork (22% for ciprofloxacin) compared to domestic products (0 % for ciprofloxacin). Several human *S.* Typhimurium outbreaks occurred in 2009; the two largest consisted of 212 and 83 cases, respectively. The occurrence of resistance in human domestically acquired *S.* Typhimurium cases was in general lower than the occurrence in both Danish and imported pork and might at least in part be explain by the outbreaks. A significantly higher occurrence of nalidixic acid and ciprofloxacin resistance was observed in travel associated cases when compared with domestically acquired cases.

Salmonella **Enteritidis** is relatively rare in the Danish poultry production; in 2009, it was isolated only from imported broiler meat. Among the isolates tested, notably the occurrence of resistance to ciprofloxacin and nalidixic acid increased, with 49% of the isolates resistant to nalidixic acid and ciprofloxacin. Resistance to ampicillin, ciprofloxacin and nalidixic acid was significantly more frequent in travel associated cases compared to those acquired in Denmark.

From 2008 to 2009, no significant changes in occurrence of resistance were observed among ***Campylobacter jejuni*** from Danish broilers and cattle or ***Campylobacter coli*** from pigs. In Danish chicken meat, ciprofloxacin and nalidixic acid resistance in *C. jejuni* decreased significantly, with no resistant isolates

in 2009. Imported chicken meat contained *C. jejuni* with significantly higher levels of resistance to ciprofloxacin (56%), nalidixic acid (56%) and tetracycline (52%) compared to Danish broiler meat. In human campylobacter cases, resistance to ciprofloxacin, nalidixic acid and tetracycline was significantly higher in cases associated with travel than in cases acquired in Denmark.

Overall, the monitoring programme for the food borne zoonoses shows that even though there is reason to keep an eye on the development within the country, the Danish status with regard to antimicrobial resistance is better than in many other countries within and outside Europe.

Pigs at slaughter and retail meat (pork, beef and broiler meat) was investigated for the prevalence of MRSA. Thirteen % of the pigs at slaughter were positive for MRSA and 93% of these were CC398. In Danish meat MRSA was found in 4.6%, 1.4% and 0% of pork, beef and broiler meat, respectively. In imported meat the occurrence was 7.5%, 0% and 18% in pork, beef and broiler meat, respectively. Based on human data, meat are not suspected as a source of infection.

Resistance in indicator bacteria (main report pp 67-71)

Indicator bacteria were included in the programme to provide information about the general levels of resistance in healthy food animals.
From 2008 to 2009, a significant increase in occurrence of ampicillin resistance was detected in both ***Enterococcus faecium*** **and** ***Enterococcus faecalis*** from pigs, presumable as a result of increased usage of penicillins. For some antimicriobials, the occurrence of resistance in pork was lower than that found in the live pigs.
In Enterococci from broilers, the occurrence of resistance to four antimicrobials increased significantly from 2008 to 2009. Compared to isolates from broilers, significantly lower occurrence of resistance was found in the broiler meat regarding salinomycin, ampicillin and avilamycin resistance among *E. faecium,* and tetracycline resistance among *E. faecalis*. Compared to imported broiler meat, resistance was significantly lower in Danish produced broiler meat for erythromycin, kanamycin, tetracycline and others.

In ***Escherichia coli*** from pigs we found increasing occurrence of resistance to a number of antimicrobial agents, with some of the trends in resistance temporally related to trends in consumption of antimicrobial agents. Similar increases were seen for some antimicrobial agents in *E. coli* from pork.
In contrast to what we found for Salmonella, there were no significant differences in resistance in *E. coli* between Danish and imported pork.
In *E. coli* from broilers, for the first time one case of resistance to ceftiofur (a cephalosporin), possibly ESBL-producing, was detected. The occurrence of resistance to at least 14 antimicrobial agents was significantly higher in imported chicken meat that in chicken meat produced in Denmark.
Among *E. coli* isolates from cattle and from Danish and imported beef, occurrence of resistance was low.

Overall, the occurrence of resistance was significantly lower within the indicator bacteria found in Danish broiler meat compared with imported broiler meat. The occurrence of resistance in Danish pork has been increasing in past years and is not significantly lower than in imported pork. However, resistance to ciprofloxacin was very low in *E. coli* from Danish pork (1%), in contrast to imported pork (6%).

Resistance in human clinical bacteria (main report pp 76-85)

Data on antimicrobial resistance in bacteria from diagnostic submissions are gathered by voluntary reporting from the DANRES group which covers the departments of clinical microbiology (DCM) in Denmark. The only exceptions are methicillin resistant *Staphylococcus aureus* and invasive *Streptococcus pneumoniae* that are notifiable. Data on these bacteria are obtained from the reference laboratories at SSI.

Among ***E. coli*** **blood isolates**, the occurrence of resistance to fluoroquinolones (ciprofloxacin) and cephalosporins (3rd generation cephalosporins) increased significantly from 2008 to 2009, reaching levels seen in several other European countries. The increase in resistance corresponds to the increase in the consumption of these antimicrobial agents seen over the years. No *E. coli* isolates from blood were carbapenem resistant.

In ***E. coli*** **urine isolates** obtained from hospitals and primary health care, resistance to ampicillin, ciprofloxacin, nalidixic acid and cefuroxime increased significantly in 2009. In *E. coli* urine isolates obtained from hospitalized patients, resistance to mecillinam and sulfonamide also increased significantly. Resistance to 3rd generation cephalosporins reached 6% in isolates from both hospitals and primary health care.

In ***Klebsiella pneumoniae* blood isolates**, 3rd generation cephalosporin (12%) (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) and gentamicin resistance (9%) was above the 2008 level in the other Nordic countries. Resistance to ciprofloxacin was 18%; however, a significantly larger occurrence was seen in Eastern Denmark (23%) than in Western Denmark (9%). No *K. pneumoniae* isolates from blood were carbapenem resistant. The occurrence of multi-resistant isolates (3rd generation cephalosporins, quinolones and gentamicin) increased from 1% in 2006 to 8% in 2009. Also, a 24% increase in the number of *K. pneumoniae* blood isolates since 2006 was observed.

In this DANMAP report, resistance in ***K. pneumoniae* urine isolates** obtained from hospitals and primary health care was reported for the first time. Ciprofloxacin resistance was 17% in isolates from hospitals and 13% in isolates from primary health care, 3rd generation cephalosporin resistance (reported as ceftazidime, cefpodoxime or cefotaxime) was13% in isolates from hospitals and 8% in isolates from PHC. Carbapenem (meropenem) resistance was present in these isolates; however, the occurrence of antimicrobial resistance in this species in not mandatory reportable and no calculation of the frequency of carbepenem resistance could be made.

The occurrence of resistance in ***Pseudomonas aeruginosa*** isolates obtained from blood was low for all the tested antimicrobial agents.

Resistance to penicillins and erythromycin in ***Streptococcus pneumoniae*,** Group **A, B, C** and **G streptococci** remained low in 2009. In *S. pneumoniae* isolates, macrolide resistance (4%) decreased significantly in 2009.

Resistance to ampicillin was high (87%) in ***Enterococcus faecium*** isolates from blood. The occurrence of vancomycin resistance was 1.6% in the *E. faecium* and less than 1% in the ***E. faecalis*** blood isolates. Only one of the DCMs tested all enterococci from bloodstream infections for high level gentamicin resistance (HLGR). Here, 34% of the tested *E. faecalis* isolates were HLGR, as were 56% of the tested *E. faecium* isolates. Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as risk factors for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has also been observed in hospitals in Denmark during the past years and this might explain the increasing numbers of *E. faecium* bloodstream infections.

In 2009, 1466 cases of ***Staphylococcus aureus*** bacteraemia were reported, corresponding to 26.6 cases per 100,000 inhabitants. The number of methicillin resistant *S. aureus* (MRSA) was 23 (1.6%). This frequency is very low compared to the incidence in most other European countries. Resistance frequencies to other antimicrobials were at the same level as in previous years.

In 2009, the number of new cases of **MRSA** was comparable to the number in 2008. Nineteen percent of the cases were acquired abroad, 7% in hospitals and 10% of the cases were persons with recent contact to hospitals or nursing homes while 61% of the cases were community acquired. Sixty percent of all cases presented with infections at the time of diagnosis. The trend observed since 2006 that most cases are acquired in the community thus continues while the number of hospital acquired cases remains the same. The clonal complex CC398 which is associated with swine and other livestock animals constitutes a minor proportion of all MRSA cases (39 cases) and so far no signs of transmission to the general population are seen. The control of MRSA in hospitals was effective while the prevalence of community acquired MRSA represents an increasing challenge.

Regarding blood and urine infections in human patients, the occurrence of multiresistance is increasing, particularly occurrence of ESBL-producing *E. coli* and *Klebsiella pneumoniae*. This may in part be driven by increasing consumption of fluoroquinolones and cephalosporins. The increased consumption of fluoroquinolones and cephalosporins may also drive the increasing occurrence of the ampicillin resistant *Enterococcus* bloodstream infections. However, the occurrence of resistance in *Pseudomonas* and Streptococci is still generally low. Also, the hospital acquired infections with MRSA remain the same, but an overall increase in MRSA infections is driven by spread in the community.

Extended spectrum beta-lactamase producing bacteria in Danish pigs, Danish and imported retail meat and human patients

Background: Extended spectrum beta-lactamase (ESBL) producing bacteria are one of the fastest emerging resistance problems worldwide. ESBL-producing *E. coli* have been observed in animals, healthy humans and patients, whereas ESBL-producing *Klebsiella pneumoniae* was found in patients. There is a strong relationship with consumption of broad spectrum antimicrobial agents such as third generation cephalosporins and fluoroquinolones and the emergence of ESBL-producing bacteria. ESBL-producing *K. pneumoniae* have had a tendency to spread as epidemics in hospitals [Lester *et al.* 2010. ECCMID. Poster 1251]. The origin of ESBL-producing bacteria is as far unknown, but food producing animals has recently been discussed as a reservoir.

The use of cephalosporins in pig production in Denmark has increased during 2001–2007 and may select for ESBL-producing *E. coli* in pigs. However, consumption of ciprofloxacin and second and third generation cephalosporins has also been on constant increase in Danish hospitals for the last 10 years, causing increased selection pressure further promoting ESBL-producing bacteria in the hospitals. In 2009, investigations have been undertaken both in the veterinary field (i.e. to investigate the prevalence of ESBL-producing *E. coli* in pigs at slaughter and in meat at retail), as well as among patients (i.e. a country-wide prevalence study of ESBL-producing *E. coli* and *K. pneumoniae* from clinical samples) [EPI-NEWS 15/2010].

Materials and Methods:

Animals and food: During 2009, fecal samples from pigs (n=786) were taken at slaughter and 866 meat samples (Danish pork (153), Danish broiler meat (121), Danish beef (142), imported pork (173), imported broiler meat (193), and imported beef (84)) were collected in retail stores and outlets. The fecal samples were randomly selected and only one pig from each farm was included each month (one to three pigs were sampled per farm in the entire sampling period). The meat samples were collected randomly in all regions of Denmark. *E. coli* was isolated from 1 g of feces or 5 g of meat after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL resistance was revealed by use of PCR, array and DNA sequencing.

Humans: In October 2009, 14 of the 15 Danish departments of clinical microbiology tested all *E. coli* and *K. pneumoniae* isolates obtained from urine and blood for ESBL-production. Screening for ESBL-production was done using a cephalosporin-disc/tablet on the primary plate, and confirmation was done by demonstrating inhibition with clavulanic acid in combination with cefotaxime and/or ceftazidime using the disc-combination method or by Etests. Further confirmatory tests were carried out, e.g. at SSI. Prevalences of ESBL-producing bacteria were calculated with the total number of the same species tested at each site as the denominator.

Results:

Animals and food: Eleven percent (86/786) of the pig fecal samples contained ceftriaxone resistant *E. coli*. Among these isolates, 66% contained CTX-M-1 but other genotypes were found as well (ampC up-regulation (17%), CTX-M-14 (7%), CTX-M-15 (2%), CTX-M-2 (2%), SHV-12 (1%), TEM-20 (1%) and unknown (3%)) **(Figure 1).** From meat samples, the highest prevalence of ceftriaxone resistant *E. coli* was found among imported broiler meat (36%), in the other meat categories 0.7-3.3% ceftriaxone resistance was observed. The ceftriaxone resistant *E. coli* from imported broiler meat (69 isolates) contained CMY-2 (48%), CTX-M-1 (25%), SHV-12 (16%), CTX-M-2 (3%) and other mechanisms (TEM-20, TEM-52, ampC up-regulation) (3%). Among the other meat categories, CMY-2, CTX-M-1, CTX-M-2, CTX-M-14, TEM-52 and ampC up-regulation were found **(Figure 2).**

Humans: The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* isolated from blood or urine, the latter divided into hospital or community derived urine cultures, is shown in **Table 1**, where the ESBL-prevalences obtained in 2009 are compared with data from a similar study performed in 2007. The prevalences of ESBL-producing bacteria increased significantly for all categories (except for *E. coli* from blood). The increase seen in *K. pneumoniae* in blood to14.6% producing ESBL is highly significant.

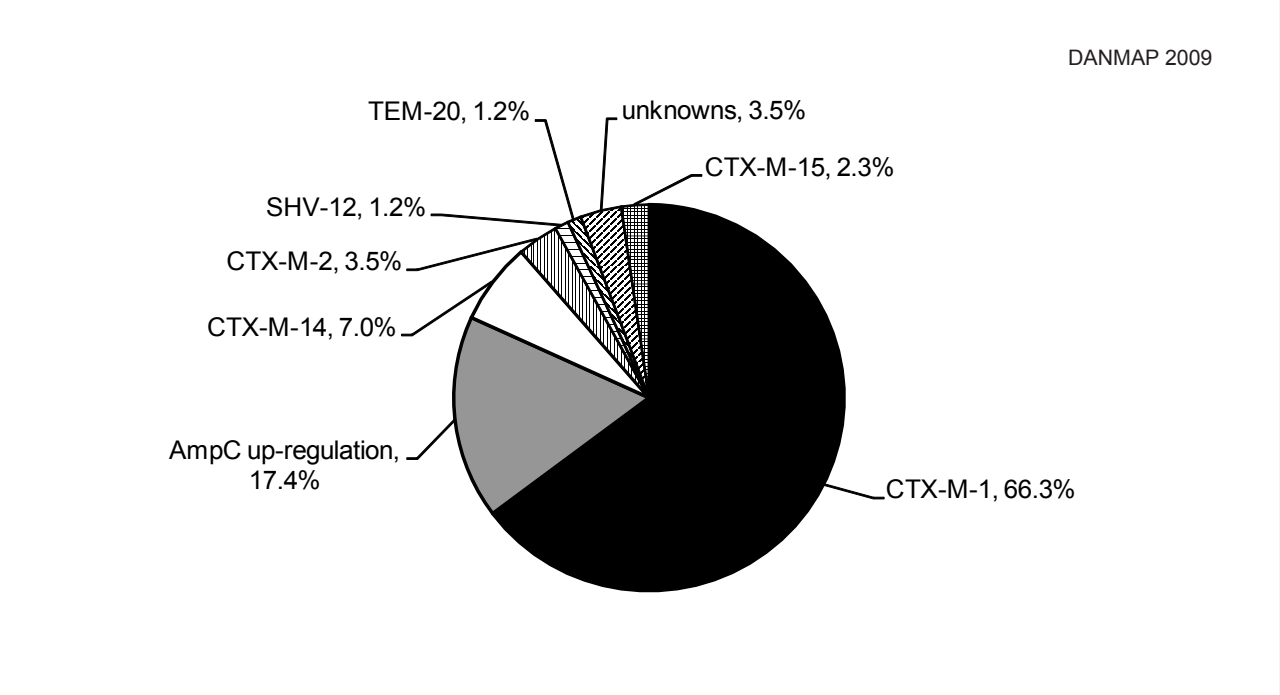

Figure 1. ESBL gene distribution in pigs

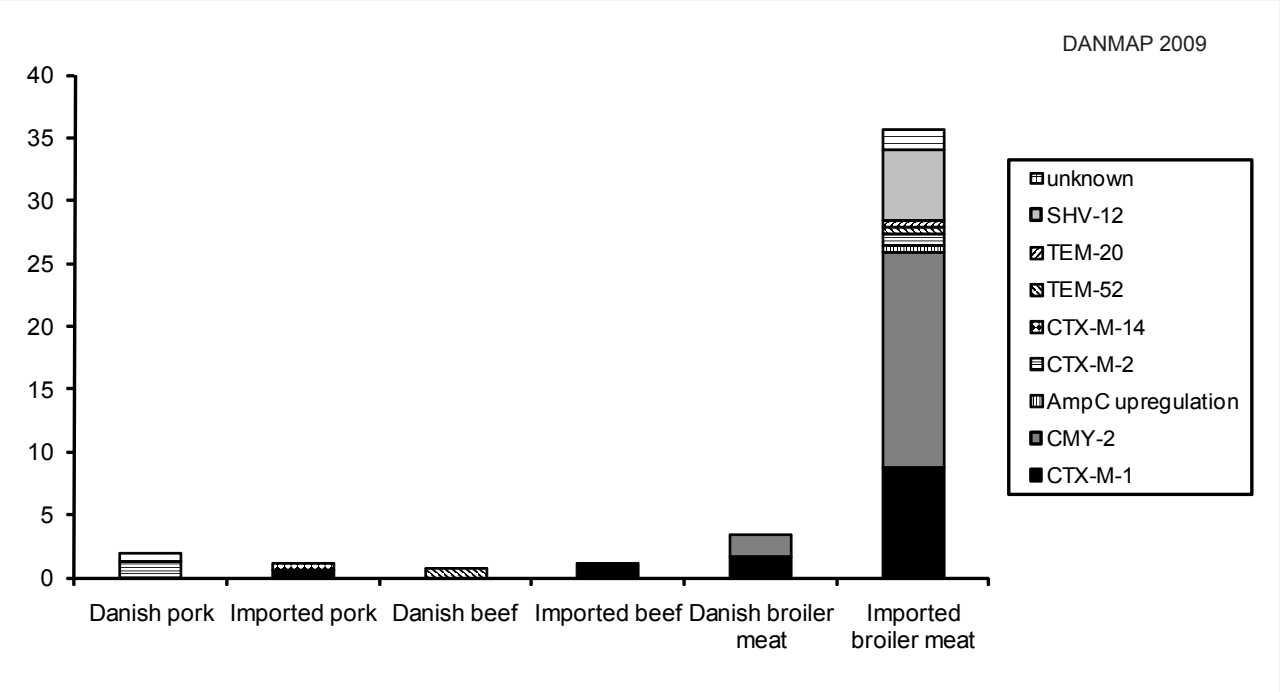

Figure 2. Frequency of ESBL types in various types of meat products

Focus area

Table 1. Prevalence of Extended Spectrum Beta-Lactamase (ESBL) in E. coli *and* K. pneumoniae *isolated from blood and urine cultures, September-October 2007 and October 2009*

DANMAP 2009

Sample type	Period	Total no. of cultures	*E. coli* findings	Of these, ESBL (%)	*K. pneumoniae* findings	Of these, ESBL (%)
Blood	2007	18259	625	26 (4.2)	160	8 (5.0)
	2009	11532	356	25 (7.0)	89	13 (14.6)
Urine, hosp.	2007	*	6791	157 (2.3)	1078	71 (6.6)
	2009	16536	4004	152 (3.8)	675	78 (11.1)
Urine, GP	2007	*	4966	74 (1.5)	513	14 (2.7)
	2009	12574	3392	74 (2.3)	385	26 (6.8)

*) For the 2007-study, no information was provided on the distribution of the in all 47,504 urine cultures performed in hospitals and general practices

Discussion and conclusions: For animals and food, the use of selective enrichment with ceftriaxone revealed ESBL-producing *E. coli* in pigs which were not found by standard monitoring of indicator *E. coli* in pigs. The occurrence of ESBL-producing *E. coli* may increase due to the increased consumption of cephalosporins in the pig production. Even though ESBL *E. coli* are present in Danish pigs at slaughter, the meat source with the paramount highest fraction of ESBL *E. coli* was imported broiler meat. As certain genotypes were dominant in *E. coli* from certain sources the genotype may be valuable for source attribution.

For humans, there was a 40-50% increase in the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in both urine and blood from 2007 to 2009.

The isolates from 2009 have not been genotyped for ESBL type yet, but in 2007 roughly 70% of the ESBL-producing *E. coli* belonged to CTX-M-15, while the CTX-M-1 type comprised around 5% [Hansen *et al.* 2010. ECCMID. Poster 1617]. The most common ESBL-type among pig isolates was CTX-M-1 (66%) and only 2% belonged to CTX-M-15. Among the isolates from imported broiler meat, CMY-2 was most often present. The distribution in ESBL types is therefore not congruent among animals and humans. However, the parallel increase in prevalence of ESBL-producing bacteria in both humans and animals indicate that antimicrobial selection takes place in both reservoirs, and food derived spread of ESBL-producing bacteria may be the origin in at least part of the human cases.

Infection with ESBL-producing bacteria frequently entails prolonged admission periods at hospitals with ensuing human and financial costs. The mortality for septicaemia caused by *E. coli* and *K. pneumoniae* susceptible to antibiotics is approx. 20%, but this figure may increase in case of ESBL-producing bacteria. Alternatively, empirical treatment on suspicion of septicaemia should be changed to a carbapenem (meropenem). Carbapenem is the last resort antibiotic to very resistant Gram-negative bacteria, and we should not expect new and more effective antibiotics to be introduced in the next decade. Furthermore, patients colonized with carbepenem resistant *K. pneumoniae* have already been observed in Denmark. The two patients had been hospitalised in Greece and were both carrier of *K. pneumoniae* with the transferable KPC-2 gene [Hammerum *et al.* Int. J. Antimicrob.Agents 2010. 35: 610-612]. Consequently, infections may occur in Denmark for which no treatment options exist.

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Demographic data - general information

Demographic data

Demographic information is presented in order to show the magnitude of animal and human populations in which antimicrobial agents were used during 2009.

The production of food animals (including animals for live export), meat, and the population of dairy cattle is shown in Table 1. Regarding pigs, the export of fattening pigs (15-50 kg) is shown; pigs at this age have used a large amount of antimicrobial agents relative to their bodyweight at export, while treatment after export is not included in this statistics.

Table 2 provides information on the distribution of the human population in Denmark and on the Danish health care system by region. The trends in numbers of occupied bed-days and discharges from somatic hospitals 2000–2009 are shown in Figure **3**. The five Danish health care regions are displayed in Figure 4.

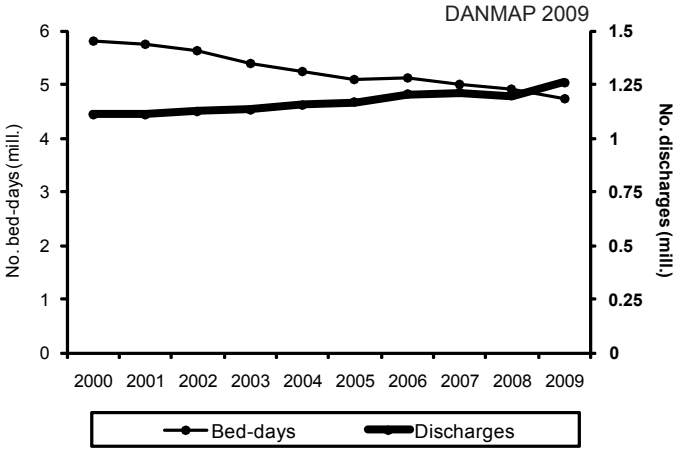


Figure 3. Number of beddays and discharges in somatic hospitals, Denmark

Antimicrobial agents in Denmark

Table 4 shows the antimicrobial agents which are registered for use to treatment of bacterial infections in animals and humans. Antimicrobial agents used for both humans and animals are shown in italic; furthermore, the growth promoters, which are no longer used for animals in Denmark, are shown in parentheses. Most of the antimicrobial agents used for growth promotion in Denmark had effects on Gram-positive bacteria. The indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance to the growth promoters since 1995.

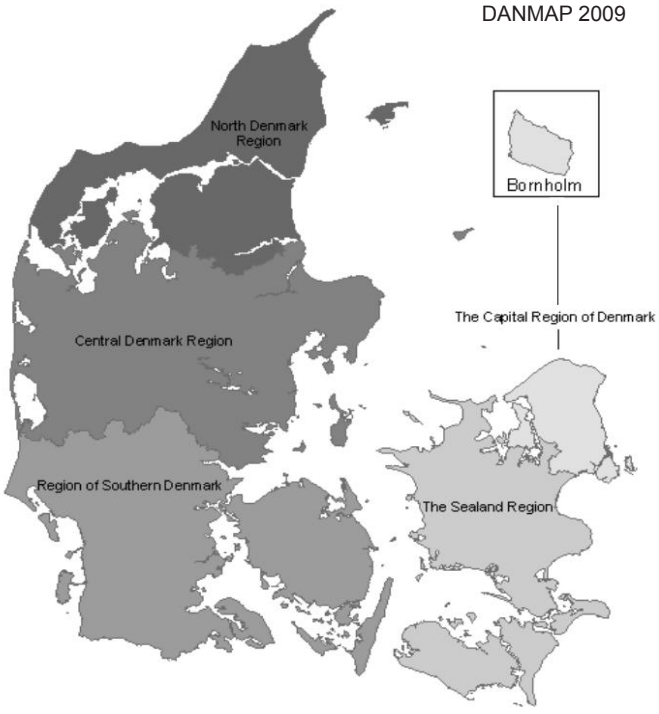


Figure 4. The five health care regions of Denmark

Table 2. Production of food animals and the production of meat and milk, Denmark

DANMAP 2009

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish	
												Fresh water	Salt water
	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	a) 1,000 heads	Export b) 1,000 heads	mill. kg	mill. kg	mill. kg
1990	94,560	116	571	2.5	789	219	753	4,542	16,425	-	1,260	-	-
1992	107,188	137	761	5.4	862	236	712	4,405	18,442	-	1,442	35	7
1994	116,036	152	1,091	8.6	813	210	700	4,442	20,651	-	1,604	35	7
1996	107,895	149	961	9.3	789	198	701	4,494	20,424	-	1,592	32	8
1998	126,063	168	1,124	11.6	732	179	669	4,468	22,738	-	1,770	32	7
2000	133,987	181	1,042	10.3	691	171	636	4,520	22,414	-	1,748	32	7
2001	136,603	192	1,086	13.2	653	169	623	4,418	23,199	-	1,836	31	8
2002	136,350	190	1,073	12.8	668	169	611	4,455	24,203	-	1,892	32	8
2003	129,861	181	777	11.2	625	161	596	4,540	24,434	-	1,898	34	8
2004	130,674	181	1,086	19.6	632	165	569	4,434	25,141	1,712	1,967	34	9
2005	122,179	180	1,237	17.4	549	145	559	4,449	25,758	2,720	1,988	31	8
2006	106,182	163	785	11.3	509	140	556	4,492	25,763	3,204	1,957	29	8
2007	107,952	178	1,009	14.4	512	141	545	4,515	26,311	3,522	2,046	31	10
2008	107,595	186	1,068	12.3	509	138	559	4,575	27,078	4,943	1,985	30	10
2009	108,851	181	1,175	11.1	507	137	569	4,733	27,603	6,642	1,898	-	-
Increase[c] (%)	1	-3	10	-10	0	-1	2	3	2	34	-4	-	-

Source: Statistics Denmark (www.dst.dk) and The Danish Directorate for Fisheries. All data include export of live animals for slaughter
a) Including export of all age groups (not only for slaughter)
b) Export of 15-50 kg pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside Denmark
c) Increase from 2008 to 2009

Table 3. Distribution of the human population and health care structure by region, Denmark

DANMAP 2009

Region name	No. inhabitants	No. inh./km2	No. inh./ GP a)	GP contacts/1000 inhabitant-days	No. bed-days b)	No. discharges b)
The Capital Region of Denmark	1,662,285	649	1519	18.8	1,679,240	428,690
The Sealand Region	821,252	113	1582	20.7	679,626	189,106
Region of Southern Denmark	1,199,667	98	1487	20.8	936,100	256,289
Central Denmark Region	1,247,732	95	1498	20.1	939,846	266,619
North Denmark Region	580,515	73	1613	20.0	492,436	118,652
Denmark c)	5,511,451	128	1525	19.9	4,727,248	1,259,356

a) GP, general practitioner
b) Excluding private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices
c) Compared to the previous year no. inhabitants have increased by 0.7%, no. bed-days have decreased by 3.5% and no. discharges have increased by 5.3% (the number of discharges was affectedly low in 2008 due to a major hospital strike)

Table 4. Systemic antimicrobial agents marketed for the use in animals (incl. intrammamary) and humans, Denmark

DANMAP 2009

ATC / ATCvet codes [a]	Therapeutic group	Antimicrobial agents within the therapeutic groups [b]	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, *doxycycline, oxytetracycline, tetracycline*	*Doxycycline,* lymecycline, *oxytetracycline, tetracycline,* tigecycline
J01BA / QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	*Ampicillin, amoxicillin*	*Ampicillin,* pivampicillin, *amoxicillin,* pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	*Benzylpenicillin, phenoxymethylpenicillin,* procaine penicillin, penethamate hydroiodide	*Benzylpenicillin, phenoxymethylpenicillin*
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins, incl. beta-lactamase inhibitors	*Amoxicillin/clavulanate*	*Amoxicillin/clavulanate,* piperacillin/tazobactam
J01DB / QJ01DB,QJ51DA	First-generation cephalosporins	*Cefalexin,* cefadroxil, cefapirin	*Cefalexin*
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DA	Third-generation cephalosporins	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone
J01DE / QJ51DA	Fourth-generation cephalosporins	Cefquinome	
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, doripenem
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ,QJ51R	Short-acting sulfonamides	Sulfadimidine, sulfathiazole	Sulfamethizole
J01EE / QJ01EW	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, acetylisovaleryltylosin, tulathromycin	Erythromycin, roxithromycin, clarithromycin, azithromycin
J01FF / QJ01FF	Lincosamides	*Clindamycin,* lincomycin	*Clindamycin*
J01FG / QJ01XX	Streptogramins	(Virginiamycin) [c]	
J01G,A07AA / QJ01G,QA07AA [d]	Aminoglycosides	Streptomycin, dihydrostreptomycin, *gentamicin,* neomycin, apramycin	Tobramycin, *gentamicin*
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, danofloxacin, marbofloxacin, difloxacin, ibafloxacin	Ofloxacin, ciprofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ	Quinoxalines	(Carbadox, olaquindox)	
J01XA	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
J01XB,A07AA / QA07AA [d]	Polypeptides (incl. polymyxins)	*Colistin,* (bacitracin)	*Colistin*
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB / QJ01XD [d]	Imidazole derivatives	*Metronidazole*	*Metronidazole*
J01XE / QJ01XE	Nitrofurane derivatives	*Nitrofurantoin*	*Nitrofurantoin*
J01XX / QJ01XX,QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin
QJ01XX9	Pleuromutilins	Tiamulin, valnemulin	
QP51AH	Pyranes and hydropyranes (ionophores)	(Monensin, salinomycin)	
Not in ATCvet	Oligosaccharides	(Avilamycin)	
Not in ATCvet	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes starts with a Q
b) Antimicrobial agents that are used in both humans and animals are listed in italics, animal growth promoters used before 1999 are listed in parentheses
c) Pristinamycin and quinupristin/dalfopristin (for humans) are not used in Denmark
d) Although intestinal antiinfectives (A07AA) and nitroimidazole derivatives for protozoal diseases (P01AB) are used to treat human patients, they are not reported by DANMAP

Antimicrobial consumption

Antimicrobial consumption in animals

Since 2001, detailed data on all prescription medicines for animals have been registered in the VetStat database. VetStat is a relational database, meaning that information on e.g. a given item, animal species or the defined daily dose is registered on separate tables in the database. Some of the recorded codes refer to other relational databases, e.g. the Central Husbandry Register and the register of authorized veterinarians. In 2009, a thorough revision of the data on medicinal products and the defined animal daily doses (ADD) in VetStat was performed. In addition, the principles for the ADD calculation was changed regarding medicinal products with prolonged effect (>24 hours) (For further information see **Text Box 1)**. At the same time it was decided that active ingredients should consistently be calculated as the molecular weight of the base; this reduced the amounts measured of tetracyclines and pleuromutilins in 2005-2008. Therefore, in this report all data back to 2001 were updated.

In DANMAP, overall antimicrobial consumption is measured in kg active substance, while analyses of trends within species are measured in Defined Animal Daily Doses compared to the animal production (Table 2). Production is measured in kg-meat-produced (pork, poultry, cattle) and number of animals slaughtered or exported (pigs).

In 2009, the total veterinary consumption of antimicrobial substance amounted to 129.7 tonnes (see details in 6), representing a 10.4% increase relative to 2008. This increase was mainly attributable to increase in consumption in pigs. The distribution of antimicrobial agents among the major species has not changed importantly from previous years. In 2009, the antimicrobial consumption in pigs and cattle comprised 80% (103.7 tonnes) and 12% (15 tonnes) of the total veterinary consumption, respectively. The consumption of antimicrobial agents in poultry increased substantially, but still comprised only 0.5% of the total consumption in 2009. The consumption of antimicrobial agents in companion animals (pet animals and horses) was unchanged at an estimated 2.2 tonnes.

For production animals in general, the consumption of antimicrobial agents has increased gradually by 59% from 2000–2009 (see details in Table 5), mainly due to an increasing consumption in pigs. During the same period, the meat production from all species has increased by 5.5%, from 2.1 billion kg to 2.2 billion kg meat, including export; the milk production has increased by 4.7% in the same period (Table 2).

Table 5. Trends in the estimated total consumption (kg active compound [b]) of prescribed antimicrobials for production animals, Denmark

DANMAP 2009

ATC_{vet} group a)	Therapeutic group	1990	1992	1994	1996	1998	2000	2002	2004	2005	2006	2007	2008	2009
QJ01AA	Tetracyclines	9,300	22,000	36,500	12,900	12,100	24,000	23,950	29,350	29,550	31,800	36,600	35,400	38,400
QJ01CE	Penicillins, b-lactamase sensitive	5,000	6,700	9,400	7,200	14,300	15,100	17,500	20,900	22,250	22,650	23,850	23,950	25,950
QJ01C/ QJ01D	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	7,300	9,750	12,500	11,900	11,200	11,200	10,850	12,250
QJ01EW	Sulfonamides + trimethoprim c)	3,800	7,900	9,500	4,800	7,700	7,000	7,800	11,500	11,950	13,400	13,800	13,300	14,950
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	900	850	750	750	700	600	445
QJ01F	Macrolides, lincosamides	10,900	12,900	11,400	7,600	7,100	15,600	13,200	16,150	15,300	14,350	16,500	15,250	17,350
QJ01XQ	Pleuromutilins d)							4,450	6,600	6,500	6,350	6,100	9,200	10,650
QJ01G/ QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	10,400	11,700	11,600	10,750	10,550	8,100	6,000	6,350
	Others c)	6,700	6,800	4,400	600	650	300	700	950	1,200	1,200	1,150	1,650	1,900
Total		53,400	73,200	89,900	48,000	57,300	80,700	90,000	110,400	110,100	112,300	118,000	116,100	128,300

1990–2000: Data based on reports from the pharmaceutical industry of total annual sales. (Data 1990–1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996–2000: Danish Medicines Agency). Data 2001–2006: VetStat. For comparability between VetStat data and previous data, see DANMAP 2000. Only veterinary drugs are included. Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded. Dermal spray with tetracycline, extensively used in production animals, is the only topical drug included.

a) Only the major contributing ATCvet groups are mentioned

b) Kg active compound rounded to nearest 50 for antimicrobial classes and 100 for totals

c) Consumption in aquaculture was not included before 2001

d) Pleuromutilins included in macrolide-group before 2001

Table 6. Antimicrobials sold (kg active compound) from pharmacies and feedmills by animal species and age group, Denmark

DANMAP 2009

Therapeutic group	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macro	Pleuro	Pen-b-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATCvet groups [a]	QJ01B	QJ01G	QJ01D	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XX	QJ01CE	QJ01CA	QJ01E	QJ01AA	QJ01X	
Pigs														
- Sows and piglets	83	2,192	84	<0.1	0	788	1,062	2,002	9,919	4,425	7,127	3,577	57	31,315
- Weaners	24	2,583	10	0	0	891	7,551	3,732	1,605	3,058	1,845	19,148	341	40,788
- Finishers	12	279	4	0	0	1,102	4,710	4,823	6,185	1,427	146	12,361	5	31,054
- Age not given	0.2	23	1	<0.1	0	17	77	58	87	67	37	168	4	540
Cattle b)										0.00				
- Cows and bulls	5	70	38	0	0	0.4	24	0	2,013	98	34	228	0.6	2,512
- Calves<12 months	245	219	1	0	0	4	39	1	287	110	356	367	4	1,633
- Heifers, Steers	5	1	0.2	0	0	<0.1	0.4	0	11	1	4	11	<0.1	35
- Age not given	2	3	1	0	0	<0.1	1	0	24	5	4	5	<0.1	46
Poultry														
- Broilers	8	0	0	0	0	0	0.2	0	0	21	0.9	11	0.3	41
- Rearing, broilers	1	1	0	0.2	0	0.3	42	0	4	100	5	78	0	231
- Layers, primatily rearing	6	0	0	4	0	0	22	0	51	105	5	32	0	224
- Turkeys	10	0.4	0	0	0	0.2	185	0	8	64	0	179	0	447
- Geese and ducks	0	0	0	0	0	0	15	0	0	0	0.6	1	0	17
- Gamebirds	0.8	4	0	0.1	0	2	3	0	0.3	13	40	9	0	73
- species unknown	0.4	1	0	0.1	0	0.6	2	0.2	0	7	11	17	<0.1	38
Other species										0				
- Small ruminants	<0.1	0.4	<0.1	0	0	0	3	0	1	4	-1	16	<0.1	24
- Fur animals	0.2	202	<0.1	1	0	98	382	0	1	944	673	498	<0.1	2,800
- Aquaculture	197	<0.1	<0.1	<0.1	758	<0.1	0	0	0.7	3	2,342	0	<0.1	3,300
- Other production animals	<0.1	2	1	0.2	0	0.9	0.9	0.1	8	3	20	2	<0.1	38
- Horses	<0.1	1	0.4	0.1	0	0.2	1	0	11	1	87	2	0.1	104
- Pet animals	0.3	6	97	5	1	16	6	1	19	117	187	20	14	489
- Farm identified c)	0.5	0.3	<0.1	<0.1	0	<0.1	0.2	0	4	0.5	0.2	2	<0.1	8
For use in vet. practice d)														
- Pet animals	2	31	251	9	0	46	5	0.1	258	538	203	70	22	1437
- Horses or pet animals	0	12	0.4	0.1	0	0.3	<0.1	0	54	4	113	5	0	189
- Pigs	0	2	<0.1	<0.1	0	0.1	0	4	11	6	2	3	0.5	29
- Cattle	87	591	39	2	9	60	259	19	5,077	1,269	1,953	1,537	3	10,904
- Small ruminants	0.1	1	<0.1	<0.1	0	<0.1	0.1	0	3	3	3	6	<0.1	17
- Fur animals	0	10	0	0	0	4	0	0	0	25	40	17	0	96
Species unknown														
- Topical drugs	1	4	0	<0.1	0	0	0	0	0	0	<0.1	51	7	62
- Intramammaries	0	31	52	0	0	4	0	0	88	120	9	0	2	308
- Micellaneous d)	1	63	4	0.1	0	12	5	6	276	96	406	52	0.2	923
Total	690	6,336	585	23	768	3,048	14,395	10,647	26,007	12,633	15,652	38,475	460	129,718

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

a) Only the ATC group contributing mostly to the antimicrobial group are mentioned. Combination drugs are divided into active compounds

b) Only 20% of the prescribed antimicrobials for cattle are purchased at pharmacies. The remaining 80% are either administered or handed out by veterinary practitioners. The proportions used in various species in practice is estimated from identification of practice type, and information from the repoting from large animal practice. The consumption in poultry practice and aquaculture is more precise, and included together with the consumption from the pharmacies

c) Sales to farmers (valid farm ID code), animal species not identifiable

d) This group contains drugs purchased mainly by veterinarians working in mixed practice

Antimicrobial consumption in pigs

In 2009, the total antimicrobial consumption in pigs was 103.7 tonnes active substance (See Table 6 for details), representing an 11% increase from 2008, while the consumption increased by 12% when measured in doses, ADD_{kg} (calculated from figures in Table 34 in Appendix 1). Relative to production, the consumption increased to 54.6 mg/kg pork produced or 4.9 ADD_{kg}/kg pork produced, which is high compared to consumption in poultry, but low compared to consumption in aquaculture (Figure 5).

Number of heads produced (slaughtered or exported) increased by 2%, while the production decreased by 4% measured in kg meat produced, i.e. slaughtered in Denmark (Table 2). This apparent divergence is mainly due to an increasing number of pigs exported around 30 kg, involving 24% of pigs produced in 2009. As a consequence, measuring antimicrobial use against kg-pork-produced tends to overestimate the increase in treatment frequency, while measuring consumption against number of pigs produced tends to underestimate the treatment incidence. This is illustrated in Figure 6, showing the development in the antimicrobial use per pig produced and per kg pork produced (including export of finishers for slaughter). In between these two graphs is a line showing an adjusted measure of consumption per pig, taking into account the export of 30 kg pigs; the adjustment is based on the assumption that pigs exported at 30 kg compared to those not exported, on average received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg (see Appendix 2).This measure appears to be more stable (independent of changes in trade pattern), and a more reliable measure of trends in antimicrobial consumption per pig. The antimicrobial use per pig produced increased in 2009 by 9.7% compared with 2008 and 33% compared with 2001, ignoring the changes in production with increasing export at 30 kg. Using the adjusted total, the consumption in pigs increased 12.7% per pig produced in 2009, compared with 2008, and 45% compared with 2001. Relative to meat production, the increase in 2009 was 17% compared with 2008, and 53% compared with 2001. During 2001–2009, the consumption increased in all age groups, but more in finishers (by 71%), and less in weaning pigs (by 34%) and sow herds (by 55%) (Figure 6). *Below, the non-adjusted figures will be given unless otherwise mentioned.*

The increasing consumption in 2009 could largely be attributed to tetracyclines, macrolides and

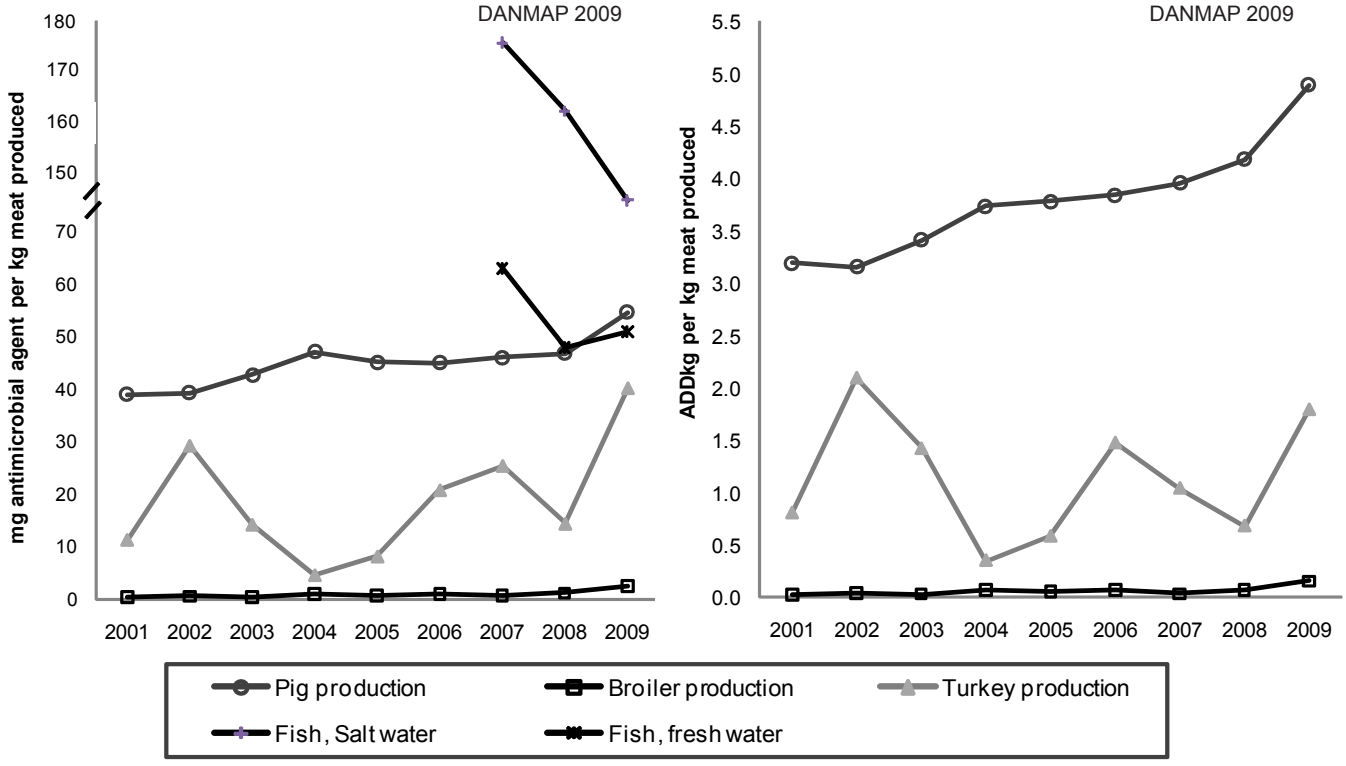


Figure 5. Trends in antimicrobial consumption per kg meat produced from pigs, broilers and turkey

Export of animals for rearing or slaughter is included. However, data for pig production is not adjusted for the increasing export of pigs at 30 kg body weight, although the body mass is included in the production (see text). Thus, the figures overestimate the increasing consumption in the pig production in particular the last three years.

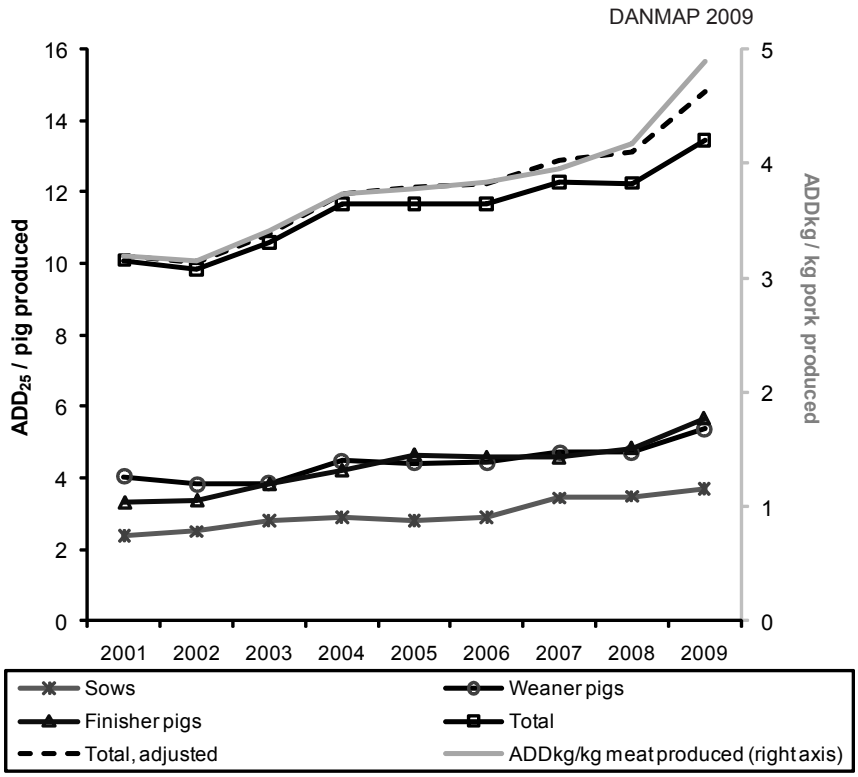


Figure 6. Trends in consumption of antimicrobials in the pigs, given as Animal Daily Doses (ADDs) relative to different measures of production, Denmark

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

pleuromutilins, which continued to be the most commonly used antimicrobial agents in pigs **(**Figure 7a**)**. These agents are mainly used for oral therapy in pigs. Since 2005, tetracyclines have been the most common antimicrobial class used in pigs, and the consumption has been increasing in particular since 2005. This change was likely related to new treatment guidelines launched by the veterinary authorities that year in an attempt to reduce the consumption of macrolides, because this class was defined as critically important by the WHO. The macrolide consumption in ADD_{kg}/pig produced decreased by 3.4% from 2005–2008, but increased by 12.4% in 2009 compared with 2008 (16% when adjusted for export pattern).
The consumption of tetracyclines per pig produced increased by 9.5% (12% adjusted) from 2008 to 2009**,** mainly due to a 15% increase in consumption in weaning pigs (Figures 7). From 2001–2009, the use of tetracyclines in ADD_{kg} per pig produced increased by 83% (100%, adjusted), a trend which has mainly been driven by increasing use in weaning pigs (Figure 7). From 2007–2009, the consumption of pleuromutilins per pig produced increased by 53%, mainly driven by reduced prices for tiamulin in 2007, making it price competitive with macrolides and tetracyclines[1] and almost as commonly used as macrolides (Figure 7a). The use of aminoglycosides (neomycin, apramycin and gentamicin) has remained at a low level since 2007, when the most commonly used aminoglycoside product (neomycin) was redrawn from the market (Figure 7a, 7b). The major aminoglycoside for oral use in weaners changed from neomycin in 2006 to apramycin in 2008. In 2009, the consumption of 3rd and 4th generation cephalosporins in pigs decreased by 25% to 99 kg in 2009; this is the first important decrease since 2001 when this class was approved for use in pigs (Figure 8). As in previous years, the majority (87%) was used in sow herds (Table 6). The decrease is mainly associated with herds receiving occasional prescription. As discussed in DANMAP 2007, a pilot study has shown that cephalosporins were commonly used for prophylactic treatment of umbilical infection in piglets as one injection within the 1st or 2nd day after birth. Prophylactic treatment is not generally allowed in Denmark. However, if a herd is known to have recurrent problems with specific diseases, it is allowed to treat before an expected outbreak.

1) Development in antibiotic consumption, expenses and prices for prescription veterinary medicine for production animals from April 1st 2005 to April 1st 2008, Report of December 15th 2008 to the Ministry of Health; The Danish Medicines Agency, the Danish Veterinary and Food Administration and the Technical University of Denmark, 2008

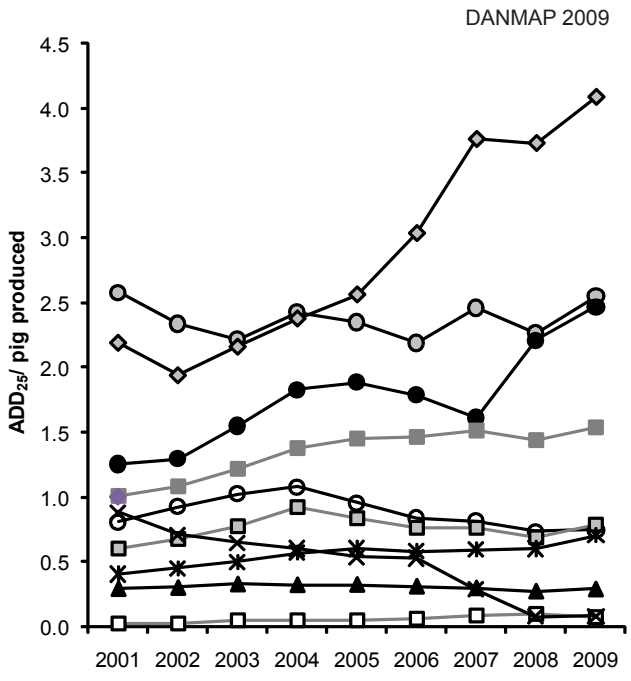


Figure 7a. Trends in antimicrobial consumption (in ADD25 [a]) in pigs, 2001–2008, Denmark

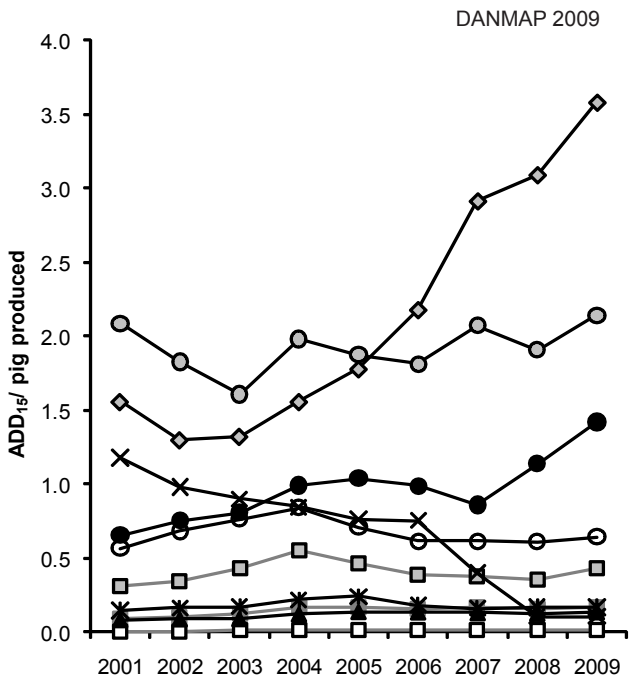


Figure 7b. Trends in antimicrobial consumption (in ADD_{15} [a]) in weaners, Denmark

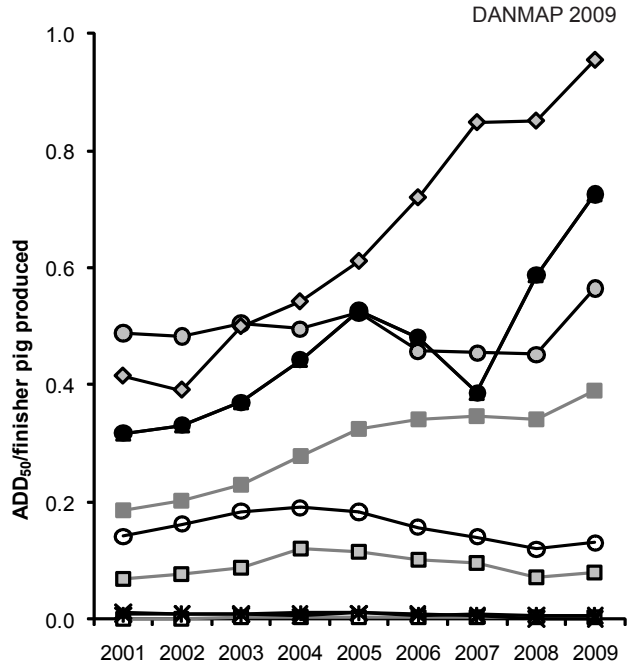


Figure 7c. Trends in antimicrobial consumption (in ADD_{50} [a]) in finishers, Denmark

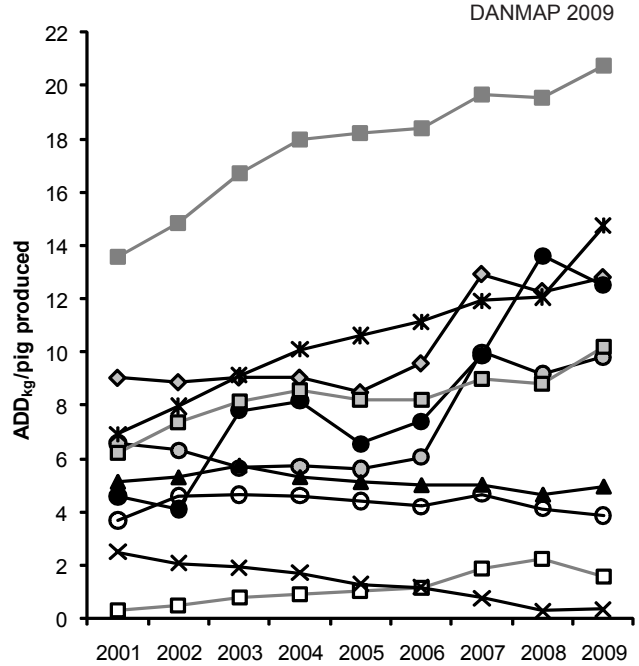
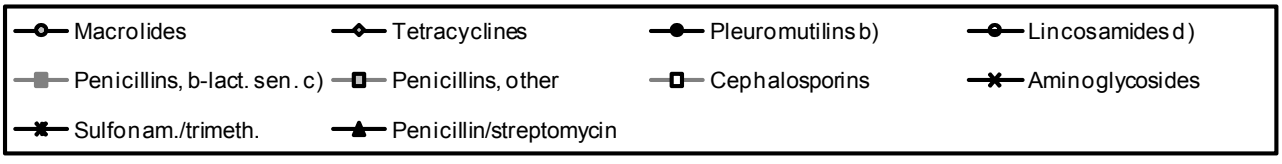


Figure 7d. Trends in antimicrobial consumption (in ADD_{kg}) in sows and piglets, Denmark



Amphenicols, colistin, fluoroquinolones, intramammaries and gynecologicals are not included in the figure. Data from veterinary practice are not included (<1% of the consumption in pigs). In Figure V11, number of pigs produced is exclusive pigs exported at 15–50 kg. In all other figures, pigs exported at 15–50 kg are included

a) ADD_{25}: doses for treatment of 25 kg pigs, to compare treatment across age groups.doses for treatment of 15 kg pigs
ADD_{15}: An assumed average dose for treatment of weaners (7.5–30 kg)
ADD_{50}:Doses for treatment of 50 kg pigs. An assumed average dose for treatment of finishers (30–110 kg). Number of pigs produced excluding pigs exported at 15–50 kg
ADDkg, doses for treatment of one kg pigs: unit used to measure antimicrobial use in sow herds; the drugs are used either in sows (bodyweight>200 kg) or in piglets (<2kg–7.5 kg)

b) Pleuromutilins comprise primarily tiamulin

c) b-lactamase sensitive penicillins

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

Antimicrobial consumption in cattle

In 2009, approximately 15 tonnes of antimicrobial substance was prescribed for cattle, as compared to 14.4 tonnes in 2008. While data on pigs and poultry have a high degree of certainty, this is not the case for cattle: For cows, a major part of the medicine is reported from veterinarian practices; in early years up to 20% of some of the antimicrobials used in practice are missing, possibly due to technical errors in the data transmission; this bias affected mainly data on cows, and lesser the data on calves, as a larger proportion of medicines for calves are handed out from the pharmacies. Based on the type of technical errors, it can be assumed that the missing data are random, and thus that the data from the veterinarians are representative for the choice of drugs.

The quality of data has been improving the last years, but trends in total consumption in cattle cannot be followed with high degree of certainty. However, from the pharmacy sales data, it can be concluded that the antimicrobial consumption in cattle has been stabile, between 14–15 tonnes during 2004–2009. During this period, the beef production has decreased by 17% and the milk production has increased by 6.7% (Table 2).

Table 35 in Appendix 1 shows the registered consumption of antimicrobials for systemic use; the numbers indicate a stable consumption in calves; the apparent increase in cows was at least in part due to the bias mentioned above. The numbers also show the changes in choice of drugs; these are depicted in Figure 9 and 10. The major drug of choice for cows was benzylpenicillin, increasing to 57% of the consumption in 2009, while tetracyclines comprised 20%. The major indication for treatment of cows was mastitis. In calves, the major drugs of choice were long-acting oxytetracycline medicines-, and since 2006, also macrolides. The major indication in calves was respiratory disease. In 2009, the consumption of cephalosporins decreased both in cows and in calves. Overall, the consumption of 3rd and 4th generation cephalosporins decreased by 10 kg for systemic use to 58 kg in 2009; for intramammary use, a 7 kg decrease to 17 kg occurred (Figure 8).

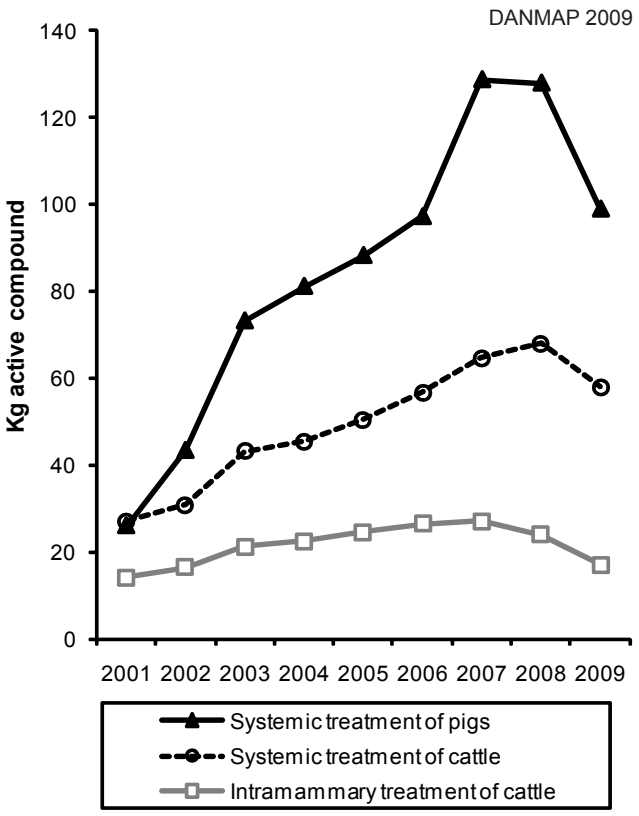


Figure 8. Trends in consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark

The consumption of penicillins for intramammary application increased by 18% from 2008 to 2009, comprising 51% of the intramammary use in 2009 (Table 7). Furthermore, combinations of benzylpenicillins (mainly dihydrostreptomycin), comprised additional19% of the consumption in 2009. From 2008 to 2009, the intramammary use of 3rd and 4th generation cephalosporins decreased by 32%, measured in ADD. This was mainly encountered by the increase in use of penicillins. These trends in choice of antimcronial agents for intramammary treatment probably reflect a sensitive response to debate, guidelines and information in recent years, about the consequences regarding development of ESBL.

Table 7. Consumption in cattle of antimicrobial agents for intramammary application, Denmark DANMAP 2009

Antimicrobial agents	ATC_{vet} classes	2005	2006	2007	2008	2009	% change 2008–2009
				ADD (1000's)[a)]			
Penicillins [b)]	QJ51CE, QJ51CF, QJ51CR	454	464	454	506	599	18
Aminoglycoside-benzylpenicillin combinations [c)]	QJ51RC	231	203	202	201	220	9
Cephalosporins, 1st generation	QJ51DA	206	197	177	169	178	5
Cephalosporins, 3rd and 4th generation	QJ51DC, QJ51DD	229	255	262	231	156	-32
Others[d)]		42	35	31	30	27	-11
Total		1163	1153	1126	1138	1180	4

a) For intramammary treatment, 1 ADD is defined as the dose to treat one teat for 24 hours
b) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations
c) Mainly dihydrostreptomycin- benzyl benicillin combinations; also combinations of penicillin/aminoglycoside with bacitracin or nafcillin
d) Lincosamides, neomycin- lincomycin combinations and trimethoprim-sulfonamide combinations

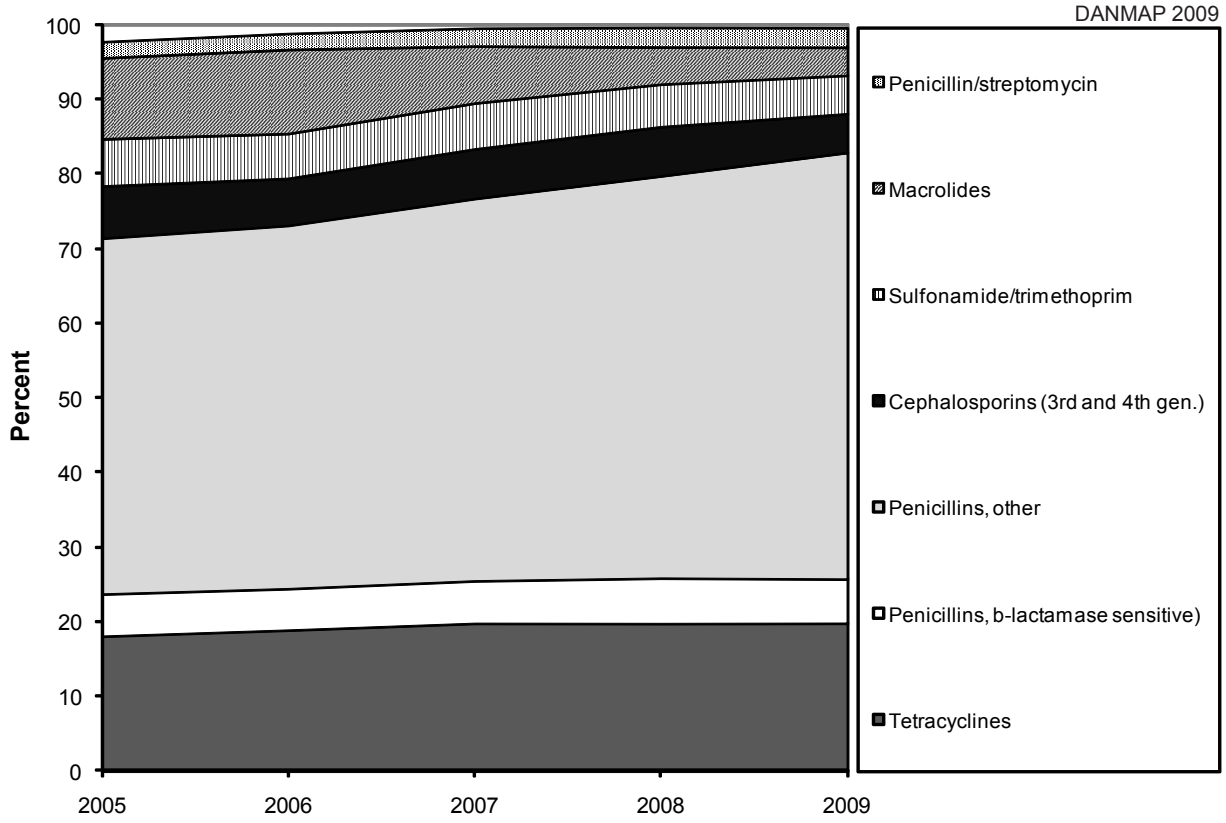


Figure 9. Relative consumption of systemic antimicrobial drugs in cows and bulls, measured in ADD; 2005–2009, Denmark

The antimicrobials not shown (amphenicols, fluoroquionolones, lincosamides, aminoglycosides, colistin and pleuromutilins), each accounts for less than 1 percent of the consumption.

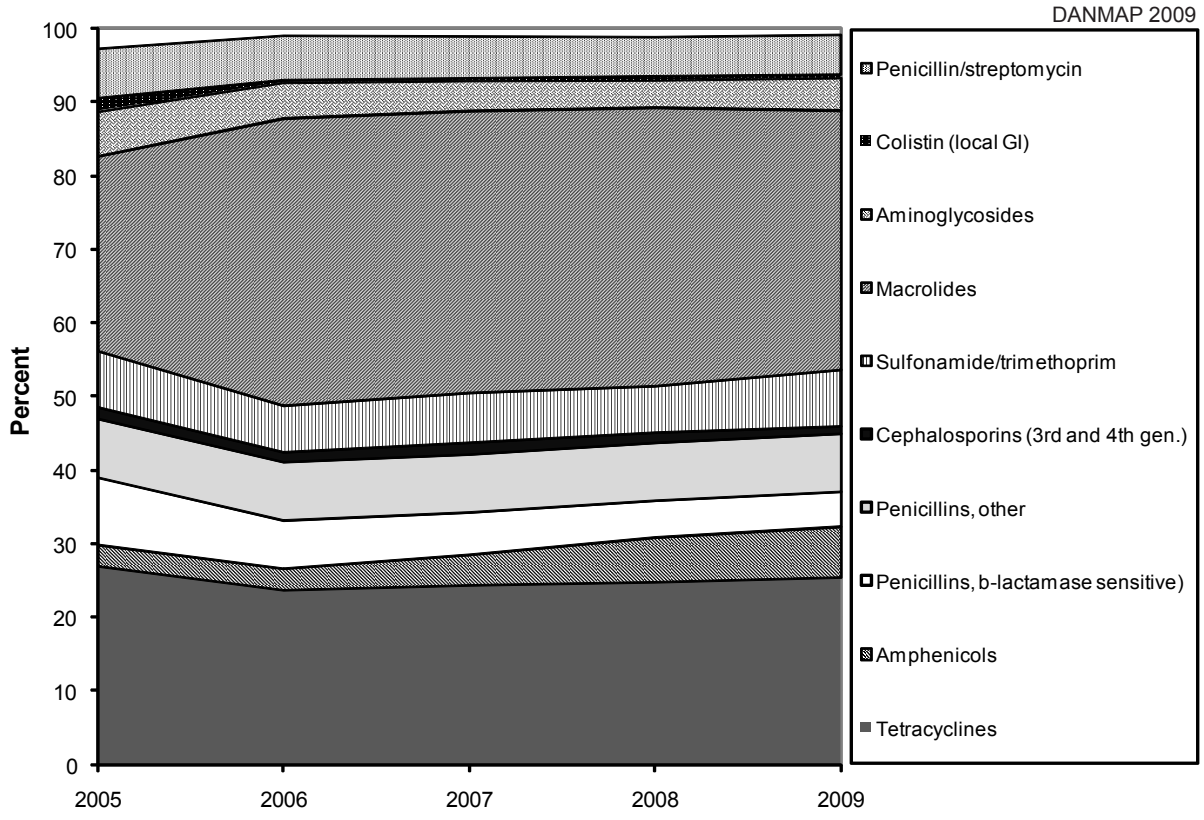


Figure 10. Relative consumption of systemic antimicrobial drugs in calves, measured in ADD, 2005–2009, Denmark

The antimicrobials not shown (fluoroquionolones, lincosamides, and pleuromutilins), each accounts for less than 1 percent of the consumption.

Antimicrobial consumption in poultry

The consumption of antimicrobial agents in poultry almost doubled, from 556 kg active substance in 2008 to 1070 kg in 2009 (Table 6). This increase was not as dramatic as it may seem because the antimicrobial consumption in poultry, particularly in domestic fowl, is generally at a very low level compared to other species (Figure 5). Therefore, disease outbreaks in a few farms affect importantly the national consumption in poultry, causing considerable fluctuations in annual consumption, between 400–1070 kg active substances per year, 2001–2009.

In the broiler production, the consumption per broiler produced increased from 0.07 ADD_{kg} in 2008 to 0.15 ADD_{kg} in 2009 (Table 8). Similar statistics are published in the Netherlands, where the consumption per broiler was 5 ADD_{kg} per broiler produced in 2008 (MARAN 2008), or 70 times more than in Denmark. In 2009, the increase in consumption in the broiler production was related to use of amoxicillin and tetracyclines; according to the poultry practitioners, the increase was mainly due to femoral head necrosis (bacterial chondronecrosis with osteomyelitis) in the chickens, but *E.coli* infections (septicaemia) have also been a problem; in addition, botulism (*Clostridium botulinum*) in some rearing and parent flocks in 2009, has contributed to increasing antimicrobial consumption in the broiler production. In the broiler production, the consumption of fluoroquinolones was reduced by 97% in 2008 compared with the level in 2006. However, in 2009, the fluoroquinolone consumption in the broiler production increased to 63% of the 2006 level, for treatment of multi resistant infections in the chickens from a specific parent flock, according to the major poultry practitioners.

In 2009, the consumption in turkeys increased to 1.8 ADD_{kg} per kg turkey, the highest level since 2002 (Figure 5). According to the poultry practitioners, the increase was due to respiratory infections (*Pasteurella*) and diarrhoea; a vaccination program has been introduced, to combat the problems with *Pasteurella* infections. The increase was related to an increase in the usage of the three major antimicrobial classes in turkeys: tetracyclines, amoxicillin, and macrolides. In turkeys, the consumption of fluoroquinolones decreased in 2008 to 17% of the 2006 level, and further to zero in 2009 (Table 8).

Table 8 shows considerable changes in choice of drug in the poultry production since 2006. Until 2007, amoxicillin comprised approximately 90% of the antimicrobial used in poultry, and fluoroquinolones were the second most used antimicrobial group**.** The major cause for this consumption pattern was that only fluoroquinolone and amoxicillin products had been approved for use in poultry. In 2008, a tetracycline product was approved for poultry, causing a steep increase in use of tetracyclines, in particular for turkeys and in the broiler production in 2008–2009. Also, other antimicrobial classes were approved for use in poultry in 2007–2008. Also, the changes in choice of drugs were partly attributed to guidelines from the supervision team (from The Veterinary and Food Administration) to take into consideration national restriction in use of fluoroquinolone together with the “cascade rule”. The use of fluoroquinolones has been low during 2007–2009, except for the consumption in turkeys in 2007 and in the broiler production flocks in 2009.

Measured on ADD_{kg} per kg poultry produced, the consumption in ducks and geese was similar to the consumption in the broiler production. Annual production data are not available for game birds. The population was estimated at 1 million pheasants, 0.5 million ducks and 0.1 million other birds in 2004. Assuming a constant population, the antimicrobial consumption in game birds has been stable around 1.1 ADD_{kg}/kg meat produced during 2002—2009 (Table 8).

Table 8. Consumption of prescribed antimicrobials in poultry given as Animal Daily Doses (ADDkg), Denmark

DANMAP 2009

ATC$_{vet}$ group	QA07AA	QJ01A	QJ01CA	QJ01CE	QJ01E / QP51AG	QJ01FA	QJ01MA	QJ01X	QA07 / QJ01			
Antimicrobial group	Aminoglycosides	Tetracyclines	Amoxicillin	Penicillins, β-lactamase sensitive	Sulfonamides a)	Macrolides	Fluoroquinolones	Pleuromutilins	Others c)	**Total**	*Production*	
	ADDkg (1000's)										*million kg or eggs d)*	ADD$_{kg}$ per kg meat produced
Broilers												
2001	0	36	2,777	0	90	16	250	13	0	**3,181**	*192*	0.03
2002	0	0	3,352	0	69	0	680	0	0	**4,101**	*190*	0.04
2003	0	70	3,052	0	8	0	270	0	0	**3,399**	*181*	0.03
2004	100	116	4,617	8	43	44	650	75	46	**5,699**	*181*	0.07
2005	0	32	3,984	22	58	3	661	0	100	**4,860**	*180*	0.05
2006	0	0	3,356	6	40	0	620	0	6	**4,029**	*163*	0.06
2007	0	0	1,718	0	168	289	130	0	36	**2,341**	*178*	0.03
2008	0	429	4,086	0	83	133	20	0	80	**4,830**	*186*	0.07
2009	0	5,200	6,988	439	75	560	20	60	80	**13,422**	*181*	0.15
Rearing for broiler production												
2001	0	0	1,392	0	30	0	230	0	0	**1,652**	-	
2002	0	88	2,025	0	96	0	660	0	0	**2,869**	-	
2003	0	0	1,361	0	0	0	80	0	0	**1,441**	-	
2004	0	0	6,464	0	0	0	490	0	0	**6,954**	-	Included in broiler production above
2005	0	0	3,348	0	0	0	400	0	0	**3,748**	-	
2006	0	0	6,238	0	15	0	114	0	0	**6,367**	-	
2007	0	0	2,659	0	43	22	190	0	0	**2,914**	-	
2008	0	400	6,913	0	100	322	0	0	10	**7,745**	-	
2009	0	2,067	7,738	2,851	80	289	440	0	290	**13,754**	-	
Layers and layer rearing												
2001	0	19	434	0	196	16	50	13	0	**727**	*69*	0.01
2002	0	285	670	0	171	0	100	0	0	**1,226**	*70*	0.02
2003	0	540	350	0	328	0	0	0	0	**1,218**	*69*	0.02
2004	0	2	819	2	215	6	30	0	230	**1,303**	*72*	0.02
2005	0	8	680	4	243	0	0	3	30	**967**	*69*	0.01
2006	0	28	376	0	140	11	0	0	0	**555**	*67*	0.01
2007	0	0	1,150	0	96	0	0	0	150	**1,396**	*67*	0.02
2008	0	12	2,563	0	100	0	0	0	70	**2,745**	*68*	0.04
2009	0	713	1,475	0	15	2	0	0	488	**2,693**	*61*	0.04
Turkeys												
2001	0	0	10,477	0	0	0	90	0	0	**10,567**	*13.2*	0.80
2002	0	0	26,829	0	0	0	0	0	0	**26,829**	*12.8*	2.10
2003	0	0	10,900	0	58	0	360	4,568	0	**15,885**	*11.2*	1.42
2004	200	0	4,873	0	76	16	1,560	0	0	**6,725**	*19.6*	0.34
2005	150	60	8,963	0	68	0	780	0	0	**10,020**	*17.4*	0.58
2006	100	150	15,193	0	45	0	1,160	0	0	**16,648**	*11.3*	1.47
2007	518	1,654	6,788	278	0	2,547	2,430	0	728	**14,941**	*14.4*	1.04
2008	0	5,767	1,038	0	4	811	190	0	531	**8,340**	*12.3*	0.68
2009	0	11,771	4,563	491	0	2,538	0	0	536	**19,899**	*11.1*	1.79

Table 8. Consumption of prescribed antimicrobials in poultry given as Animal Daily Doses (ADDkg), Denmark (Continued)

DANMAP 2009

ATC_{vet} group	QA07AA	QJ01A	QJ01CA	QJ01CE	QJ01E / QP51AG	QJ01FA	QJ01MA	QJ01X	QA07 / QJ01			
Antimicrobial group	Aminoglycosides	Tetracyclines	Amoxicillin	Penicillins, β-lactamase sensitive	Sulfonamides a)	Macrolides	Fluoroquinolones	Pleuromutilins	Others c)	**Total**	*Production*	
	ADDkg (1000's)										*million kg meat or eggs d)*	ADD_{kg} per kg meat produced
Ducks and geese												
2001	0	2	0	0	1	11	50	3	0	**67**	*4.5*	0.01
2002	0	12	36	0	0	30	0	0	0	**77**	*4.9*	0.02
2003	0	8	257	0	0	0	0	0	0	**265**	*4.2*	0.06
2004	0	14	400	0	13	11	150	3	0	**591**	*4.2*	0.14
2005	0	0	525	0	0	14	0	3	0	**542**	*4.1*	0.13
2006	0	0	1,125	0	0	0	0	0	0	**1,125**	*4.5*	0.25
2007	0	0	100	0	0	0	0	0	2	**102**	*2.4*	0.04
2008	0	36	250	0	1	0	0	0	0	**287**	*2.6*	0.11
2009	0	24	0	0	10	200	0	0	0	**234**	*2.2*	0.11
Game birds												
2001	0	77	768	0	205	146	85	5	84	**1,370**	-	-
2002	125	177	1,466	0	346	289	10	10	94	**2,518**	-	-
2003	150	128	923	0	318	273	1	933	0	**2,725**	-	-
2004	250	148	1,003	0	460	113	30	18	0	**2,022**	-	-
2005	160	98	1,939	0	403	177	0	13	14	**2,803**	-	-
2006	110	86	1,863	0	258	39	11	5	42	**2,413**	-	-
2007	2	126	1,425	0	542	37	0	0	73	**2,203**	-	-
2008	110	80	1,825	0	256	39	11	0	38	**2,360**	-	-
2009	0	270	901	18	664	46	10	0	172	**2,080**	-	-
Production type unknown e)												
2001	1	155	3,814	0	441	306	475	10	18	**5,219**	-	-
2002	29	95	2,909	0	315	272	93	5	0	**3,718**	-	-
2003	300	91	2,370	0	348	186	391	5	0	**3,690**	-	-
2004	450	106	3,654	0	440	90	131	3	4	**4,878**	-	-
2005	0	58	2,978	0	192	3	121	5	46	**3,403**	-	-
2006	50	144	3,059	0	182	4	110	0	0	**3,549**	-	-
2007	0	140	1,321	72	518	118	34	8	58	**2,267**	-	-
2008	0	374	863	0	263	148	3	3	39	**1,692**	-	-
2009	2	794	486	0	182	22	11	5	56	**1,557**	-	-

ADDkg: the dose necessary for treating 1 kg body-weight
a) Includes sulfaclozin (a coccidiostat/antibacterial) and sulfonamide/trimethoprim combinations
b) Lincomycin, including combinations with spectinomycin
c) Includes QA07AA10 (colistin), QJ01FF (lincosamides), QJ01B (amphenicols) and QJ01R (penicillin/streptomycin combinations)
d) For layers and layer rearing, only the production of egg for consumption is included
e) includes prescription with erraneaus farm id or farms with more than one poultry species

Antimicrobial consumption in fur animals, aquaculture and pet animals

In 2009, the production of fur animals included 14 million mink, 34,000 chinchillas and a minor production of foxes, as in 2008, except for a small increase in chinchilla production. The consumption of antimicrobial agents in fur animals increased by 28% - from 2,270 kg in 2008 to 2,896 kg in 2009. In 2009, aminopenicillins remained the most commonly used antimicrobial class in fur animals, comprising 33% of the antimicrobial consumption (kg active compound) in 2009; macrolides, tetracyclines and sulfonamide-trimethoprim combinations comprised another 56%. Fluoroquinolones and cephalosporins comprised less than 0.1% (1.4 kg) of the consumption in fur animals.

The antimicrobial consumption in aquaculture decreased by 4% to 3,300 kg in 2009 compared with 2008. The decrease was possibly related to the introduction of a recent vaccine against furunculosis. The antimicrobial consumption for fish in salt water decreased by 11% from 2008 to 146 mg/kg fish in 2009, possibly due to marketing of a vaccine against furunculosis (Figure 5). Regarding fish produced in fresh water, the consumption increased by 3% from 2008 to 51 mg/kg in 2009; (both changes, assuming an unchanged production in 2009 compared with 2008, Table 2). Unusually warm summers are the most likely reason why the use of antimicrobial agents in aquaculture was importantly higher in 2007 and 2008 as compared with previous years, and still at a high relatively high level in 2009. The major class of antimicrobial was sulfonamide/trimethoprim, comprising 71% of the consumption in aquaculture. The consumption of quinolones (oxolinic acid) comprised 23% of the consumption in aquaculture in 2009, and has increased importantly (by 131%) since 2007.

In companion animals, an estimated 2.2 tonnes of antimicrobial substance was used in 2009, which is similar to 2008 (Table 6). The majority was used in pet animals. The major antimicrobial agent used in pet animals, amoxicillin in combination with clavulanic acid, increased by 6% to 525 kg in 2009, as part of a continuous increase, at least since 2004. Other frequently used drugs were cephalosporins (348 kg), mainly first generation for oral use; and sulfonamide/trimethoprim (estimated 400 kg), and narrow spectrum penicillin (estimated 300 kg), both mainly for parenteral use. Cephalosporins used in pet animals are predominantly first generation (cefadroxil and cefalexin), while 4th generation cephalosporin comprised an estimated 3.5 kg active compound, corresponding to 1% of the cephalosporins used in pet animals in 2009, a 25% increase compared with 2008. In pet animals, the consumption of 3rd and 4th generation cephalosporin was an estimated 2.4 kg, corresponding to 1.4% of the veterinary consumption of these antimicrobials. The use of fluoroquinolones in pet animals was estimated 14 kg in 2009 and this corresponded to 61% of the total veterinary use of fluoroquinolones in Denmark, 2009.

Textbox 1

The assignment of the National Defined Daily Doses for animals (ADD)

Over time and between populations large variability may occur in choice of drug group (ATC_{vet} 3rd or 4th level), but also of active compound within antimicrobial group. The dosages of various antibacterial drugs may vary considerably depending on e.g. potency, pharmacokinetic characteristics, formulation, MIC-values and disease. Accordingly, shifts in choice of drugs (without change in disease occurrence and treatment frequency), may be misinterpreted as a change in total consumption when measured in kg-active-compound on group level.
The Defined Animal Daily Dose (ADD) is a quantitative measure, solving the above mentioned problems. Neither the kg-active-compound nor the ADD is a measure of treatment frequency, as the applied daily dose and the prescribed daily dose may vary both within country and between countries. These limitations should be taken into account for the interpretation of data both when using the kg-active compound or the ADD as the chosen metric.
Based on an EU expert recommendation, the Danish government decided in 1998 to establish a national program for monitoring use of veterinary medicines (VetStat). Valid data were available from 2001, and a National defined daily doses system was developed in 2002. Fundamentally, the ADD is defined as a National veterinary parallel to the international Defined Daily Dose in human pharmaco-epidemiology. The Animal Daily Dose was defined for the specific domestic species (i.e. cattle, swine, poultry, sheep, goats, dogs/cats). In 2009, the definition of the Animal Daily Dose was changed regarding formulations with prolonged effect.

Definition of ADD_{kg} and ADD

- *ADD_{kg} is defined as the assumed average maintenance dose per day for treatment of one kg animal for the main indication in a specified species.*
- *The ADD is defined as the assumed average maintenance dose per day for the main indication in a specified species and "standard" body weight for the species and age group.*

The ADD_{kg} is used as the basis for calculating the exposure in a specified species by multiplying with" standard" body weights and for measuring the consumption in populations comprising various age groups and large variation of body weights.
An ADD_{kg} was assigned for each veterinary medicinal product (VMP) for each relevant species, both the species for which the approval is given, and for other species for which the drug is prescribed. The ADD_{kg} is also set for human drugs and for commonly used extemporaneously prepared medicinal products, identified in VetStat as prescribed for animals. The ADD_{kg} has been assigned for all systemic antibacterial drugs identified as prescribed for animals, drug for local gastrointestinal, intramammary or intra uterine use. For most topical preparations, general and local analgesics, anesthetics, neuroleptics, and contrast media, an ADD_{kg} is not feasible.

Assigning ADD_{kg} and calculating ADDs

The daily dose is initially defined per kg bodyweight (ADD_{kg}) and species, and subsequently an ADD per animal species and age class is calculated by multiplication with a defined standard animal bodyweight for the age-group within species.
An ADD_{kg} is principally calculated as the mean-range value of the recommended maintenance dosage per day for the active ingredient of the VMP. The range and treatment frequency are taken from the recommendations approved by the Danish Medicines Agency (the SPC's). Where synonymous VMPs have been marketed but with differing dosage recommendations, a common ADD_{kg} for the active ingredient is defined and applied for these VMPs. In such cases, the ADD_{kg} is set as the most frequent maintenance dose recommended among these drugs, i.e taking into consideration which VMPs are used more.
Regarding drugs for which only one administration is required (i.e. long acting drugs), the ADD_{kg} was

initially set as the (initial) recommended dose, although the concentration maintains at a therapeutic level for several days. Thus, special attention had to be directed towards the use of long-acting drugs in consumption statistics as described in DANMAP 2007. However, this definition was not logical, considering that for drugs applied more than once daily, the ADD_{kg} is defined as the sum of doses applied per 24 h. Therefore, in 2009 the definition of the ADD_{kg} for long-acting drugs was changed accordingly: An ADD_{kg} is defined as the initial dose divided by the minimum duration of therapeutic effect (hours) and multiplied by 24 (hours). When the therapeutic duration is uncertain, the drugs are assigned the same ADDs (mg active compound) as the ordinary dosage forms. Accordingly changes affected of the ADD_{kg} included drugs within the macrolide- and cephalosporin classes, oxytetracycline and benzylpenicillin. The dose for long acting ampicillin formulations was identical to the dose set for the ordinary formulations and therefore not changed.

The “standard body-weight”, used to calculate the ADD from the ADD_{kg}, was set as the assumed average weight at treatment for each age-group. The standard body-weights for pigs and cattle were defined following consultations with a group of specialized practitioners for each species. For goats and sheep, researchers in close contact with the producers of these species were consulted. For poultry a standard weight was not defined i.e. only the ADD_{kg} is applied, because the growth rate is extremely high compared to other food animals and because in these food production systems, dosing is based on the total population biomass. For single-dose formulations, usually formulated for a specific age-group but independent of bodyweight, only the ADD — and not the ADD_{kg} — is defined.

The defined animal standard weight will only give an estimate of the true average of the animal weight at treatment in the subpopulation. However, measuring drug usage in ADDs for “standard animal weight”, always leaves an opportunity for recalculating the figures to the more precise quantitative measure, the $ADD_{kg,}$ (when the age-group or weight is not known), or using other defined body weights when relevant for specific purposes and populations.

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Textbox 2

Antimicrobial resistance and antimicrobial consumption in conventional and alternative pig production systems

More than 95.5% of close to 19 million finisher pigs slaughtered in Denmark are produced in conventional indoor production systems, yet a couple of alternative animal production concepts do exist. The major alternative systems are the organic finisher pig production and the production of non-organic free range pigs both with access to outdoor pens. The free-range and organic systems respectively produced approximately 86.000 and 80.000 finisher pigs yearly in 2008–2009.
According to the legislation (2007-2008, when the described study was conducted) the use of antimicrobials in the husbandry production in Denmark must be based on a veterinary diagnosis in the herd. Preventive antibiotic treatment is prohibited, but it is allowed to treat animals before onset of disease if infection with well-defined illness is suspected. If the herd has a Health Agreement Contract (HAC) with a veterinarian, prescription of antimicrobials for expected disease treatments the following 35 days is allowed. In herds without a HAC, antibiotics may only be prescribed for the following five days. The HAC comprise a veterinary advisory visit (incl. relevant examinations and diagnostic work) in the herd approx. monthly (12 times annually). HAC was optional for conventional herds until July 2010 and in general larger conventional pig herds have a HAC. According to the EU-legislation the use of antibiotic growth promoters is prohibited.

Specific regulation of the use of antimicrobials in

Organic pig production
- Pigs receiving antimicrobial treatment more than once loose the organic status
- Each antimicrobial treatment must be based on a veterinary diagnosis and prescription is specified for the actually diseased pigs – contact with the veterinarian is needed in each case
- In the herd, only antimicrobials for ongoing treatments may be stored
- The withdrawal period from treatment with antimicrobial agents to slaughter is twice the length in conventional herds

Non-organic outdoor pig production
- A Health Agreement Contract (HAC) with a veterinarian is mandatory.
- Continuous use of antibiotics is not allowed in any part of the production
- The withdrawal period from treatment with antimicrobial agents to slaughter is twice the length in conventional herds

In the project QUALYSAFE, conducted 2007–2008, the antimicrobial resistance and the antimicrobial consumption on herd level was studied for finisher pigs (pigs from 30 kg to slaughter at app. 107 kg) in 146 conventional herds, 51 organic herds and 27 non-organic free range herds. Data on consumption of antimicrobial agents was obtained from the Vetstat data base, and resistance in *E. coli* to 16 antimicrobials (DANMAP standard panel) was tested by use of sensititre.
Overall, there was a marked difference in the use of antimicrobial agents (Figure 11); only about half of the organic herds used antimicrobials, as compared to more than 90% of free range herds and conventional herds. Both consumption of antimicrobial agents and occurrence of antimicrobial resistance (Figure 12) were higher in conventional herds and lower in organic herds. As an example, out of the total of 868 isolates available from all 224 farms, 205 isolates were resistant to tetracycline (organic: 8%, free range: 25% conventional: 32%). The organic farms had the lowest average consumption of tetracycline with 0.04 doses per slaughter pig followed by conventional farms and free range farms with 1.3 and 1.6 doses per slaughter pig, respectively. Thirty-three percent of the free range farms, 38% of the conventional farms and 76% of the organic farms had no tetracycline consumption in the year 2007. A strong significant association

between resistance to tetracycline in *E. coli* isolates and the use of tetracycline to finisher pigs in the herd (increased risk of resistance from use of tetracycline) as well as the herd type (increased risk of resistance in *E. coli* isolates from conventional and non-organic free range herds compared to organic herds) was demonstrated (using the procedure NLMIXED in SAS. Significance of variables were tested using the the likelihood ratio test with a significance level of 5%). The antimicrobial resistance was higher in winter/spring when the antimicrobial consumption is known to increase[1] than in summer/autumn, and the resistance pattern in free range herds resembled the pattern in organic herds in summer and the pattern in conventional herds in winter (Figure 12). The results indicate that consumption of antimicrobial agents is a major driving factor for the occurrence of antimicrobial resistance, although other herd characteristics may be involved. Further studies into the reason for differences in antimicrobial consumption may reveal new ways to limit the antimicrobial consumption and resistance in the pig production. The report may be accessed at http://www.bioethics.kvl.dk/tekster/0911koedsikkerhed.pdf, [in Danish]

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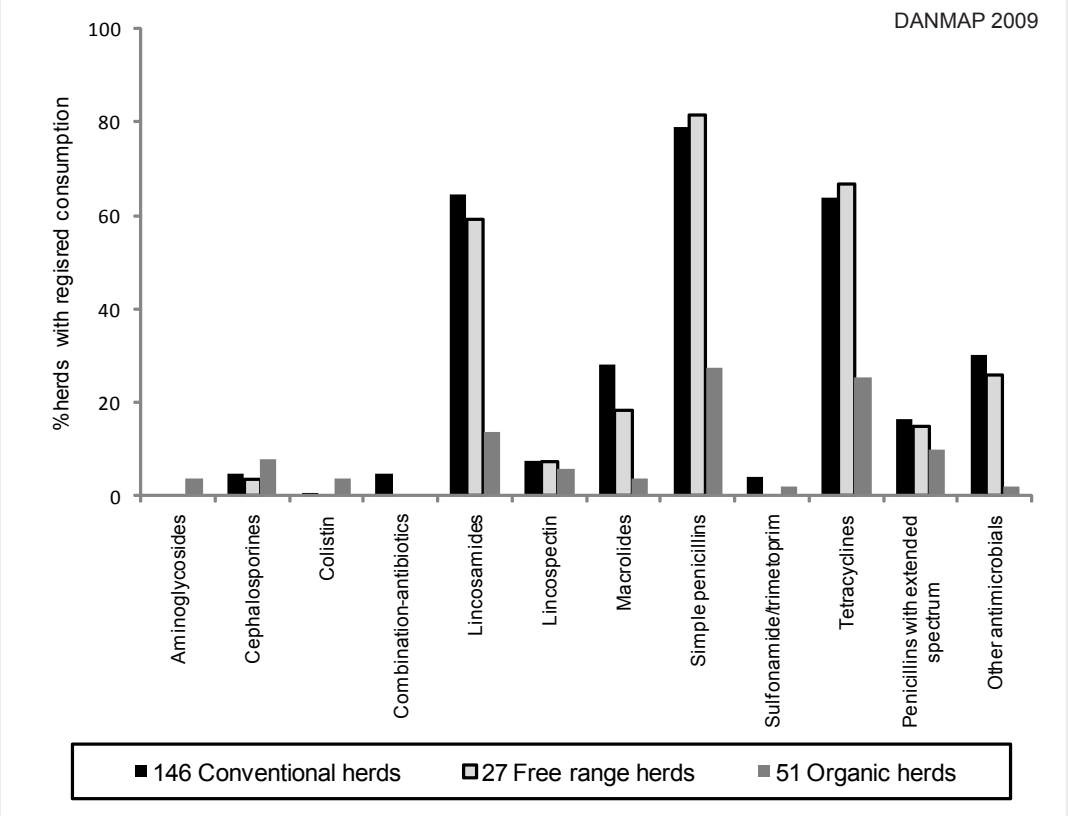

Figure 11: Proportion of finisher pig herds within study herd type with registered consumption of different types of antimicrobial agents in 2007 (Source: Vetstat). Only antimicrobial agents prescribed for finishers is included.

[1] Jensen VF, Enøe C, Wachmann H, Nielsen EO. Antimicrobial use in Danish pig herds with and without postweaning multisystemic wasting syndrome. Prev Vet Med. 2010 May 12. [Epub ahead of print]

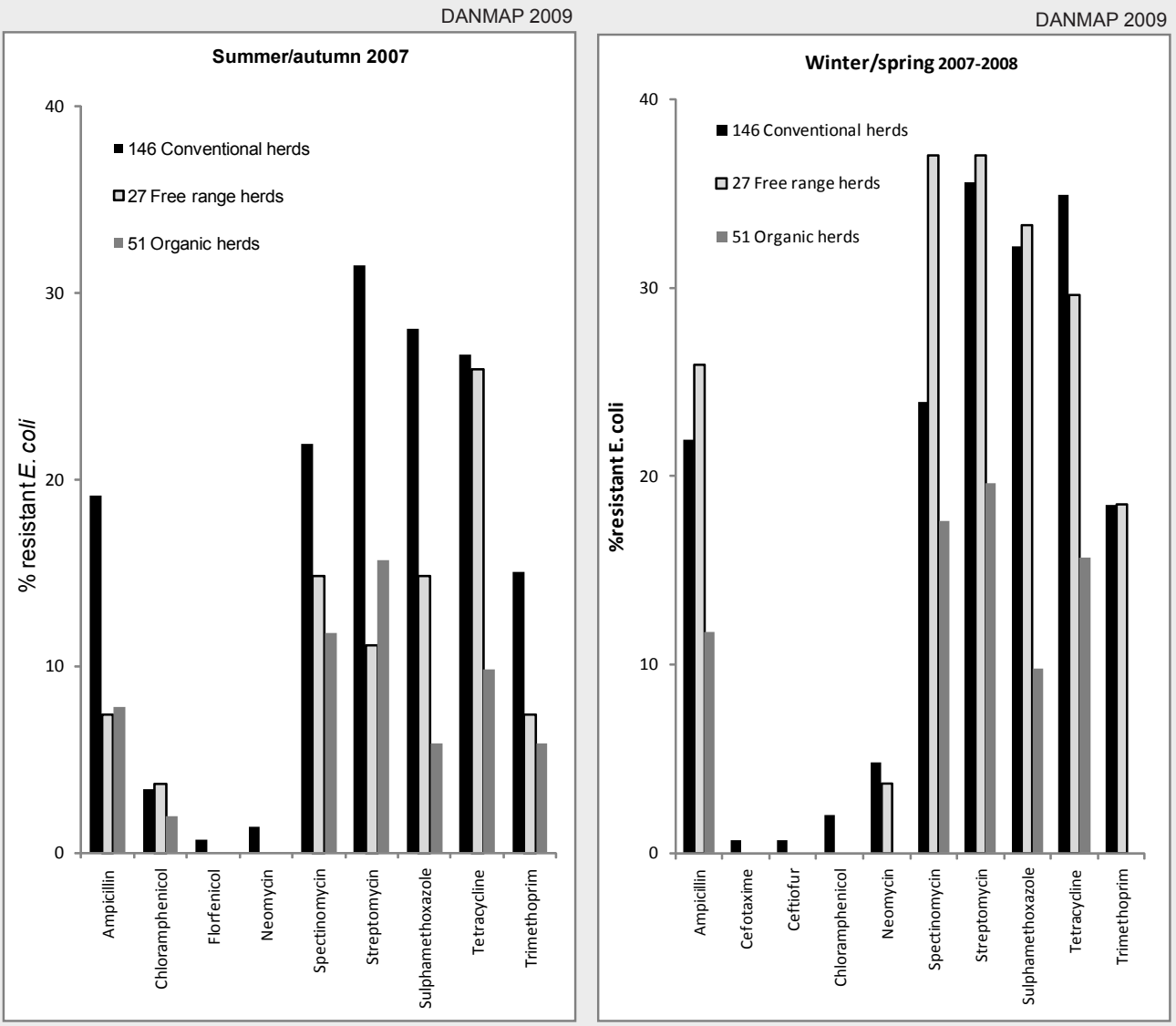

Figure 12: Resistance to antimicrobial agents in E.coli *from 224 caecal samples from finisher pigs conventional , organic and non-organic free range herds in summer/autumn 2007 (first* E. coli *isolated from each herd) and winter/spring 2008 (last* E. coli *isolated from each herd) . Among these isolates none were resistant to Axoxicillin+Clavulansyre, Apramycin, Ciprofloxacin, Colistin, Gentamicin and Nalidixan. (Source: the QUALYSAFE project)*

Antimicrobial consumption in humans

Introduction and definitions

The consumption of 2009 is compared with the consumption of 2008 and of 2000, respectively. Antibacterial agents used for systemic treatment in humans (and in animals) are listed in Table 4.
Defined Daily Dose (DDD) is the unit for measuring antibacterial consumption. The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that DDD is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose [http://www.whocc.no].

Primary health care: consumption should preferably be presented as numbers of DDD per 1000 inhabitants per day (DID). Data presented in DDD per 1000 inhabitants per day provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DDDs per 1000 inhabitants per day indicates that 1% of the population on average gets a certain treatment daily. Consumption measures at treated patient level are achievable, using DDDs/patient or DDDs/package. Also, consumption measured by the number of packages per 1000 inhabitants and by the number of treated patients per 1000 inhabitants is displayed in the appendix (Tables 36, 37).

Hospitals: consumption should preferably be presented as both DDD per 100 occupied bed-days (DBD) and DDD per 100 discharges to include the activity in hospitals, and as the number of DID when comparing to primary health care.
Due to procedural rearrangements of certain chemical substances for infusion, the reporting of sales (consumption) by the hospital pharmacies to the Danish Medicines Agency has been inaccurate for some groups. Consumption of cephalosporins, carbapenems and combinations of sulfonamides and trimethoprim incl. derivatives has been corrected, as in 2005–2008. In 2009, national data were not accessible for the consumption of combinations of sulfonamides and trimethoprim, incl. derivatives. Instead, data from eighteen hospitals within three regions are reported (The capital region of Denmark, Region of Southern Denmark and Central Denmark region).
The number of occupied bed-days and discharges of 2008 obtained from the National Board of Health has been updated. This update has led to minor changes in the reported consumption.

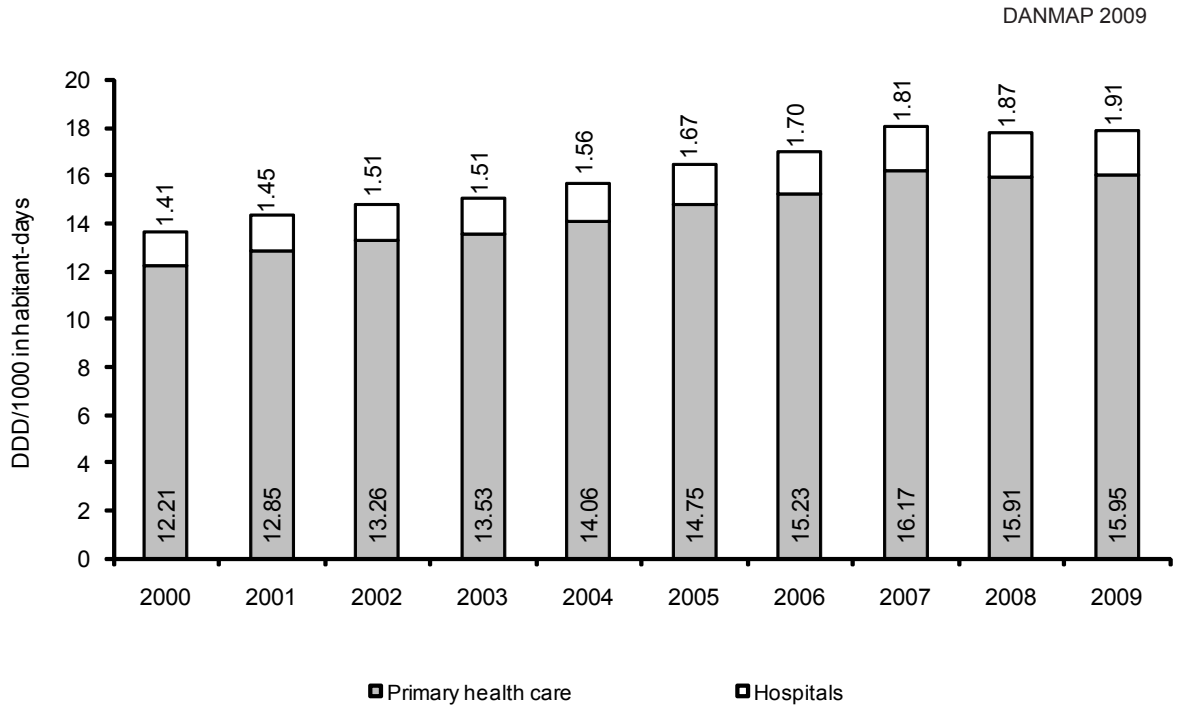

Figure 13. Total consumption of antibacterial agents (J01) in humans, Denmark

Total consumption in both primary health care and hospitals

In 2009, the total consumption of antibacterial agents for systemic use (primary health care and hospitals) amounted to 17.9 DDDs per 1000 inhabitants per day (DID) compared with 17.8 DID in 2008. Consumption has increased by 4.2 DID (31.1%) since 2000 (Figure 9). To view the detailed distribution of DIDs among antibacterial groups in primary health care and hospitals, please refer to Table 10 and Table 38 in Appendix 1), respectively. The percentage of DDDs prescribed in primary health care represented 89% of the total human consumption. In all previous years, 2000–2008, the percentage of DDDs prescribed in primary health care represented 90% of the total human consumption.

Figure 14 shows the distribution of the total number of DIDs of antibacterial agents between primary health care and hospitals. For example, penicillins with extended spectrum (J01CA) and fluoroquinolones (J01MA) had a ratio of consumption in primary health care vs. consumption in hospitals of around 9/1 and 2/1, respectively.

To allow comparison with consumption of antibacterial agents in animals, total human consumption is also presented in kilograms (Table 9). In 2009, 48.5 tonnes of antibacterial agents for systemic use were used in humans in Denmark representing a decrease of 0.1 tonnes (0.1%) compared with 2008, but an increase of 8.4 tonnes (20.8%) compared with 2000.

Table 9. Consumption of antibacterial agents for systemic use in humans (kg active substance), Denmark. Includes data from both primary health care and hospitals and has been recalculated from original data expressed as DDDs. For monitoring in human primary health care and hospitals, the recommended way of expressing consumption is DDDs per 1000 inhabitant-days and DDDs per 100 occupied bed-days (see Tables 10, 12 and 13) DANMAP 2009

ATC group a)	Therapeutic group	Year									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	1486	1475	1501	1542	1636	1748	1835	1855	1884	2039
J01B	Amphenicols	0	1	0	0	0	0	0	0	0	0
J01CA	Penicillins with extended spectrum	5141	5385	5356	5295	5346	5561	5722	6188	6061	6076
J01CE	Beta-lactamase sensitive penicillins	19,749	20,730	21,263	21,630	22,230	22,520	22,760	24,003	22,466	21,744
J01CF	Beta-lactamase resistant penicillins	2655	3230	3738	4075	4377	4565	4842	5037	5183	5250
J01CR	Comb. of penicillins, including beta-lactamase inhibitors	93	146	249	336	480	534	724	1012	1348	1836
J01D	Cephalosporins and related substances d)	692	739	811	830	894	1582	1778	2285	2530	2740
J01EA	Trimethoprim and derivatives	262	280	293	307	334	359	382	402	402	399
J01EB	Short-acting sulfonamides	3142	3113	3092	3064	3067	2987	2865	2565	2273	2200
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	291	289	288	273	185	208	208	148	183	193
J01FA	Macrolides b)	4040	4089	4150	3876	3743	3775	3542	3434	3164	2966
J01FF	Lincosamides d)	29	37	40	45	53	52	66	78	94	113
J01G	Aminoglycosides	32	30	31	28	31	31	27	27	25	23
J01MA	Fluoroquinolones d)	344	398	451	611	722	866	979	1162	1351	1371
J01XA	Glycopeptides	37	36	42	43	46	51	56	61	64	86
J01XC	Steroid antibacterials (fusidic acid)	70	59	59	58	52	62	65	67	64	62
J01XD	Imidazoles	155	168	179	191	195	206	198	202	191	160
J01XE	Nitrofuran derivatives (nitrofurantoin)	151	155	163	166	171	180	185	190	192	201
J01XX05	Methenamine d)	1788	1637	1662	1590	1473	1107	1076	1060	1087	1047
J01XX08+09	Linezolid, daptomycin	0	0	3	4	5	10	14	12	14	14
J01	Antibacterial agents for systemic use (total) c)	40,157	41,997	43,371	43,964	45,040	46,404	47,324	49,788	48,579	48,518

a) From the 2009 edition of the ATC classification system
b) When two different DDDs of an antimicrobial agent existed for different presentations an average DDD was used. Estimates using the lowest and the highest calculated limit are 2363–3568 for 2009
c) Does not include polymyxins
d) Since 2005, the kg active substance was estimated taking into account the DDD for each route of administration, e.g. cefuroxime parenteral DDD=3 g and cefuroxime oral DDD=0.5 g. From 1997 to 2004, it was estimated with a DDD corresponding to an average for the various routes, e.g. for cefuroxime: 1.75 g

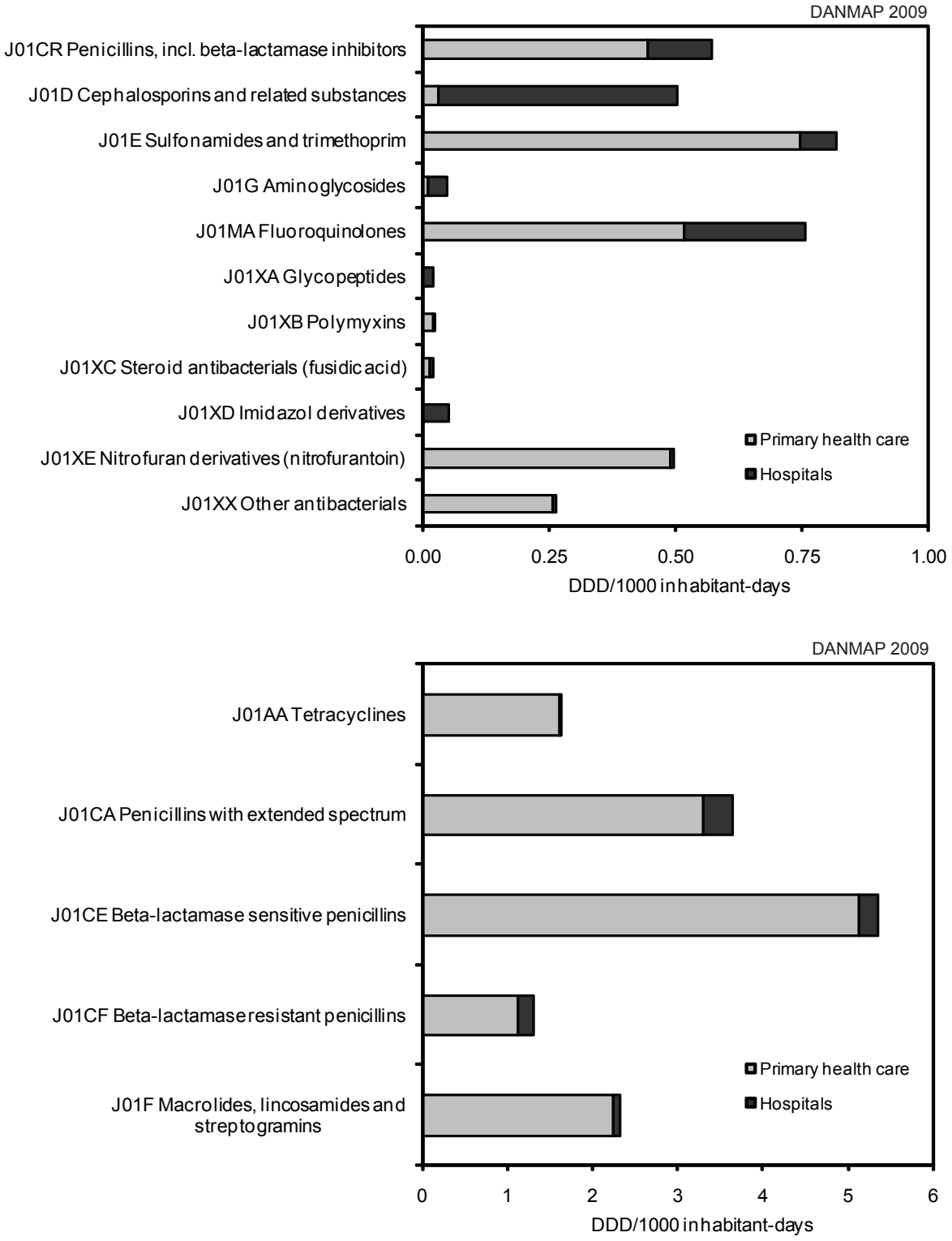

Figure 14. Distribution of DIDs between primary health care and somatic hospitals, Denmark

Primary health care

Total consumption

In 2009, the total consumption of antibacterial agents for systemic use (J01) in primary health care to 15.95 DID compared with 15.91 DID in 2008 (Table HuAB_P1). Consumption increased in six groups: tetracyclines 0.07 DID (4.5%); penicillins with extended spectrum 0.03 DID (1.0%); beta-lactamase resistant penicillins 0.01 DID (1.1%); combinations of penicillins, including beta-lactamase inhibitors 0.18 DID (65.9%); fluoroquinolones 0.01 DID (0.8%); and nitrofuran derivatives 0.02 DID (3.7%). In five groups consumption decreased: beta-lactamase sensitive penicillins 0.18 DID (3.4%); trimethoprim and derivatives 0.01 DID (1.4%); short-acting sulfonamides 0.01 DID (3.7%); macrolides 0.07 DID (3.4%); and methenamine 0.01 DID (4.0%).

Beta-lactamase sensitive penicillins still represented the largest group of antibacterial agents consumed (32%) followed by penicillins with extended spectrum (21%) and macrolides (14%) (Figure 15).
Antibacterial consumption (J01) increased by 31% ($p<0.0001$) during 2000–2009 (Table 10). For all leading groups of antibacterial agents, consumption was higher in 2009 than 10 years before and for most groups the trend in consumption has been a steady increase year by year (Figure 16). Only short-acting sulfonamides (J01EB) and 'other antibiotics' (J01XX) were at a lower reported level in 2009 compared with 2000.

Table 10. Consumption of leading antibacterial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

DANMAP 2009

ATC group a)	Therapeutic group	Year									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	0.98	0.99	1.04	1.07	1.17	1.28	1.38	1.48	1.54	1.61
J01CA	Penicillins with extended spectrum	2.29	2.47	2.51	2.52	2.63	2.79	2.95	3.25	3.26	3.29
J01CE	Beta-lactamase sensitive penicillins	4.69	4.91	5.00	5.07	5.20	5.28	5.40	5.67	5.30	5.12
J01CF	Beta-lactamase resistant penicillins	0.52	0.65	0.77	0.85	0.92	0.97	1.05	1.09	1.12	1.13
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.02	0.03	0.04	0.05	0.06	0.08	0.12	0.19	0.27	0.45
J01D	Cephalosporins and related substances	0.02	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.33	0.35	0.36	0.38	0.41	0.44	0.47	0.49	0.49	0.48
J01EB	Short-acting sulfonamides	0.37	0.36	0.36	0.36	0.36	0.35	0.35	0.31	0.28	0.27
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.03	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.02	2.10	2.15	2.13	2.23	2.41	2.31	2.42	2.28	2.21
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03
J01GB	Aminoglycosides	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.15	0.17	0.18	0.25	0.28	0.33	0.37	0.44	0.51	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	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.38	0.39	0.41	0.42	0.43	0.45	0.46	0.47	0.47	0.49
J01XX05	Methenamine	0.36	0.33	0.34	0.32	0.30	0.28	0.27	0.26	0.27	0.26
J01XX08	Linezolid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01	Antibacterial agents for systemic use (total)	12.21	12.85	13.26	13.53	14.06	14.75	15.23	16.17	15.91	15.95

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

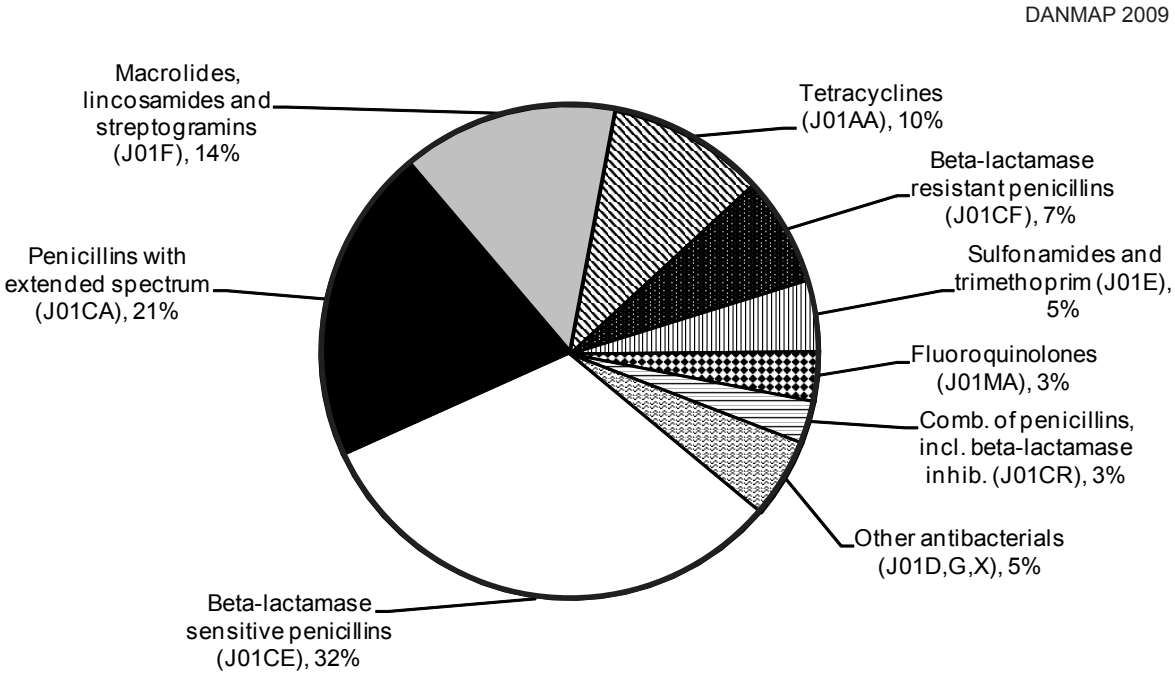

Figure 15. Distribution of the total consumption of antibacterial agents in primary health care, Denmark

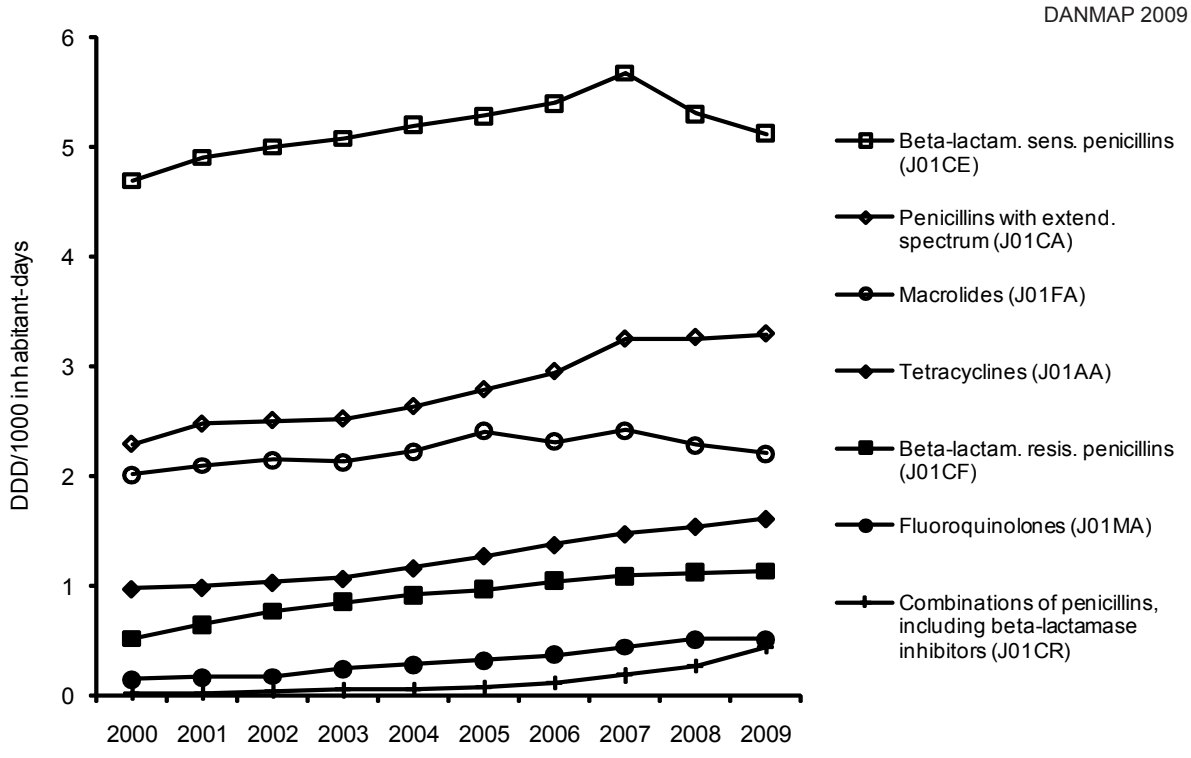

Figure 16. Consumption of leading antibacterial groups for systemic use in primary health care, Denmark

Measures at treated patient level

The total number (J01) of DDDs per treated patient increased from 18.9–19.2 compared with 2008. For all leading substances, DDDs per treated patient ranged from 11–21 in 2009 with the exception of tetracyclines (45.2) (Table 11). During 2000–2009, trends displayed an increasing number of DDDs per treated patient and DDDs per prescribed package. Tetracyclines; comb. of penicillins, including beta-lactamase inhibitors; and fluoroquinolones showed the largest increases.

Tetracyclines: the available packages (size and strength) have not been altered over the last decade; prescriptions of packages with higher numbers of tablets could be explanatory for this trend as pointed out in DANMAP 2008.

Penicillins with extended spectrum: a few low-strength packages (for children) are no longer marketed and national guidelines have increased the recommended dosage of certain substances over the last decade [DANMAP 2008].

Beta-lactamase sensitive penicillins: some low-strength packages (for children) are no longer marketed while packages with higher strength have been introduced; recommended dosage has increased for certain indications over the last decade [DANMAP 2008].

Beta-lactamase resistant penicillins: no changes in packages have occurred over the last decade, but national guidelines have introduced new indications of treatment for certain infections over the last decade [DANMAP 2008].

Combinations of penicillins, including beta-lactamase inhibitors: the proportion of children (<15 years) receiving these substances have decreased and been replaced by adults as pointed out in the DANMAP 2007 report. As a consequence, low-strength packages have presumably been replaced by packages with higher strength.

Macrolides: the packages have not changes over the last decade, but national guidelines have increased the recommended treatment dosage for certain indications over the last decade [DANMAP 2008].

Fluoroquinolones: no changes in packages have occurred over the last decade, and national guidelines only recommend these substances as second line choices for a limited number of indications.

Trends in indicators—measures of antibacterial consumption in primary health care—showed DDD as the indicator that increased the most during the last 10 years (Figure 17). Because codes of indication are incomplete, as pointed out in DANMAP 2008, making assumptions of the nature of these changes in trends are difficult. Consumption measured as the number of packages per 1000 inhabitants and the number of treated patients per 1000 inhabitants is displayed in Tables 36, 37 in Appendix 1.

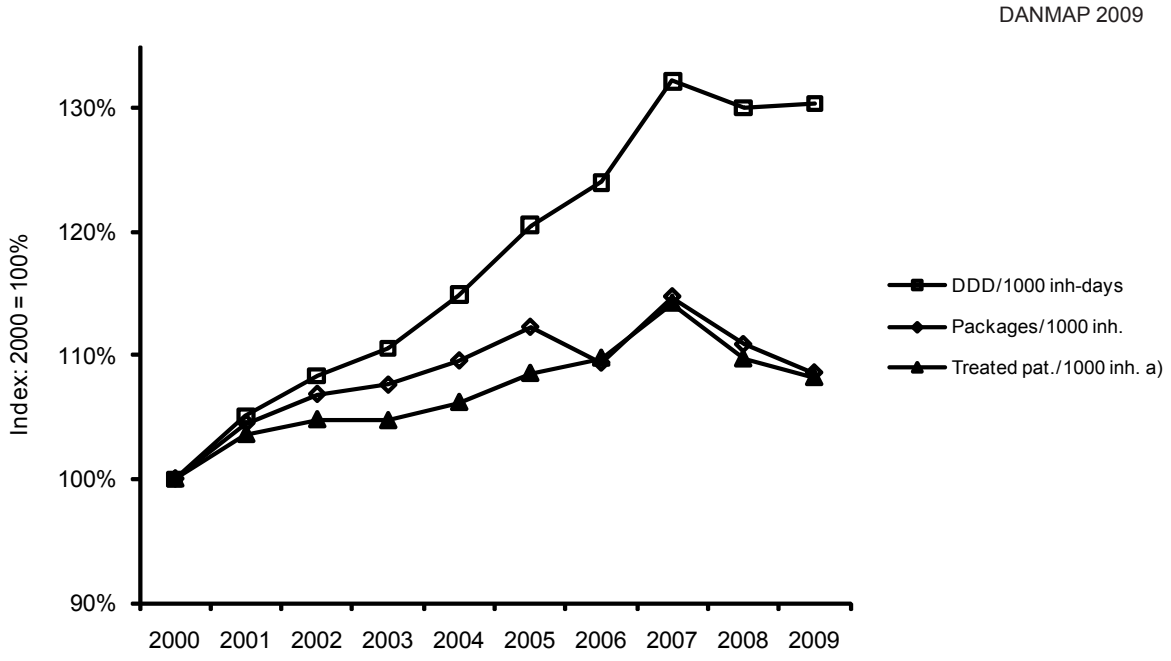

Figure 17. Trends in indicators of antibacterial consumption (J01) in primary health care, Denmark

a) Cumulated number of patients treated with anbacterials (ATC-4 level). The Danish Medicines Agency counts the first treatment within each ATC-group for each patient, each year

Table 11. Number of DDDs per treated patient and per package among leading groups of antibacterial agents in primary health care, Denmark

DANMAP 2009

ATC group a)	Therapeutic group	Indicator	Year									
			2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	DDDs / patient	29.8	30.6	33.0	34.4	36.9	39.0	40.9	43.0	44.4	45.2
		Packages / patient	1.9	1.9	1.9	1.9	1.9	2.0	1.9	2.0	2.0	2.0
		DDDs / package	15.7	16.1	17.5	18.1	19.0	19.6	21.0	22.0	22.7	22.7
J01CA	Penicillins with extended spectrum	DDDs / patient	12.8	13.0	13.2	13.4	13.6	13.9	14.2	14.4	14.7	14.8
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
		DDDs / package	8.1	8.1	8.2	8.2	8.4	8.5	8.9	9.0	9.2	9.2
J01CE	Beta-lactamase sensitive penicillins	DDDs / patient	10.2	10.3	10.5	10.7	11.1	11.3	11.5	11.7	11.8	11.8
		Packages / patient	1.4	1.4	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4
		DDDs / package	7.1	7.1	7.2	7.3	7.5	7.7	8.0	8.2	8.2	8.4
J01CF	Beta-lactamase resistant penicillins	DDDs / patient	12.2	12.4	11.8	11.8	12.4	12.7	13.0	13.4	13.7	13.9
		Packages / patient	1.5	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5
		DDDs / package	7.9	7.9	7.5	7.4	7.8	8.0	8.6	8.7	9.0	9.1
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDDs / patient	11.8	15.9	14.7	16.6	17.2	16.8	19.3	19.1	19.9	20.4
		Packages / patient	1.8	1.7	1.7	1.8	2.0	2.0	1.8	1.6	1.6	1.5
		DDDs / package	6.7	9.1	8.6	9.1	9.1	9.3	10.7	11.7	12.4	13.3
J01FA	Macrolides	DDDs / patient	11.3	11.3	11.7	12.1	12.4	12.4	12.6	12.4	12.5	12.5
		Packages / patient	1.5	1.5	1.5	1.6	1.6	1.6	1.5	1.5	1.5	1.5
		DDDs / package	7.6	7.5	7.6	7.8	7.9	8.0	8.3	8.1	8.1	8.1
J01MA	Fluoroquinolones	DDDs / patient	7.8	8.3	8.6	10.3	9.5	9.6	10.3	10.6	11.0	11.2
		Packages / patient	1.4	1.4	1.4	1.6	1.5	1.5	1.5	1.5	1.5	1.5
		DDDs / package	5.7	5.9	6.0	6.6	6.4	6.5	6.9	7.0	7.5	7.6
J01	Antibacterial agents for systemic use (total)	DDDs / patient	15.3	15.6	16.0	16.4	17.0	17.5	17.9	17.3	18.9	19.2
		Packages / patient	2.0	2.0	2.0	2.1	2.1	2.1	2.0	1.9	2.1	2.1
		DDDs / package	7.7	7.8	7.8	7.9	8.1	8.3	8.7	8.9	9.1	9.3

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Tetracyclines (J01AA) – drug names listed in Table 4

In 2009, consumption of tetracyclines increased by 0.07 DID (4.5%) compared with 2008 (Table 10). Tetracycline (0.68 DID (43%)) was the most used of the tetracyclines in 2009 followed by doxycycline (0.54 DID (33%)), lymecycline (0.33 DID (21%)) and oxytetracycline (0.07 DID (4.2%)), respectively (Figure 18). Within the group, the only substance to decrease in 2009 was doxycycline.

Since 2000, an extensive increase in the consumption of tetracyclines (0.63 DID (65%)) has been observed (Table 10). As previously pointed out in the DANMAP 2007 report, a large part of the consumption of tetracyclines are prescribed for teenagers and young adults, and a binary pattern of consumption has been observed (tetracycline and lymecycline with peak values in the spring and autumn, doxycycline with peak values in January and June).

Penicillins (J01C) – drug names listed in Table 4

In 2009, consumption of beta-lactamase sensitive penicillins decreased by 0.18 DID (3.4%) compared with 2008. Nonetheless, an equal increase in penicillins, including beta-lactamase inhibitors of 0.18 DID (66%) balanced the total consumption of penicillins between 2008 and 2009 (Table HuAB_P1).

In both the group of penicillins with extended spectrum and the group of beta-lactamase resistant penicillins consumption increased slightly.

The decreasing consumption of phenoxy-methylpenicillin and amoxicillin in 2009 (and 2008) could be the result of a decreased disease burden after the introduction of a heptavalent conjugate pneumococcal vaccination in the Danish Childhood Immunization Programme in October 2007 (Figure 19). However, without accurate indication codes on the prescriptions this connection is difficult to demonstrate [DANMAP 2008].

Within the group of penicillins with extended spectrum, the increase in consumption was due to an increasing consumption of pivmecillinam; the only substance to increase in 2009 (Figure 19). Since 2007, pivmecillinam has been a first-line antibiotic for the treatment of urinary tract infections as has sulfamethizole [DANMAP 2008].

Over the last decade (2000–2009), the consumption of penicillins with extended spectrum has increased by 1.01 DID (44%), beta-lactamase sensitive penicillins has increased by 0.43 DID (9.2%), beta-lactamase resistant penicillins has increased by 0.61 DID (117%) and combinations of penicillins, including beta-lactamase inhibitors has increased by 0.42 DID (1711%), respectively (Table 10). Phenoxymethylpenicillin is still by far the most consumed penicillin, but among the other penicillins the order has changed during the last decade (Figure 19).

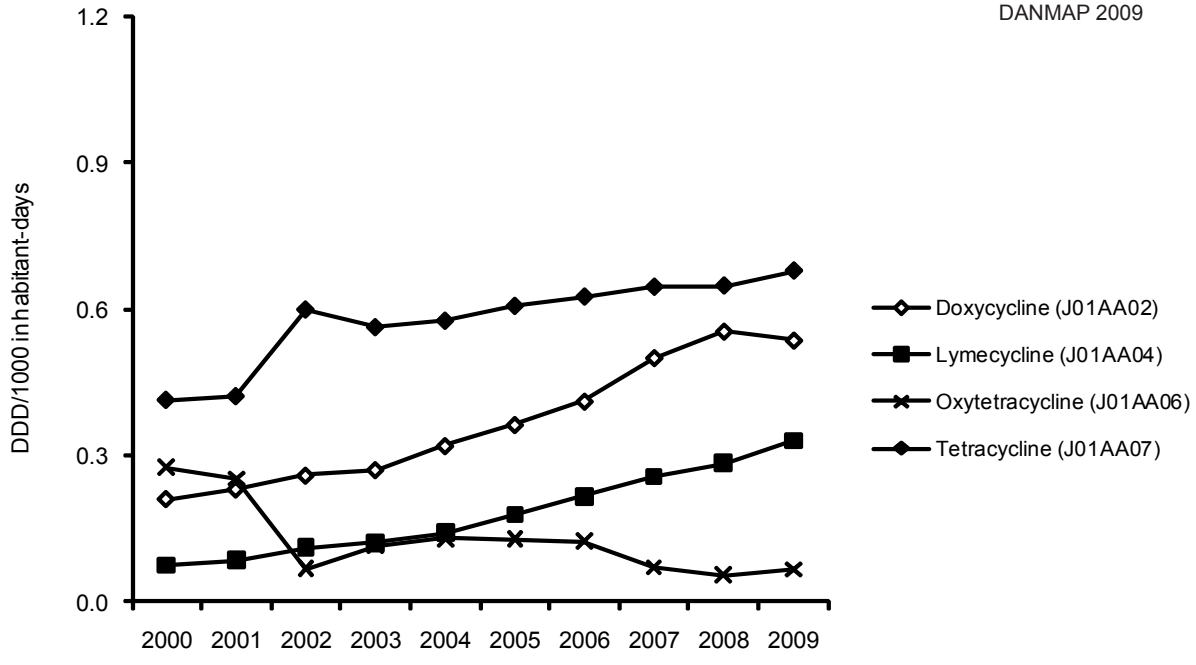

Figure 18. Consumption of tetracyclines in primary health care, Denmark

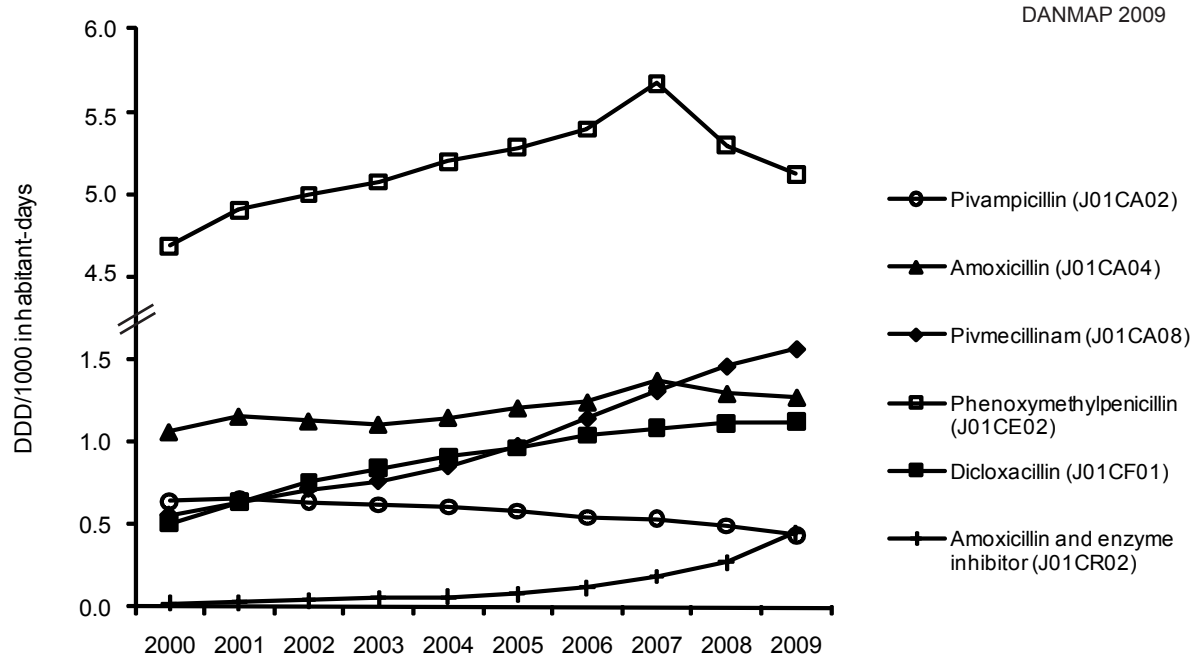

Figure 19. Consumption of leading penicillins in primary health care, Denmark

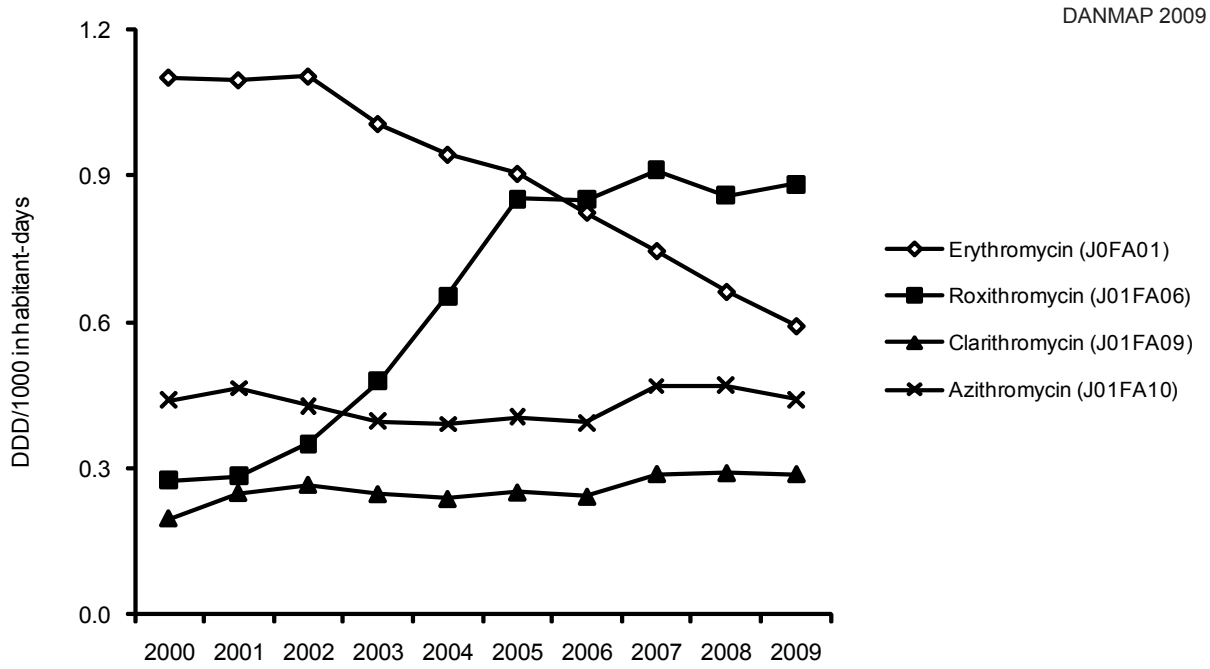

Figure 20. Consumption of macrolides in primary health care, Denmark

Macrolides (J01FA) – drug names listed in Table 4

A decrease of 0.07 DID (3.4%) occurred in the consumption of macrolides 2008–2009 (Table 10). Within the group of macrolides, the only substance to increase in 2009 was roxithromycin (Figure 20). Theoretically, a decreased disease burden after the introduction of a heptavalent conjugate pneumococcal vaccination in the Danish Childhood Immunization Programme in October 2007 could influence the consumption of macrolides in 2008 and 2009. However, such a trend has not become evident.

Over the last decade (2000–2009), roxithromycin (0.6 DID) consumption, and to some extend clarithromycin (0.1 DID) and azithromycin (<0.1 DID) consumption, has increased while erythromycin (0.5 DID) consumption has decreased (Figure 20). These changes are not in complete agreement with changes in national guidelines for primary health care from erythromycin towards first roxithromycin (2004 version), and subsequently clarithromycin (2007 version) as first-choice macrolide [DANMAP 2008]. Throughout the last decade, azithromycin has been recommended for urethritis/cervicitis and epididymitis. In 2004 and 2005, part of the increase in roxithromycin consumption was likely due to an outbreak of *Mycoplasma pneumoniae* [DANMAP 2005].

Fluoroquinolones (J01MA) – drug names listed in Table 4

Consumption of fluoroquinolones increased by 0.01 DID (0.8%) compared with 2008 (Table 10). Ciprofloxacin accounted for 94% of the total fluoroquinolone consumption in 2009; ofloxacin and moxifloxacin each accounted for 3% (Figure 21). Thus, the trend of increasing fluoroquinolone consumption seems to have ceased. Nevertheless, the consumption of fluoroquinolones has increased by 0.37 DID (243%) during 2000–2009 (Table 10). The continuously increasing consumption of ciprofloxacin began when the price of ciprofloxacin dropped markedly following the introduction of generics onto the Danish marked in December 2001 [Jensen *et al.* 2010 Antimicrob. Chemother. 65: 1286-91].

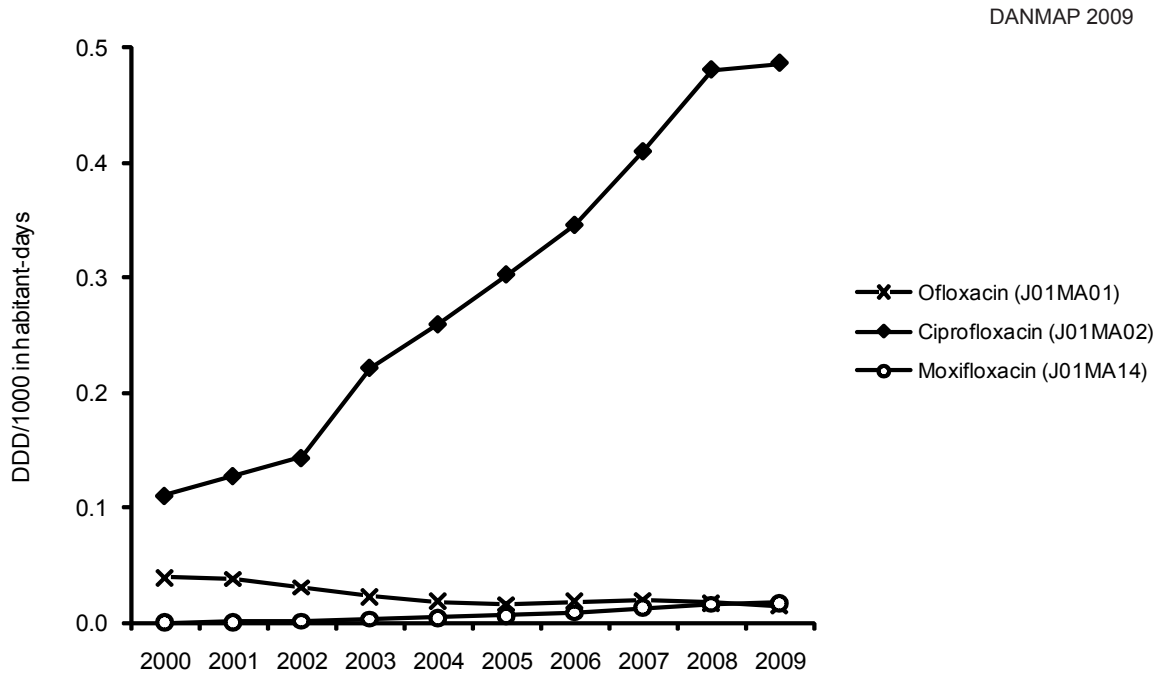

Figure 21. Consumption of leading fluoroquinolones in primary health care, Denmark

Hospitals

Due to a major hospital strike during spring 2008 that particularly affected the number of discharges and to a lesser extent the number of bed-days, the reported hospital consumption (DDD/100 discharges and to some extent DDD/100 bed-days) of 2008 was presumably higher than expected [DANMAP 2008]. Therefore, comparing the hospital consumption to 2008 using DDD/100 discharges is misleading. Instead, the consumption of 2009 is compared with 2007 as well as 2000.

DDD per 100 occupied bed-days

Total consumption (J01) in somatic hospitals increased by 3.57 (4.8%) DDD/100 bed-days (DBD) from 2008 to 2009 (Table 12). Two groups dominated the increase in consumption: second-generation cephalosporins 1.43 DBD (11%) and combination of penicillins, including beta-lactamase inhibitors 1.28 DBD (32%), but the consumption of all the major antibacterial groups increased with the exception of beta-lactamase sensitive penicillins, aminoglycosides and imidazole derivates.

During 2000–2009, the total consumption (J01) in somatic hospitals increased by 31.2 DBD (66%) (Table 12). This increase was due to a 40% increase in the number of DDDs, and a concurrent 19% decrease in the total number of hospital bed-days (trends in hospitalisation patterns have changed towards more patients being hospitalised with shorter length of stay). Cephalosporins accounted for 21% of the total consumption in somatic hospitals. Penicillins with extended spectrum (18%), fluoroquinolones (13%) and beta-lactamase sensitive penicillins (12%) were the other major contributing antibacterial groups in 2009 (Figure 22).

Table 12. Consumption of antibacterial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark
Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were exluded.

DANMAP 2009

ATC group a)	Therapeutic group	Year									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	0.29	0.28	0.32	0.31	0.35	0.34	0.40	0.63	0.78	0.97
J01CA	Penicillins with extended spectrum	11.57	11.61	11.52	11.92	11.79	13.01	13.09	13.42	13.96	14.38
J01CE	Beta-lactamase sensitive penicillins	10.03	10.65	11.43	12.12	12.31	12.26	10.74	10.79	9.98	9.26
J01CF	Beta-lactamase resistant penicillins	5.35	6.02	6.28	6.68	6.99	6.76	6.55	6.70	6.81	6.92
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.09	0.17	0.31	0.50	0.85	1.17	1.85	2.95	4.00	5.28
J01DB	First-generation cephalosporins	0.10	0.12	0.14	0.14	0.17	0.15	0.14	0.13	0.18	0.12
J01DC	Second-generation cephalosporins	4.74	5.21	5.85	6.39	7.06	8.46	9.44	12.31	13.32	14.72
J01DD	Third-generation cephalosporins	0.67	0.65	0.65	0.67	0.68	0.83	0.84	1.03	1.25	1.33
J01DF	Monobactams	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.04	0.07	0.06
J01DH	Carbapenems	0.39	0.42	0.60	0.69	0.86	1.16	1.39	2.13	2.70	2.94
J01EA	Trimethoprim and derivatives	0.37	0.43	0.42	0.44	0.42	0.41	0.42	0.44	0.44	0.42
J01EB	Short-acting sulfonamides	1.23	1.25	1.24	1.18	1.08	0.99	0.76	0.34	0.35	0.32
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	1.40	1.34	1.46	1.54	1.83	2.12	2.13	1.52	1.95	2.13
J01FA	Macrolides	3.28	3.26	3.23	3.09	2.94	2.91	2.85	3.08	3.06	3.20
J01FF	Lincosamides	0.16	0.17	0.19	0.19	0.23	0.24	0.31	0.35	0.41	0.47
J01GB	Aminoglycosides	2.13	1.85	1.77	1.74	2.03	1.97	1.82	1.79	1.64	1.46
J01MA	Fluoroquinolones	2.31	2.84	3.52	3.96	4.98	6.19	6.78	8.16	9.53	10.02
J01XA	Glycopeptides	0.33	0.32	0.37	0.42	0.47	0.52	0.56	0.63	0.68	0.93
J01XB	Polymyxins	0.04	0.03	0.03	0.03	0.06	0.12	0.12	0.05	0.05	0.06
J01XC	Steroid antibacterials (fusidic acid)	0.23	0.20	0.19	0.22	0.22	0.26	0.28	0.28	0.26	0.29
J01XD	Imidazole derivatives	1.79	1.96	2.11	2.37	2.47	2.64	2.80	2.62	2.55	2.20
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.29	0.29	0.28	0.28	0.28	0.30	0.29	0.28	0.29	0.34
J01XX05	Methenamine	0.14	0.13	0.12	0.08	0.10	0.08	0.11	0.09	0.10	0.08
J01XX08	Linezolid	0.00	0.00	0.04	0.04	0.07	0.11	0.20	0.16	0.21	0.20
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02
J01	Antibacterial agents for systemic use (total)	46.95	49.21	52.10	55.00	58.24	63.08	63.90	69.94	74.56	78.13

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 23 illustrates the steady shift towards increasing consumption of newer, 'broad-spectrum' antibacterial agents - defined as: combinations of penicillins, including beta-lactamase inhibitor (J01CR), cephalosporins (J01DB, DC, DD), carbapenems (J01DH) and fluoroquinolones (J01MA) - in Danish somatic hospitals. In 2000, consumption of penicillins with extended spectrum and beta-lactamase sensitive penicillins represented 25% and 21% of total somatic hospital antibacterial consumption in Denmark, respectively. These shares had decreased to 18% and 12% in 2009. In the penicillins with extended

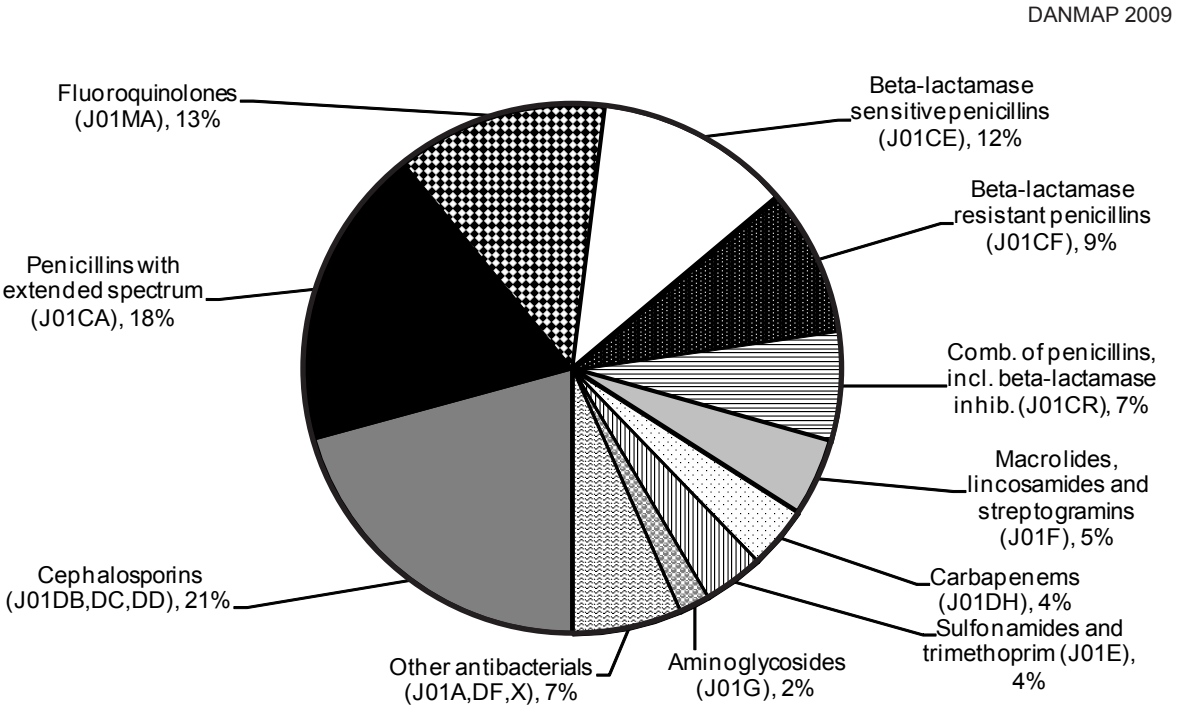

Figure 22. Distribution of the total consumption of antibacterial agents in somatic hospitals, Denmark

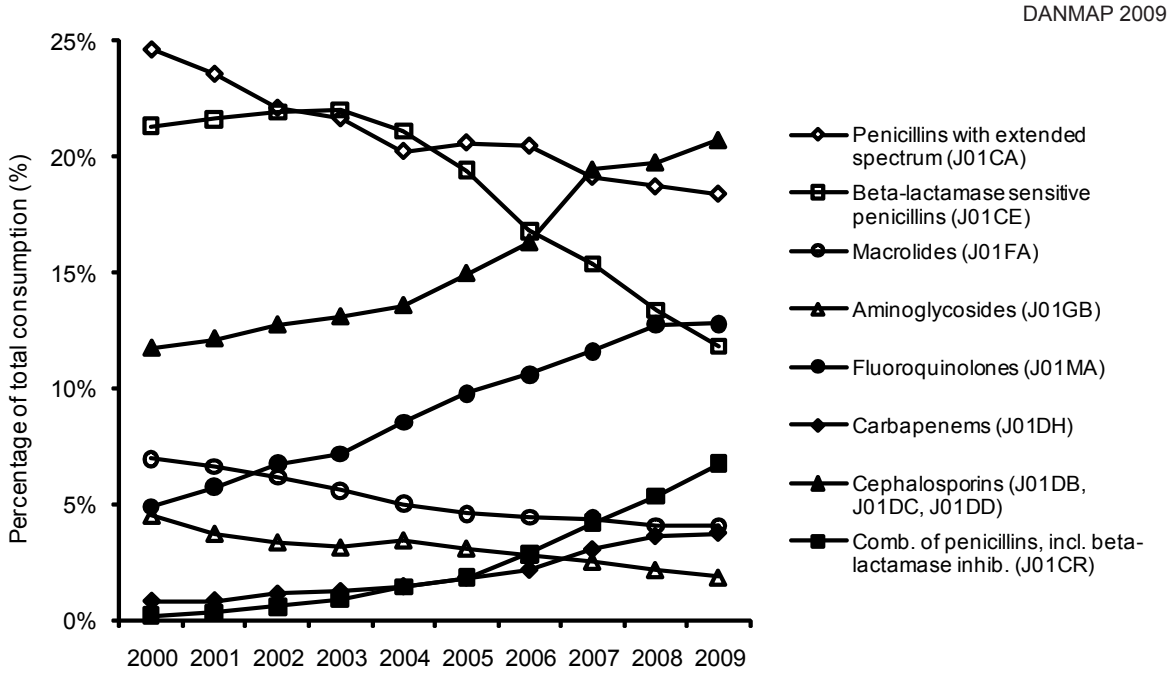

Figure 23. Percentages of total somatic hospital consumption by leading groups of antibacterial agents for systemic use (J01), Denmark

spectrum group, the decrease mainly concerned ampicillin/pivampicillin whereas consumption of mecillinam/pivmecillinam has increased. Consumption of cephalosporins represented 12% of total somatic hospital antibacterial consumption in 2000, rising to 21% in 2009. The consequences of these changes in the pattern of antibacterial consumption could be an empirical treatment with a wider coverage of pathogens responsible for infection. Nevertheless, this potential gain seems to be rapidly counterbalanced by the emergence of resistance towards newer classes of antibacterial agents.

DDD per 100 discharged patients

The total consumption (J01) in somatic hospitals increased by 4.59 (1.6%) from 2007–2009 when expressed as the number of DDD/100 discharges, and by 45.5 (18.4%) during 2000–2009 (Table 13). As this increase was partly due to a 40% increase in the number of DDDs, it also illustrates that the number of discharges has increased by 13% during the last decade as a consequence of changes in hospitalisation patterns (trends in hospitalisation patterns have changed towards more patients being hospitalised with shorter length of stay). When expressed in DDD per 100 discharges, second-generation cephalosporins and combination of penicillins, including beta-lactamase inhibitors dominated the increase in consumption from 2007–2009. However, six groups presented with a decrease in consumption, 2007–2009: penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, macrolides, aminoglycosides and imidazole derivates. This difference between trends in consumption measured by DBD or DDD per 100 discharges illustrates that the interpretation of the measures of consumption is highly dependent on the denominator as well as the nominator (DDD) and that one indicator is not enough to express hospital consumption.

Table 13. Consumption of antibacterial agents for systemic use in somatic hospitals (DDD/100 discharged patients), Denmark
Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were excluded.

DANMAP 2009

ATC group a)	Therapeutic group	Year									
		2000	2001	2002	2003	2004	2005	2006	2007	***2008 b)***	2009
J01AA	Tetracyclines	1.54	1.48	1.63	1.48	1.58	1.46	1.68	2.59	***3.19***	3.64
J01CA	Penicillins with extended spectrum	61.05	60.49	57.80	56.62	53.52	56.71	55.22	55.39	***57.18***	53.97
J01CE	Beta-lactamase sensitive penicillins	52.91	55.50	57.35	57.59	55.85	53.46	45.33	44.55	***40.90***	34.75
J01CF	Beta-lactamase resistant penicillins	28.25	31.37	31.49	31.73	31.70	29.48	27.65	27.64	***27.89***	25.97
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	0.49	0.89	1.56	2.36	3.85	5.11	7.79	12.17	***16.37***	19.82
J01DB	First-generation cephalosporins	0.52	0.61	0.72	0.68	0.77	0.67	0.60	0.55	***0.72***	0.46
J01DC	Second-generation cephalosporins	25.02	27.15	29.36	30.36	32.05	36.88	39.83	50.81	***54.55***	55.33
J01DD	Third-generation cephalosporins	3.56	3.40	3.25	3.20	3.09	3.64	3.54	4.24	***5.10***	5.00
J01DF	Monobactams	0.09	0.05	0.02	0.02	0.02	0.02	0.00	0.18	***0.27***	0.21
J01DH	Carbapenems	2.06	2.19	2.99	3.27	3.88	5.08	5.87	8.78	***11.08***	11.05
J01EA	Trimethoprim and derivatives	1.95	2.26	2.10	2.10	1.90	1.79	1.78	1.81	***1.80***	1.56
J01EB	Short-acting sulfonamides	6.49	6.49	6.22	5.58	4.92	4.34	3.19	1.41	***1.43***	1.21
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	7.36	7.00	7.32	7.31	8.32	9.26	8.99	6.28	***7.98***	7.99
J01FA	Macrolides	17.31	17.01	16.19	14.67	13.33	12.70	12.03	12.70	***12.53***	12.01
J01FF	Lincosamides	0.85	0.90	0.95	0.90	1.04	1.05	1.31	1.46	***1.69***	1.75
J01GB	Aminoglycosides	11.25	9.64	8.86	8.28	9.19	8.59	7.69	7.39	***6.71***	5.47
J01MA	Fluoroquinolones	12.18	14.81	17.67	18.80	22.60	27.00	28.63	33.66	***39.04***	37.60
J01XA	Glycopeptides	1.72	1.66	1.88	1.99	2.12	2.29	2.38	2.61	***2.77***	3.49
J01XB	Polymyxins	0.21	0.15	0.17	0.15	0.27	0.54	0.53	0.22	***0.21***	0.24
J01XC	Steroid antibacterials (fusidic acid)	1.21	1.02	0.97	1.05	1.02	1.11	1.20	1.17	***1.05***	1.09
J01XD	Imidazole derivatives	9.45	10.20	10.60	11.25	11.21	11.53	11.83	10.83	***10.46***	8.26
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.55	1.50	1.42	1.31	1.28	1.29	1.24	1.17	***1.19***	1.27
J01XX05	Methenamine	0.75	0.67	0.61	0.39	0.46	0.36	0.46	0.38	***0.43***	0.31
J01XX08	Linezolid	0.00	0.00	0.22	0.21	0.33	0.64	0.86	0.68	***0.84***	0.77
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	***0.06***	0.06
J01	Antibacterial agents for systemic use (total)	247.76	256.46	261.33	261.30	264.28	275.00	269.65	288.70	***305.46***	293.29

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) The number of discharges was affectedly low in 2008 due to a major hospital strike

Textbox 3

Female patients in primary health care consume more antimicrobial agents than male patients

A few reports have documented women more likely to receive antibacterial agents than men and receiving higher doses (DID) [Vaccheri A *et al.* 2002. J. Antimicrob. Chemother. 50: 989–997; Straand J *et al.* 1998.Scand. J. Prim. Health Care. 16: 121–7.]
From statistical data of 2008 (Statistics Denmark) it is known that women consult practitioners more often than men, regardless of the purpose of their visit (Figure 24). The fact that women consult practitioners more frequently has also been demonstrated in other European countries [Fleming DM. 1989. J. R. Coll. Gen. Pract. 39: 68–72; André M *et al.* 2008. Scand J Infect Dis. 40: 648–54; Akkerman AE *et al.* 2004. J. Antimicrob. Chemother. 54: 1116–21].
The aim of the present study was to investigate if antimicrobial consumption in primary healthcare was higher in female patients compared to male patients.
We obtained prevalence data of antibacterial agents from the Danish Medicines Agency. Firstly, data were classified by sex and antibacterial groups (ATC-4 level). Secondly, we classified data by sex and age (1-year groups) for the leading antibacterial agents (ATC-5 level).
Women had a higher prevalence of consumption in all antibacterial groups (ATC-4 level) except for beta-lactamase resistant penicillins (J01CF) compared with men. Penicillins with extended spectrum (J01CA), with a difference in prevalence of 52 treated patients/1000 inhabitants/year between the sexes, showed the largest difference followed by short-acting sulfonamides (J01EB), beta-lactamase sensitive penicillins (J01CE) and macrolides (J01FA). Not surprisingly, the antibacterial groups with the largest relative difference were those including substances used for the treatment of urinary tract infections - Penicillins with extended spectrum including pivmecillinam (J01CA), Trimethroprim and other derivatives (J01EA), Short-acting sulfonamides (J01EB) and Nitrofuran derivates (J01XE) (Figure 25).
Among the antibacterial agents that - besides other indications - are used in the treatment and prevention of urinary tract infections (sulfamethizole, pivmecillinam, trimethoprim, nitrofurantoin, pivampicillin, and ciprofloxacin), the prevalence was highest in older ages for both sexes. Consumption of sulfamethizole and pivmecillinam - the first line drugs of uncomplicated urinary tract infection in Denmark - displayed a higher prevalence in women compared with men of all ages with a crest in adolescent and in young women. A similar crest was seen in the prevalence of both ciprofloxacin and pivmecillinam, but the higher prevalence in women did not sustain to the older ages (Figure 26b).
The macrolides displayed very different trends in prevalence. Azithromycin had the highest prevalence among adolescent and young adults (women higher than men), clarithromycin had a more equally distributed prevalence (both ages and sexes), erythromycin showed the highest prevalence in the youngest ages (0–2 years) and no striking difference between men and women, whereas with roxithromycin the prevalence increased with age and markedly more adolescent and adult women than men were treated. Since indication codes are incomplete the reasons for these differences are not well understood [DANMAP 2008].
The tetracyclines (doxycycline and tetracycline) showed some interesting trends. Both had the highest prevalence among adolescents and young adults (women with a higher prevalence than men – overall), but where young men (15–20 years) were more frequently given tetracycline, more young women received doxycycline (Figure 26a).
Among the penicillins, dicloxacillin was most prevalent among men and the prevalence increased with age, whereas amoxicillin was by far the most prevalent antimicrobial agent among the youngest ages (0–2 years). Amoxicillin/clavulanic acid displayed a bimodal distribution with peaks among the youngest ages (0–2 years) and the older ages (>70 years), both dominated by men. Phenoxymethylpenicillin showed peaks among the youngest ages (0–2 years), among women aged 15–40 years and among persons older than 80 years. Men were more prevalent from age 0–7 years and again from age >70

years (Figure 26). Interestingly, the phenoxymethylpenicillin curve resembles the curve of doctor visits per 1000 inhabitants per year (Figure 24).
To conclude, women consult practitioners more often and receive more antibacterial agents, but the prevalence of antibacterial consumption in primary health care is highly dependent on the antibacterial agent prescribed and the sex and age of the patients.

Ulrich Stab Jensen

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Figure 24. Age and gender specific prevalence's of visits to general practitioners in 2008, Denmark
Data source: Statistics Denmark

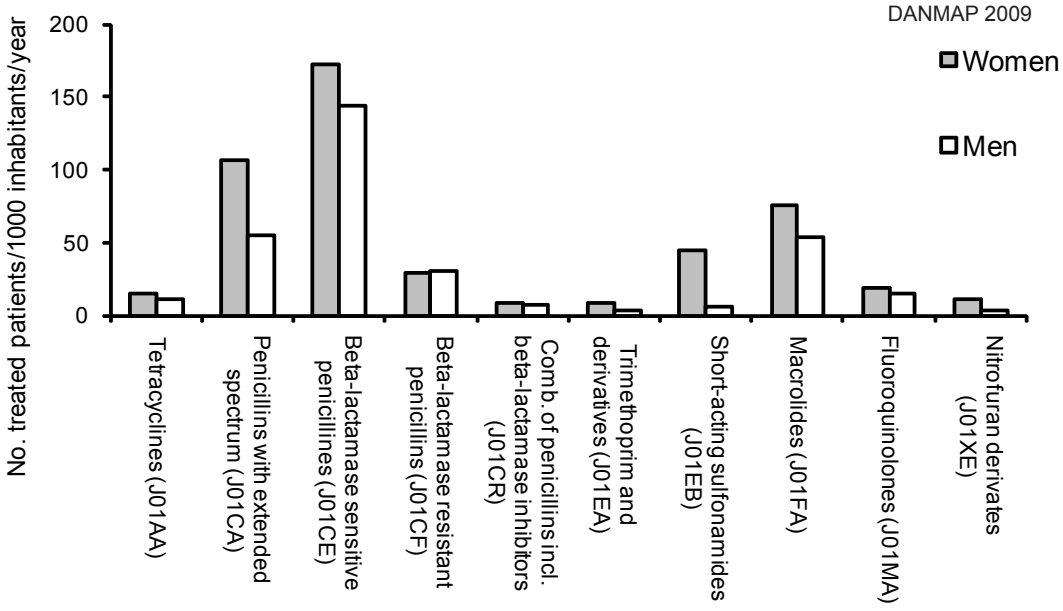

Figure 25. Prevalence of leading antibacterial groups by sex in primary health care, Denmark

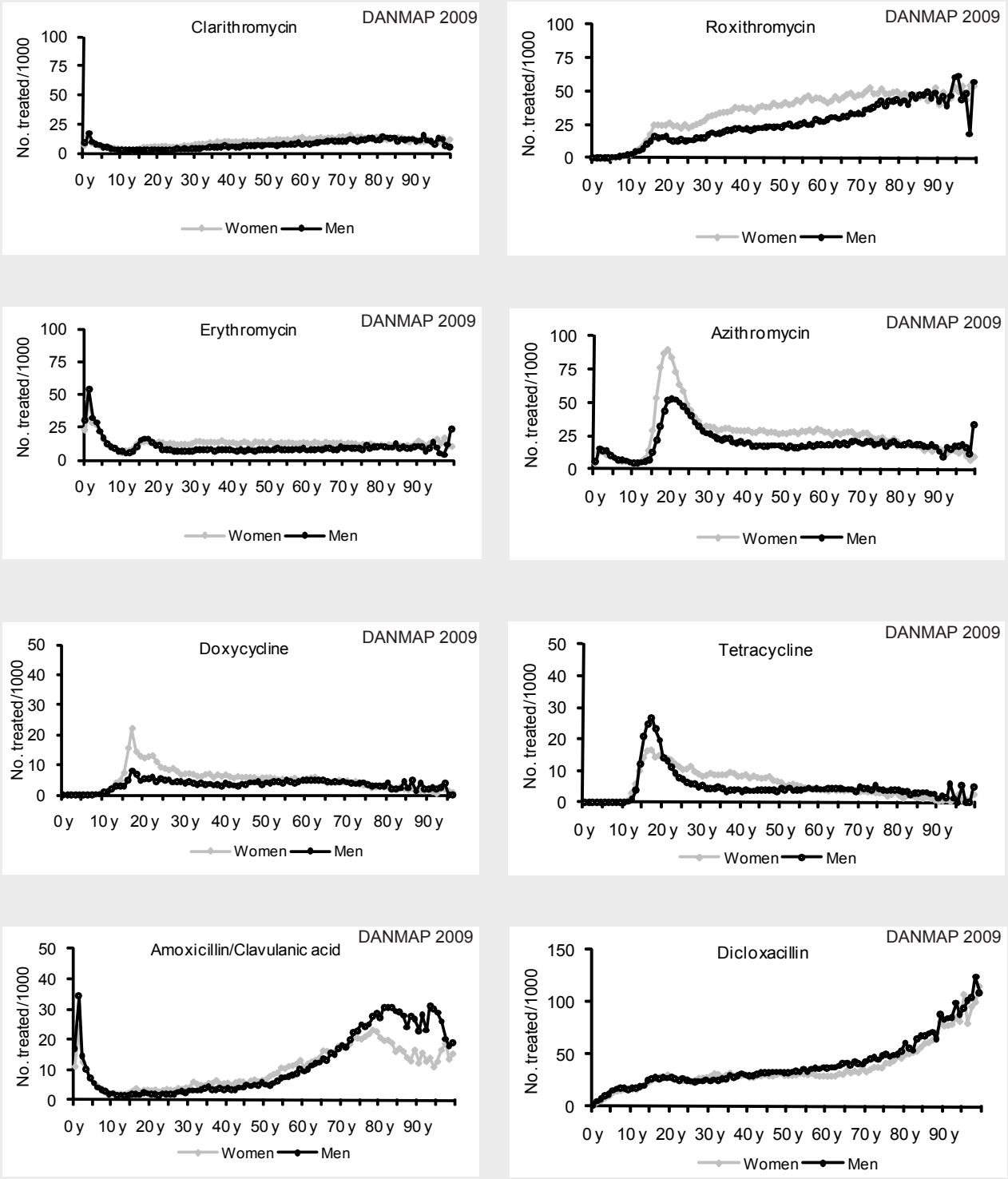

Figure 26a. Sex and age specific prevalence (no. persons treated/1000 inhabitants) of the consumption of leading antibacterial agents in primary health care, Denmark. Age is depicted on the X-axis and prevalence on the Y-axis

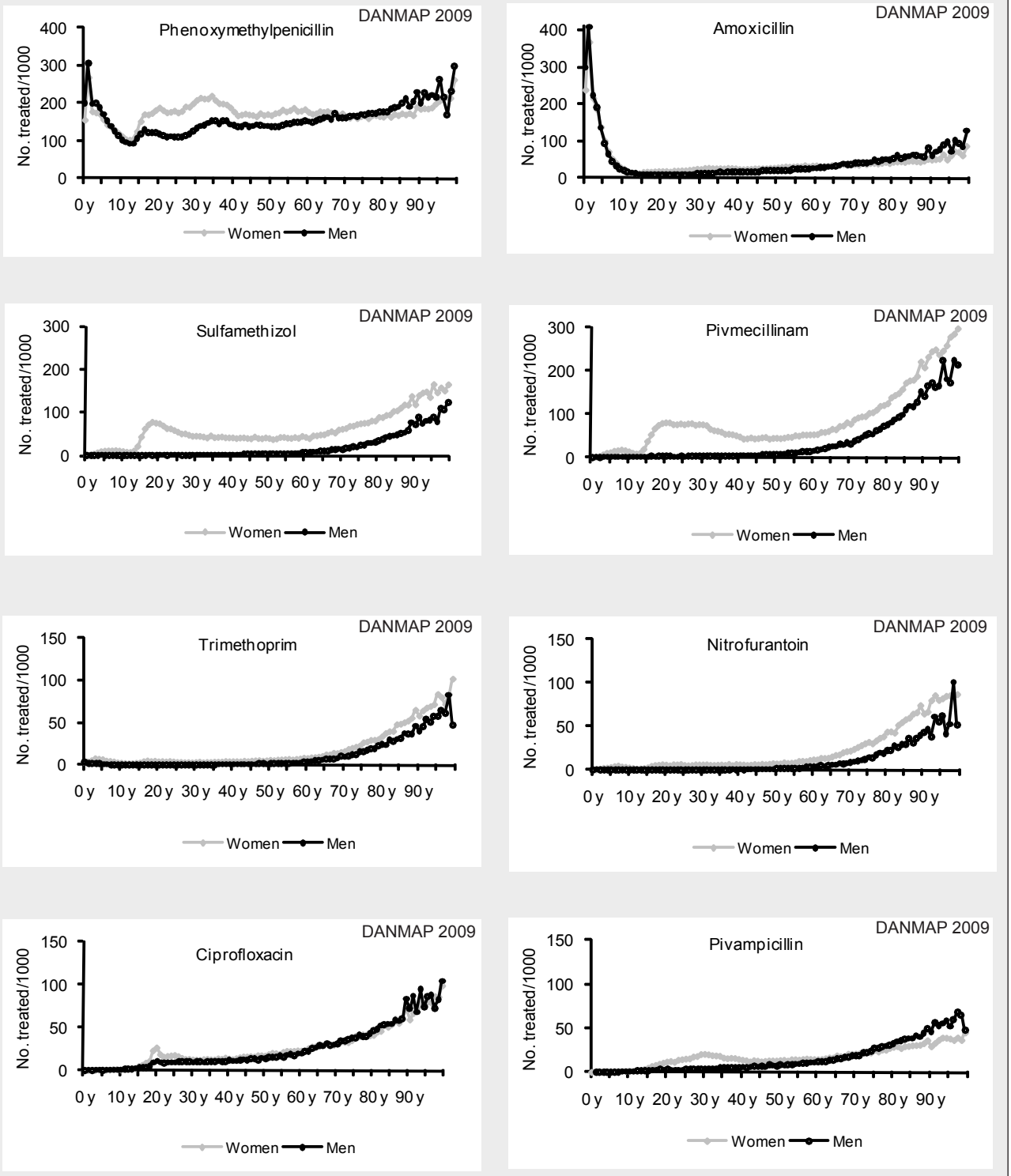

Figure 26b. Sex and age specific prevalence (no. persons treated/1000 inhabitants) of the consumption of leading antibacterial agents in primary health care, Denmark. Age is depicted on the X-axis and prevalence on the Y-axis

Resistance in zoonotic bacteria

Salmonella

In 2009, the total number of human cases of salmonellosis decreased to 2129 (39 per 100,000 inhabitants) compared to 3654 human cases in 2008. *Salmonella* Typhimurium accounted for 767 cases and *Salmonella* Enteritidis for 600 cases in 2009 [EPI-NEWS 2010, no. 12: http://www.ssi.dk/sw64290.asp]. The remaining 762 cases were caused by 129 other serotypes. In 2009, SSI collected travel information by phone interviews from all *Salmonella* patients. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. Patients who had travelled were furthermore asked about their destinations. The case was categorised as "domestically acquired" if the patient had not travelled one week prior to the onset of infection whereas the case was categorised as "travel abroad reported" if the patient had travelled one week prior to the onset of infection. Cases who had not reported travel to the general practitioners or not been interviewed by phone were categorised as "unknown origin". Information was obtained from 80% of the *Salmonella* cases.
In total, 352 of the 594 (59%) *S*. Enteritidis isolates and 739 of the 762 (97%) *S*. Typhimurium isolates were analysed for antimicrobial resistance (Table 42 and 43) in Appendix 1.

Salmonella surveillance programmes are running on broiler flocks, pigs (at herd level), Danish pork, and Danish beef. Details of the programmes are described in the Annual Report on Zoonoses in Denmark. In 2009, a total of 4991 pooled samples (estimated 14,733 single samples) were collected from pork, and *Salmonella* was isolated from 157 samples. From beef, a total of 1606 pooled samples (estimated 4438 single samples) were tested and *Salmonella* was isolated from 13 (only one *S*. Typhimurium) beef samples. For pigs, high risk herds were appointed based on *S*. Typhimurium serosurveillance at the slaughterhouses and in all breeding herds. In 2009, *S*. Typhimurium was isolated from 351 of these herds. In addition, 21 *S*. Typhimurium from clinical porcine samples were included in the report. In Denmark, all broiler flocks are sampled, and in 2009, 7081 samples (3707 flocks) were analysed, of which 33 were positive for *Salmonella*. None of these isolates were *S*. Enteritidis and only a few *S*. Typhimurium isolates were observed.

Data on Danish broiler meat and imported meats originate from the so-called case-by-case risk assessment program (at the Danish Veterinary and Food Administration), referring to risk assessment of individual batches; for each tested batch, 12 pooled samples (each 1–60 single samples) from each batch are tested for *Salmonella*. In 2009, 100 batches of Danish broiler meat were sampled with no positive batches found. Regarding imported meats, 736 batches of broiler meat (30 positive), 342 batches of turkey meat (62 positive), 301 batches of pork (37 positive) and 125 batches of beef were tested (5 positive). Results of resistance testing for animals and food isolates with MIC values are shown in table 39 and 40 in Appendix 1.

Phage types and outbreaks

In pigs and pork, the most common serotype is *S*. Typhimurium (See Annual Report on Zoonoses in Denmark). The most prevalent *S*. Typhimurium phage type in pigs and Danish pork in 2009 was DT120 (29% and 24%, respectively). The second most common phage type was DT12 in pigs (10%) and DT170 in Danish pork (18%) (Table 14). The most common serotype in imported pork was also *S*. Typhimurium with the most common phagetype DT193 followed by DT120, U302 and DT104 (24%, 19%, 19% and 16%, respectively). As observed in the previous years, the decrease in phage type DT12 and increase in DT120 continued for pigs and Danish pork (Figure 27); furthermore, the imported pork showed a tendency of decrease in DT104 and increase in DT120. In 2009, an increase in phage type DT170 was seen in the Danish pork, while phage type DT170 was not detected in the imported pork. In imported turkey meat, phage type DT193 was the most common *S.* Typhimurium phage type, as in imported pork.

All *Salmonella* Typhimurium and Enteritidis isolates from human patients were routinely real-time typed for surveillance using MLVA (multiple locus variable number tandem repeat analysis). When clusters were detected and epidemiological investigations initiated, a cluster was defined as an outbreak. Using this definition, 406 of the 560 cases of the domestically acquired human *S.* Typhimurium infections were part of outbreaks. The 406 cases were distributed among eight different outbreaks. The two major outbreaks detected in 2008 with phage types U292 and DT135 also

Table 14. Distribution (%) of Salmonella *Typhimurium phage types from food animals, pork of Danish and imported origin and human cases categorized as acquired domestically, reported as associated with travel abroad or unknown origin among the isolates selected for susceptibility testing, Denmark*

DANMAP 2009

Phage type	Broilers	Cattle	Pigs	Pork		Broiler meat	Turkey meat	Humans a)		
	Danish %	Danish %	Danish %	Danish %	Imported %	Imported %	Imported %	Domestically acquired %	Travel abroad reported %	Unknown origin %
3	-	-	<1	1	0	-	-	5	5	4
12	-	-	10	7	3	-	-	4	0	9
15a	-	14	2	-	-	-	-	0	0	0
17	-	-	7	5	0	-	-	2	0	2
41	100	-	-	-	-	-	-	<1	5	2
104/104b/104c	-	29	5	7	16	-	19	3	9	9
120	-	14	29	24	19	-	33	8	5	14
135	-	-	<1	-	-	-	-	16	4	6
170	-	-	8	18	0	-	-	4	0	10
193	-	-	7	8	24	-	38	3	11	9
U288	-	-	4	8	0	-	-	0	0	2
U292	-	-	<1	-	-	-	-	36	16	<1
U302	-	-	3	4	19	-	-	1	4	4
U312	-	-	<1	1	0	-	-	6	0	2
Others including non-typeable	-	43	22	17	19	100	10	11	42	28
Number of isolates	1	7	372	74	37	4	21	559	57	118

a) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'

represent the two largest outbreaks in 2009 consisting of 212 and 83 cases, respectively. Outbreak-related phage types in *S*. Typhimurium were U292, DT135, U312, DT3, DT120, DT170, and DT17 (Table 14), of which U292, DT135 and DT17 isolates were fully susceptible to all tested antimicrobial agents.
In 2009, all cases of *S.* Enteritidis were MLVA typed. Of the 173 domestically acquired *S.* enteritidis cases, 123 were part of outbreaks. The largest outbreak consisted of 80 cases. Outbreak-related phage types for *S*. Enteritidis were PT13a, PT8, PT6a, and PT11 (Table 15).

Data with and without the outbreaks are presented in Table 42 and 43 in Appendix 1. All isolates are included in Figure 28 and Table 16. A large part of the outbreaks in recent years consisted of fully sensitive phagetypes, the inclusion of the outbreaks giving an importantly lower occurrence of resistance in human cases in recent years, concurrent with increased prevalence of Salmonella.

Table 15. Distribution (%) of Salmonella *Enteritidis phage types from imported broiler meat and human cases categorized as acquired domestically, reported as associated with travel abroad or unknown origin among the isolates selected for susceptibility testing, Denmark*

DANMAP 2009

Phage type	Broiler meat	Humans a) b)		
	Imported %	Domestically acquired %	Travel abroad reported %	Unknown origin %
1	30	2	14	13
2	-	0	<1	0
4	37	2	11	5
4b	-	<1	1	1
6	-	1	8	7
6a	7	2	6	4
8	3	54	10	23
13a	-	25	2	1
14b	-	2	7	11
21/21b	3	3	11	9
Others including non-typeable	20	8	29	25
Number of isolates	30	312	193	75

a) Not all isolates selected for phage typing were susceptibility tested
b) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'

Comparison of resistance in *Salmonella* Typhimurium isolates from pigs, pork and human domestically acquired infections

No significant changes were observed in the occurrence of resistance in the Danish pigs in 2009 compared with 2008. Since 2001, a continuous significant increasing trend was observed in occurrence of resistance to sulfonamide, ampicillin and streptomycin, related to a shift in phage types (Figures 27, 28, see also DANMAP 2007). An increasing trend was also observed in occurrence of tetracycline resistance during 2001–2007; however, a significant decrease occurred from 2007–2009, despite a continuous increase in consumption of tetracylines throughout the decade. This decreasing trend in tetracycline resistance can not be explained by changes in the sampling procedure as no changes were introduced in that period 2007-2009. The number of farms appointed for sampling based on the serotype surveillance was about 900 in 2007, about 500 in 2008 and 466 farms in 2009 resulting in 575, 497 and 372 *S*. Typhimurium isolates, respectively. The decrease in occurrence of tetracycline resistance seem to be caused by a shift in phage types from resistant ones to more tetracycline sensitive phage types, (incl. a decrease in DT104, an increase in U288 and new occurrence of phage types sensitive to tetracycline) and partly by a decrease in the proportion of tetracycline resistance within certain phage types (including DT120, DT170, DT104). At this stage, it has not bee ruled out that the decrease in tetracycline resistance may be related to a lower consumption of tetracyclines in the sampled herds.

From 2008 to 2009, the occurrence of neomycin and chloramphenicol resistance in Danish pork showed a significant increase. Chloramphenicol is not used for pigs in Denmark but the resistance can be co-selected with other antimicrobials. The resistance in the Danish pork was very similar to the resistance in the pigs; the only significant difference was the occurrence of neomycin resistance which was 12% among isolates from Danish pork and 4% in isolates from pigs.
A significantly higher occurrence of resistance to ampicillin, ciprofloxacin, nalidixic acid and sulfonamide was seen in *S*. Typhimurium in imported pork compared to Danish pork. The use of fluoroquinolones has been very low in the Danish pig production since 2003 (Table 34 in Appendix 1), explaining the low occurrence of resistance compared to *Salmonella* from imported pork. No isolates with phenotype of transferable quinolone resistance (ciprofloxacin MIC>0.06 and naldixic acid MIC<32) or resistance to 3rd or 4th generation cephalosporins were observed in pigs and pork.

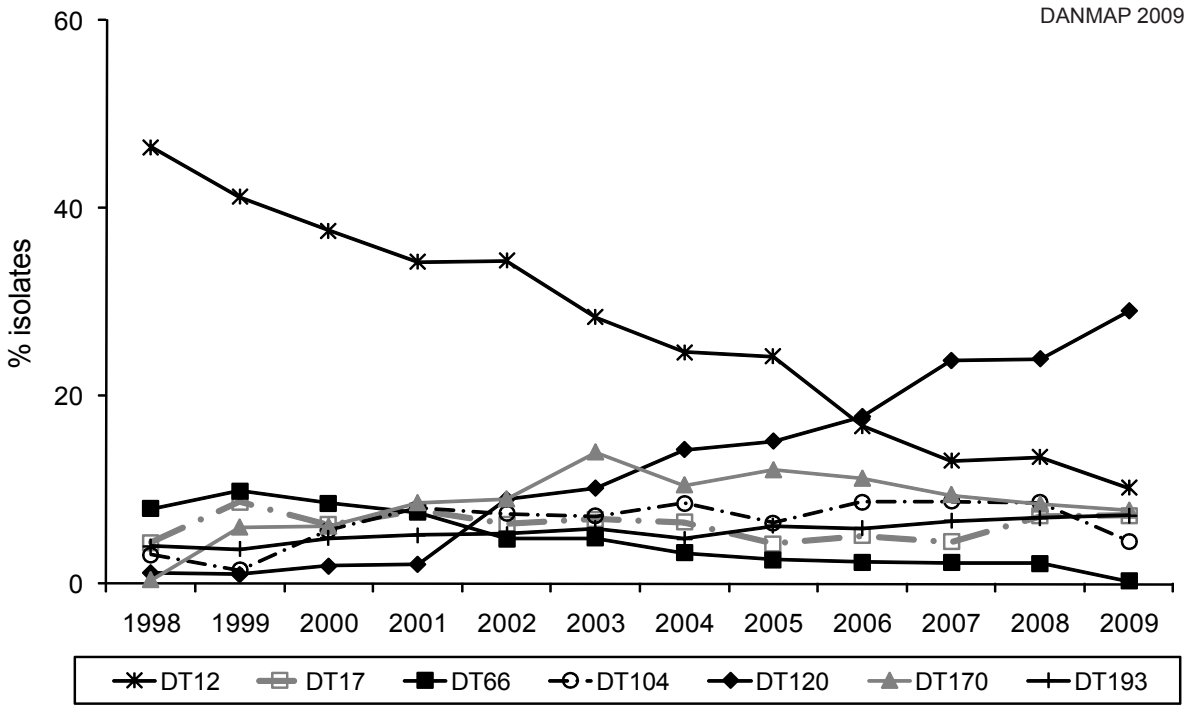

Figure 27. Changes in the distribution of the most prevalent Salmonella *Typhimurium phage types isolated from Danish pig farms from 1998 to 2009. Source: Danish* Salmonella *surveillance programme in pigs 1998-2009*

The occurrence of resistance in human domestically acquired infections was generally lower than the occurrence in both Danish and imported pork. This might in part be explained by the outbreaks of phage type U292 and DT135, which were fully sensitive *S.* Typhimurium strains. Also, various types of meat contribute to the domestically acquired *S.* Typhimurium infections, even though these are most often related to pork [Annual report on Zoonoses in Denmark 2008, http://www.food.dtu.dk/Default.aspx?ID=9606].

Occurrence of resistance in *Salmonella* Typhimurium isolates from imported turkey meat

The vast majority of Danish turkeys are exported for slaughter; therefore no *S.* Typhimurium isolates were available for susceptibility testing from turkeys or Danish turkey meat in 2009. Twenty-one isolates of *S.* Typhimurium were obtained from imported turkey meat (Table 16). In *S.* Typhimurium isolates from turkey meat, a significantly lower occurrence of resistance was found to chloramphenicol and florfenicol, ciprofloxacin and nalidixic acid, spectinomycin and sulphonamide in 2009 compared to 2008. In 2008, 34% of the isolates were resistant to ciprofloxacin. In 2009, no isolates with phenotype of transferable quionolone resistance (ciprofloxacin MIC>0.06 and naldixic acid MIC<32) or resistance to 3rd or 4rd generation cephalosporins were observed in imported turkey meat.

Comparison of resistance in *S.* Typhimurium isolates from patients with travel associated infections and domestically acquired infections

The occurrence of resistance to ampicillin, chloramphenicol, tetracycline, sulfonamides, spectinomycin, and streptomycin, was significantly higher in *S.* Typhimurium isolates from patients with travel associated infections compared to isolates from all domestically acquired infections. The difference could in part be explained by the outbreaks with sensitive *S.* Typhimurium (Table 16 and Figure 28). A significantly higher occurrence of nalidixic acid and ciprofloxacin resistance was observed in travel associated isolates compared with domestically acquired isolates, even without inclusion of the outbreaks (Tabel 16). Thus, the differences in resistance problably reflects differencies in the consumption of veterinary antimicrobial agents between Denmark and the countries to which the patients have travelled.

Table 16. Comparison of resistance (%) among Salmonella *Typhimurium from food animals, pork of Danish and imported origin and human cases acquired domestically a), reported as associated with travel abroad or with an unknown origin, Denmark*

DANMAP 2009

Substance	Pigs	Pork		Turkey meat	Humans a)		
	%	Danish %	Imported %	Imported %	Domestically acquired b) %	Travel abroad reported % b)	Unknown origin %
Tetracycline	39	43	54	90	10	38	36
Chloramphenicol	8	14	24	19	6	16	12
Florfenicol	4	5	24	19	3	3	9
Ampicillin	41	32	84	76	10	31	33
Ceftiofur	0	0	0	0	<1	0	<1
Cefotaxime	0	0	0	0	<1	0	<1
Sulfonamide	51	49	76	81	16	35	41
Trimethoprim	9	8	5	29	6	7	7
Apramycin	2	0	0	0	0	0	0
Gentamicin	2	0	0	0	<1	2	2
Neomycin	4	12	0	0	1	5	3
Spectinomycin	13	16	27	24	6	17	15
Streptomycin	46	49	57	57	12	28	36
Ciprofloxacin	0	0	22	0	1	14	7
Nalidixic acid	0	0	22	0	<1	9	7
Colistin	0	0	0	0	<1	0	0
Number of isolates	372	74	37	21	560	58	121

a) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'
b) The higher occurrence of resistance to ciprofloxacin compared to resistance to nalidixic acid was in part due to qnr genes.

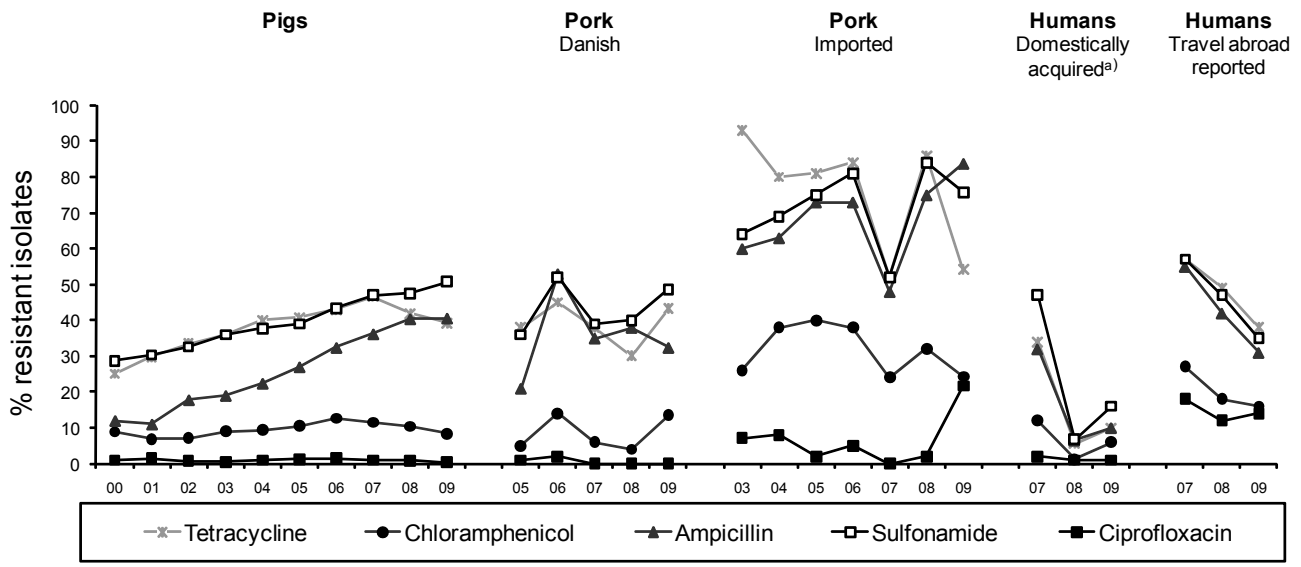

Figure 28. Trends in resistance to selected antimicrobial agents among Salmonella *Typhimurium isolated from pigs, pork and from human cases, Denmark*

The number of isolates varies between years and between source. For pigs, the annual number of isolates were between 216 and 736. For Danish pork, number of isolates was between 64 and 99, 2005-2009; data before 2005 not shown due to low number of isolates. For imported pork, the number of isolates varied between 21 and 56, except in 2007 when only 21 isolates were available

a) Until 2007, includes cases where origin of infection is not documented; therefore only data from and after 2007 are included

The occurrence of resistance to ciprofloxacin was higher than the occurrence of resistance to nalidixic acid among the human isolates due to the occurrence of the plasmid-borne *qnrS* gene, which confers resistance to ciprofloxacin only.

In 2009, two of the tested human *S.* Typhimurium isolates were resistant to cefotaxime, one from a patient with a domestically acquired infection and another from a patient with an unknown origin of infection. Both isolates had an ESBL and AmpC phenotype.

Occurrence of resistance in *Salmonella* Enteritidis from imported broiler meat and human clinical infections

Consumption of eggs is generally considered to be the most common source of human *Salmonella* Enteritidis infections [Mølbak and Neiman. 2002. Am. J. Epidemiol. 156: 654-61; Greig and Ravel. 2009. Int. J. Food Microbiol. 130: 77-87; Annual Report on Zoonoses in Denmark, 2007 (http://www.food.dtu.dk/Default.aspx?ID=9202). In 2008, 0.2% of the Danish layer flocks were positive for *Salmonella* in the surveillance program and eggs from these flocks were pasteurised. No information was available on imported eggs (Annual Report on Zoonoses in Denmark, 2008). In 2009, no *S.* Enteritidis was isolated neither from 7081 samples from Danish broilers (3707 flocks) nor from 100 batches (1200 pooled samples) of Danish broiler meat. However, 30 isolates of *S.* Enteritidis were obtained from imported broiler meat (Table 17). The most prevalent serotype among these isolates was phage type 4 followed by phage type 1. In 2009, the occurrence of resistance in *S.* Enteritidis from imported broiler meat was significantly lower for ampicillin, ceftiofur (from two to zero isolates) and cefotaxime compared to 2008, while it has been increasing for streptomycin, ciprofloxacin and nalidixic acid. Almost half of the isolates were resistant to nalidixic acid and ciprofloxacin, compared to 37% in 2008. No isolates with phenotype of transferable quinolone resistance (ciprofloxacin MIC>0.06 and naldixic acid MIC<32) or resistance to 3rd or 4th generation cephalosporins was observed in broiler meat.

The occurrence of resistance to ampicillin, ciprofloxacin and nalidixic acid was significantly higher in travel associated human *S.* Enteritidis isolates compared to domestically acquired isolates (Table 17).

As for *S.* Typhimurium, the higher occurrence of resistance in travel associated *S.* Enteritidis isolates compared to domestically acquired isolates, probably reflects differences in the use of veterinary antimicrobial agents, such as fluoroquinolones in broilers and layers, between Denmark and the countries to which the patients have travelled.

Table 17. Comparison of resistance (%) among Salmonella *Enteritidis from imported poultry meat and human cases categorized as acquired domestically [a], reported as associated with travel abroad or unknown origin, Denmark*

DANMAP 2009

Substance	Broiler meat	Humans		
	Imported %	Domestically acquired %	Travel abroad reported %	Unknown origin %
Tetracycline	0	0	10	2
Chloramphenicol	0	0	0	0
Florfenicol	0	0	0	0
Ampicillin	0	<1	14	2
Ceftiofur	0	0	<1	0
Cefotaxime	0	0	<1	0
Sulfonamide	0	0	3	2
Trimethoprim	0	<1	3	2
Apramycin	0	0	0	0
Gentamicin	0	0	<1	0
Neomycin	0	0	0	0
Spectinomycin	0	0	<1	0
Streptomycin	7	0	<1	0
Ciprofloxacin	47	4	18	16
Nalidixic acid	47	4	19	16
Colistin	0	3	8	14
Number of isolates	30	173	121	58

a) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'

Campylobacter

In 2009, 3352 human laboratory confirmed cases of campylobacteriosis were reported (61 per 100,000 inhabitants). This was at the same level as in 2008 [EPI-NEWS 2009, no. 12: http://www.ssi.dk/sw73796.asp]. For the surveillance of antimicrobial resistance, the former counties of North Jutland, Funen and Roskilde were selected, representing 2.8% of all cases in Denmark in 2009.

Since 2007, SSI collected travelling information by phone interviews from all *Campylobacter* patients residing in the former counties of North Jutland, Funen and Roskilde. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease onset. Patients who had travelled were asked about their destinations. The case was categorised as "domestically acquired" if the patient had not been travelling one week prior to the onset of infection. In 2009, information was obtained from 78% of *Campylobacter* cases in the three former counties. Among the responding patients, 26% of *Campylobacter* cases were considered acquired abroad [EPI-NEWS 2009, no. 12: http://www.ssi.dk/sw73796.asp].

In 2009, 93 *Campylobacter jejuni* isolates were submitted to SSI for susceptibility testing from three former countries (North Jutland, Funen, and Roskilde), continuously over the year. The isolates were randomly selected from all *Campylobacter* isolated from stool samples in the three former counties. Among the 93 analysed isolates, 31 were from known travel-associated cases and 62 were domestically acquired.

The Danish Veterinary and Food Administration (DVFA) collected samples from meat sold at wholesale and retail outlets for *Campylobacter* testing. The total number of samples tested for *Campylobacter* included 196 samples of Danish pork and 199 of imported pork; 246 of Danish broiler meat and 159 of imported broiler meat; 152 of Danish beef and 92 of imported beef. From broiler meat, all isolates verified as *C. jejuni* (26 Danish, 62 imported) were susceptibility tested. In 2009, isolates from food were species identified in the regional laboratories of DVFA and susceptibility tested at the National Food Institute, DTU. Samples from animals were collected at slaughter for the DANMAP program and species identified and susceptibility tested at the National Food Institute, DTU. No more than one isolate was included per positive farm. For broilers, 93 isolates of *C. jejuni* were isolated from 398 samples and 75 isolates were susceptibility tested and reported. For cattle, 107 isolates of *C. jejuni* and 20 isolates of *C. coli* were isolated from a total of 188 samples; 87 isolates of *C. jejuni* were susceptibility tested and reported, *C. coli* from cattle were not included. For pigs, 137 isolates of *C. coli* and 23 isolates of *C. jejuni* were isolated from a total of 160 samples; 113 isolates of *C. coli* were susceptibility tested and reported. *C. jejuni* from pigs were not included.

Comparison of resistance in *Campylobacter jejuni* isolates from broilers, broiler meat and human clinical infections

Fresh broiler meat is the primary source of human *C. jejuni* diarrhoea infections in Denmark [Wingstrand *et al.* 2006. Emerg. Infect. Dis. 12: 280–285]. From 2008 to 2009, ciprofloxacin and nalidixic acid resistance decreased significantly (19%) in *C. jejuni* from Danish broiler meat (Figure 29). Significant differences in occurence of ciprofloxacin and nalidixic acid resistance were observed between *C. jejuni* from Danish broilers and Danish broiler meat in 2009, with higher resistance levels found in broilers (Table 18 and Figure 29). In Danish broilers, the highest occurrence of resistance was found for ciprofloxacin and nalidixic acid (13% in 2009 compared to 12% in 2008) and tetracycline

Table 18. Comparison of resistance (%) among Campylobacter jejuni *from food animals, food of Danish or imported origin and human cases categorized as acquired domestically or reported as associated with travel* DANMAP 2009

Substance	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestically acquired	Travel abroad reported
	%	%	%	%	%	%
Tetracycline	2	12	4	52	11	39
Chloramphenicol	0	0	0	0	0	0
Erythromycin	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	3
Streptomycin	5	1	0	0	2	7
Ciprofloxacin	20	13	0	56	24	61
Nalidixic acid	20	13	0	56	24	61
Number of isolates	87	75	26	62	62	31

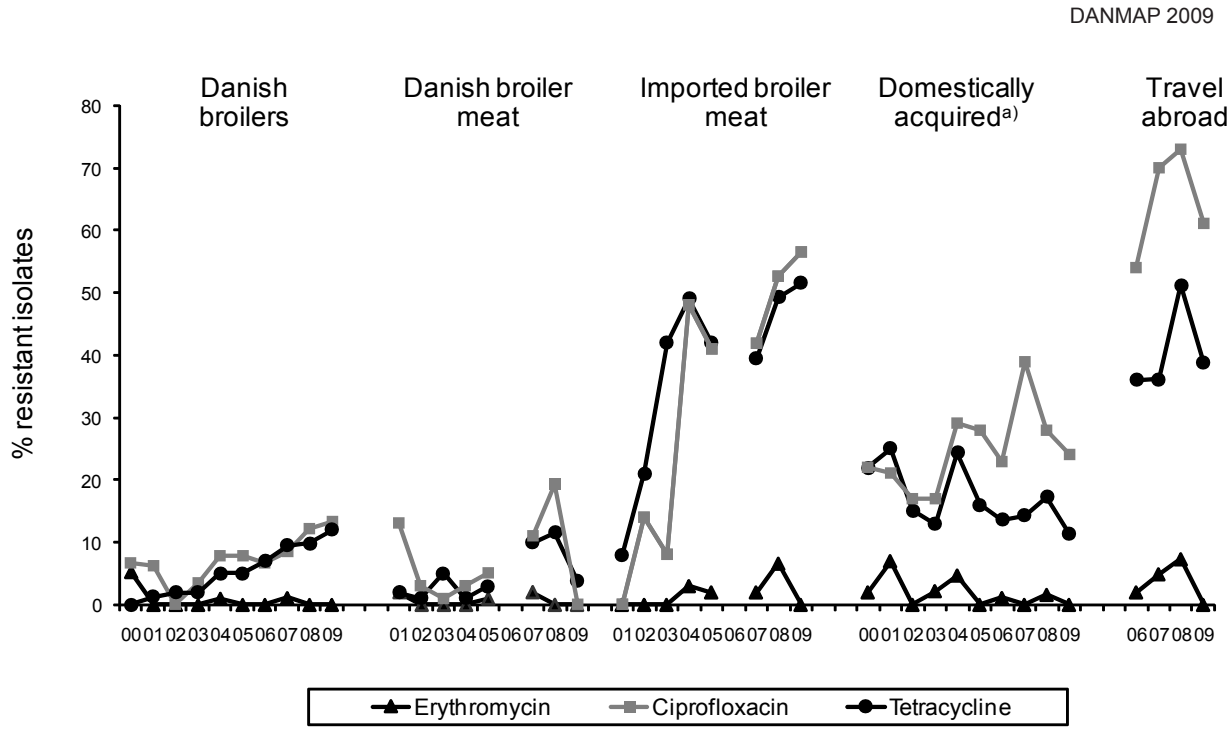

Figure 29. Trends in resistance to selected antimicrobial agents among Campylobacter jejuni *isolates from broilers, broiler meat and human cases, Denmark*

a) Until 2007, including cases where origin of infection was not documented and may therefore include isolates acquired abroad but not documented as such

(12% in 2009 compared to 10% in 2008). Among the tested antimicrobial agents, fluoroquinolones were the most commonly used antimicrobial class for broilers until 2006, but this consumption decreased by 97% from 2006–2008 which might explain the decreasing resistance in broiler meat. In 2009, the consumption of fluoroquinolones increased in chickens from specific parent flocks. The consumption of tetracyclines has increased considerable in 2008 and 2009 and was the second most used antimicrobial class next to amoxicillin in Danish broilers; this is a potential explanation for the increasing tetracycline resistance in *C. jejuni* from Danish broilers.

For *C. jejuni* from imported broiler meat, erythromycin resistance decreased significantly in 2009 to the level in Danish broiler meat. As in previous years, resistance to ciprofloxacin, nalidixic acid and tetracycline was significantly higher in *C. jejuni* from imported broiler meat compared to Danish broiler meat (Figure 29).

In 2009, the level of resistance to ciprofloxacin, nalidixic acid and tetracycline from *C. jejuni* isolates from domestically acquired human infections was in between the level of resistance for isolates obtained from Danish broiler meat and imported broiler meat, as observed in previous years. The consumption of imported broiler meat continued to increase in Denmark, from 17% in 2003 to 37% in 2009 [Annual report on zoonoses in Denmark 2009]. It is likely that imported broiler meat contributes to the high occurrence of ciprofloxacin and nalidixic acid resistance in *C. jejuni* isolates from domestically acquired human infections.

The occurrence of resistance to ciprofloxacin, nalidixic acid, and tetracycline continued to be significantly higher in travel associated *C. jejuni* isolates compared to isolates acquired domestically. For the other antimicrobial agents tested, no significant differences in the resistance level could be detected. Ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with bacterial gastroenteritis because of the activity against enteric bacterial pathogens. Fluoroquinolones are also used in animal husbandry; however, in Denmark the consumption of fluoroquinolones in animal husbandry has been restricted since 2002. Travelling to or consuming meat from countries where fluoroquinolone restrictions are not implemented can be associated with a higher risk of acquiring infection with ciprofloxacin resistant *C. jejuni.*

MIC distributions and the occurrence of antimicrobial resistance among *C. jejuni* from broilers, broiler meat of Danish and imported origin, domestically acquired human cases, and human cases associated with travel are shown in Tables 45, 46, 47 in Appendix 1.

***Campylobacter jejuni* from cattle**

Resistance to ciprofloxacin and nalidixic acid among *C. jejuni* from cattle was unchanged from 2008 to 2009, at a level around 20% (Figure 30). A significant increase in the level of fluoroquinolone resistance occurred in 2005, despite a low consumption of fluoroquinolones in cattle since 2003. Few of the fluoroquinolone resistant isolates were 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 high occurrence of fluoroquinolone resistance. As discussed in DANMAP 2007, clonal spread particularly between farms - initially in the Southern part of Jutland, or other risk factors in this area - might be an explanation. The occurrence has been moving north during 2007–2009; thus in 2009, all the ciprofloxacin and nalidixic acid resistant *C. jejuni* isolates originated from farms in Jutland, with a high prevalence in Northern Jutland.

MIC distributions among *C. jejuni* from cattle in 2009 are shown in Table 45 in Appendix 1.

Campylobacter coli

Among the human *Campylobacter* isolates selected for susceptibility testing, only a few *Campylobacter coli* isolates from domestically acquired infections and travel associated infections were detected (data not shown). Due to uncertainty about the identification of *C. coli* from imported broiler meat, these are not reported. The number of *C. coli* isolates from Danish broilers, Danish broiler meat and cattle were less or equal to 20. Therefore, only antimicrobial resistance among *C. coli* isolates from pigs is reported in this DANMAP report.

MIC distributions and the occurrence of antimicrobial resistance among *C. coli* from pigs are shown in Table 44 in Appendix 1.

From 2008 to 2009, no significant changes in resistance were observed among *C. coli* from pigs. Fluoroquinolone resistance was detected in 12% of the tested isolates (Figure 31) despite low consumption of fluoroquinolones in pigs since 2003 (Table 34 in Appendix 1). In 2009, the erythromycin resistance in *C. coli* from pigs was 12%. A continuous decrease in erythromycin resistance in *C. coli* was observed after withdrawal of the growth promoter tylosin from the Danish pig production in 1998–1999, but in the past years the prevalence of resistance has not changed significantly; since 2006, the level of resistance to erothromycin has been 10–15% (Figure 31).

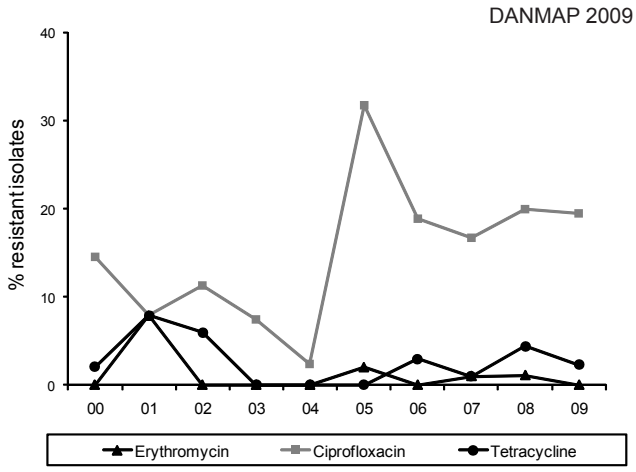

Figure 30. Trends in resistance to selected antimicrobial agents among Campylobacter jejuni *isolates from cattle, Denmark*

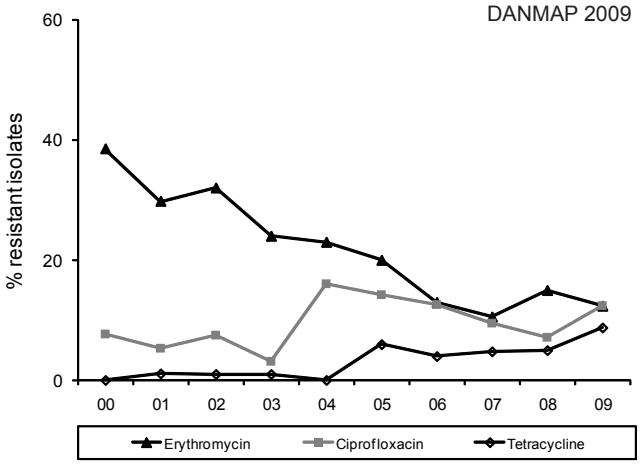

Figure 31 Trends in resistance to selected antimicrobial agents among Campylobacter coli *isolates from pigs, Denmark*

Resistance in indicator bacteria

Enterococci

Enterococcus faecium and *Enterococcus faecalis* were isolated from faecal samples from pigs and broilers. Enterococci were not collected from cattle. All samples for the DANMAP program were collected at the time of slaughter. Isolation and susceptibility testing was performed at the National Veterinary Institute for broilers and at the National Food Institute for pigs. Enterococci from food originated from meat sold at wholesale and retail outlets, collected randomly in all regions of Denmark by the Danish Veterinary and Food Administration Regional laboratories in two centrally coordinated programs. The identification and susceptibility testing was done at the National Food Institute. In 2009, no samples were collected from healthy humans. The results of susceptibility testing with MIC values are presented in Tables 48, 49, 50, 51 in Appendix 1.

Enterococci from food animals

A total of 169 *E. faecium* and 136 *E. faecalis* were isolated from 772 faecal samples obtained from pigs; of these isolates, 151 *E. faecium* and 133 *E. faecalis* isolates were susceptibility tested and reported. For broilers, enterococci were isolated from 141 of 398 samples. All *E. faecium* (43 isolates) and *E. faecalis* (19 isolates) were susceptibility tested and reported. No more than one isolate was included per positive herd or flock.

Before 1995, half of all antimicrobial agents used for production animals in Denmark were used for growth promotion. Among the antimicrobials used were avoparcin (a glycopeptide) with cross-resistance to vancomycin, tylosin (a macrolide) with cross-resistance to erythromycin, virginiamycin (a streptogramin) with cross-resistance to quinupristin/dalfopristin and avilamycin (an oligosaccharide). In 1995, the Danish government banned the usage of avoparcin as a growth promoter. Further research prompted a ban of all use of avoparcin in the European Union (EU) in December 1997. Virginiamycin was banned in Denmark in 1998, and all antimicrobial agents phased out as growth promoters in Denmark in 1998. In the EU, virginiamycin was banned in July 1999 along with tylosin, spiramycin and bacitracin. In September 1999, olaquindox and carbadox were banned in EU and all antimicrobial agents used for growth promotion were phased out in EU by the beginning of 2006. Most of the antimicrobial agents used for growth promotion in Denmark had effects on Gram-positive bacteria. Therefore, since 1995 the occurrence of resistance to these compounds and the antimicrobial group they belong to have been monitored using enterococci as indicator bacteria (Figures 32, 32; Figures 47-51 in Appendix 1.

Table 19. Occurrence of resistance (%) among Enterococcus faecium *from food animals, food of Danish and imported origin, Denmark*

DANMAP 2009

Substance	Broilers	Pigs	Broiler meat		Beef	Pork meat	
	Danish	Danish	Danish	Imported	Imported	Danish	Imported
	%	%	%	%	%	%	%
Tetracycline	16	66	14	52	7	18	14
Tigecycline	-	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0	0
Penicillin	14	35	2	23	7	6	9
Ampicillin	14	29	1	24	7	6	9
Erythromycin	23	38	16	61	13	35	32
Gentamicin	0	0	0	0	0	0	0
Kanamycin	2	30	0	13	7	12	9
Streptomycin	5	47	1	34	7	12	9
Vancomycin	0	2	0	1	0	0	0
Quinupristin/dalfopristin	7	3	3	8	0	6	5
Avilamycin	7	0	1	3	0	0	5
Salinomycin	63	0	38	14	0	0	5
Linezolid	0	0	0	0	0	0	0
Number of isolates	43	151	98	90	15	17	22

Table 20 Occurrence of resistance (%) among Enterococcus faecalis *from food animals, food of Danish and imported origin, Denmark*

DANMAP 2009

Substance	Broilers	Pigs	Broiler meat		Beef		Pork meat	
	Danish	Danish	Danish	Imported	Danish	Imported	Danish	Imported
	%	%	%	%	%	%	%	%
Tetracycline	53	88	26	58	18	15	20	49
Tigecycline	-	0	0	0	0	0	0	0
Chloramphenicol	0	19	3	9	4	0	5	1
Penicillin	0	0	0	0	0	0	0	0
Ampicillin	0	0	0	0	0	0	0	0
Erythromycin	47	49	26	50	7	3	12	8
Gentamicin	0	20	0	3	0	0	3	2
Kanamycin	5	31	3	20	7	0	4	6
Streptomycin	21	38	13	28	7	3	4	4
Vancomycin	0	0	0	0	0	0	0	0
Avilamycin	0	0	0	0	0	0	0	0
Salinomycin	11	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Number of isolates	19	133	39	88	28	33	96	109

Among *E. faecium* isolates from production animals, the highest prevalence of antimicrobial resistance was found among isolates from pigs **(**Table 19). The occurrence of resistance was highest for tetracycline (66%), followed by streptomycin (48%), erythromycin (36%) and penicillin (36%) (Figures 32, 33).
From 2008 to 2009, significant increases in the occurrence of resistance was detected for ampicillin and penicillin in *E. faecium* isolates from pigs, most likely as a result of the increased usage of beta-lactams (Figure 7a). The increase in occurrence of resistance to penicillin among *E. faecium* isolates from pigs has been observed since 2000, while the consumption has been increasing. For streptomycin, a gradual increase in occurrence in resistance was observed from 2003 (19%) to 2009 (48%) despite a constant low consumption of streptomycin in pigs. Resistance towards vancomycin and quinupristin/dalfopristin still prevails among *E. faecium* isolates more than a decade after banning the usage of streptogramins and avoparcin for growth promotion (**T**able 19) (Figures 50, 51 in Appendix 1).The persistence of vancomycin resistance in isolates from pigs has previously been associated with prevalence of resistant clones and plasmids [Hasman *et al*., 2005. Microb. Drug Res. 11: 178-84].

Among *E. faecium* isolates from broilers the highest occurrence of resistance was found for salinomycin (63%), erythromycin (23%) and tetracycline (16%). Salinomycin is widely used as a coccidiostat in the broiler production (data not shown. See DANMAP 2004). From 2008 to 2009, significant increase in the occurrence of resistance was detected for ampicillin in *E. faecium* isolates from broilers, most likely as a result of the increased usage of beta-lactams in 2009. Resistance to the growth promoter avilamycin was still present in *E. faecium* isolates from broilers (Table 19, Figure 49).

Among *E. faecalis* isolates from pigs, the highest occurrence of resistance was found for tetracycline (88%), erythromycin (49%) and streptomycin (38%) (Table 20; Figures 32, 33). A gradual increase in prevalence among isolates of porcine origin has been observed for chloramphenicol (3% in 2003 to 20% in 2009) and for erythromycin (28% in 2000 to 49% in 2009) (Figure 33). A concurrent increasing use of amfenicols in pigs has occurred since 2003 (Table 34 in Appendix 1). Macrolides has been one of the two major drugs for pigs throughout the past decade, and increased significantly in 2009, (Figure 7b-7d).
For *E. faecalis* isolates from broilers, the highest occurrence of resistance was observed for tetracycline (53%), erythromycin (47%) and streptomycin (21%) (Table 20). From 2008 to 2009, significantly higher occurrence of resistance was detected for erythromycin, streptomycin and tetracycline in isolates from broilers. An increasing consumption of tetracyclines occurred in 2008–2009, while macrolides and aminoglycosides were rarely used in the broiler production (Table 8).

Enterococci from food

For Danish meats, *E. faecium* were isolated from 8.5% (17/200) of the pork samples and 62% (98/159) of the

broiler meat samples. For imported meats, *E. faecium* were isolated from 11% (22/195) of the pork samples, 15% (15/98) of the beef samples and 37% (90/246) of the broiler meat samples.
E. faecalis were isolated from 48% (96/200) of Danish pork samples, 18% (28/158) of Danish beef samples, and 25% (39/159) of Danish broiler meat samples. For imported meats, *E. faecalis* were isolated from 56% (109/195) of the pork samples, 34% (33/98) of the beef samples, and 36% (88/246) of the broiler meat samples. All these isolates from meat were susceptibility tested.

Compared to Danish produced broiler meat, a significantly higher occurrence of resistance was found in imported broiler meat for ampicillin, erythromycin, kanamycin, penicillin, streptomycin and tetracycline. For salinomycin, a higher prevalence was found in Danish produced broiler meat when compared to imported broiler meat. The occurrence of resistance

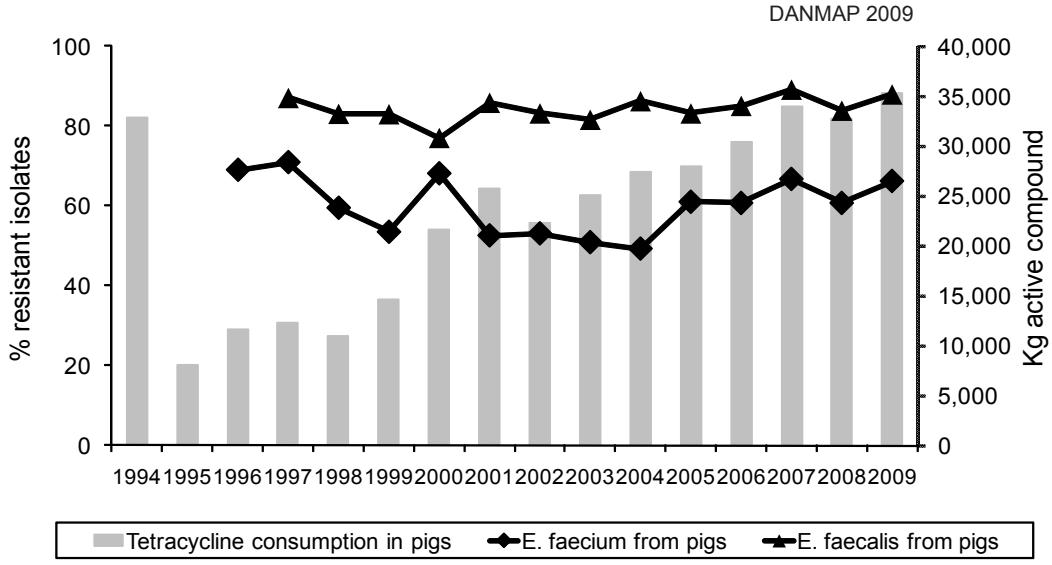

Figure 32 Trends in tetracycline resistance among Enterococcus faecium *and* Enteroccus faecalis *from pigs and the consumption of tetracycline in pigs, Denmark, 1994-2009*

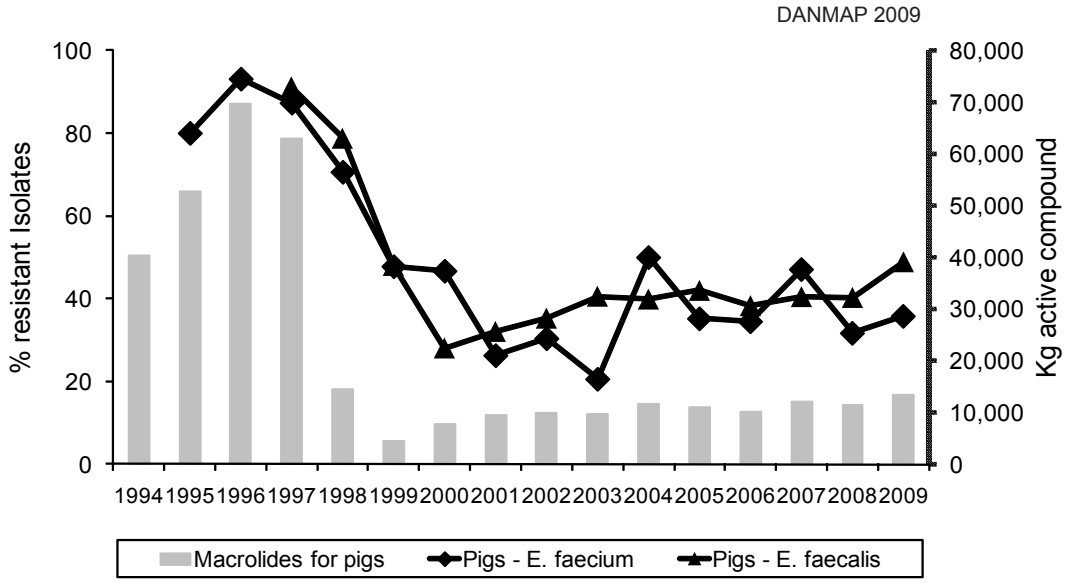

Figure 33. Trend in occurrence of resistance to erythromycin among Enterococcus faecium *and* Enterococcus faecalis *from pigs and the consumption of macrolides for pigs, Denmark, 1994-2009*

was the same in *E. faecium* isolates from Danish pork and imported pork (Table 19).
Significantly higher occurrence of resistance to tetracycline was found in isolates from imported pork compared to isolates from Danish pork, while higher occurrence of resistance to chloramphenicol was found in isolates from Danish pork, compared with isolates from imported pork (Table 20).
Compared with isolates from Danish broiler meat, significantly higher occurrence of resistance to erythromycin, kanamycin and tetracycline was found in imported broiler meat.

Comparison of occurrence of resistance in *E. faecium* from food animals and food

A comparison of resistance among *E. faecium* isolates from food animals and foods is presented in Table 19**.** Significantly lower occurrence of resistance was found for ampicillin, streptomycin and tetracycline in isolates from pork compared with pigs. Significantly lower occurrence of resistance was found for ampicillin, avilamycin and salinomycin in isolates from Danish broiler meat compared with broilers**.**

Comparison of resistance in *E. faecalis* from food animals and food

A comparison of resistance among *E. faecalis* from Danish food animals and foods is presented in Table 20. Significantly higher occurrence of resistance for chloramphenicol, erythromycin, gentamicin, kanamycin, streptomycin and tetracycline was detected in isolates from pigs compared to isolates from Danish pork. Higher occurrence of resistance to salinomycin and tetracycline was detected among isolates from broilers compared with isolates obtained from Danish broiler meat**.**

Escherichia coli

E. coli isolates from healthy food producing animals originated from samples collected for the DANMAP program at the time of slaughter. For broilers, isolation and susceptibility testing was performed at the National Veterinary Institute and for cattle and pigs at the National Food Institute. *E.coli* from food originated from meat sampled at wholesale and retail outlets, collected randomly in all regions of Denmark by the Danish Veterinary and Food Administration Regional laboratories in two centrally coordinated programs. The susceptibility testing was done at the National Food Institute. In 2009, no samples were collected from healthy humans.

Escherichia coli from food animals

E. coli was isolated from 279 out of 284 samples from pigs, 156 out of 161 samples from cattle, and 257 out of 398 samples from broilers. Not more than one isolate was included per positive farm. A randomly selected subsample of 150, 94 and 152 isolates from pigs, cattle and broilers, respectively, were susceptibility tested and reported.

Among animal species, the level of resistance in indicator *E. coli* was lowest in isolates from cattle, with 95% of the isolates being fully susceptible to all tested antimicrobial agents. Among the tested broiler isolates, 57% were fully susceptible and 8% were multi resistant (resistant to three or more antimicrobial classes). Among isolates from pigs the proportion of fully susceptible isolates decreased from 57% fully susceptible in 2008 to 43% in 2009; 37% of the isolates from pigs were multi resistant.

The occurence of resistance in 2009 is shown in Table 21.Trends in resistance to selected antimicrobial agents in isolates from production animals (2000–2009) are presented in Figure 34. The MIC distributions for 2009 are shown in Table 52 in Appendix 1**.** In general, the highest resistance level was found in *E. coli* from pigs, except for fluoroquinolone (nalidixic acid and ciprofloxacin) resistance, which was higher in *E. coli* from broilers throughout the whole study period (2000–2009). The low level of fluoroquinolone resistance in *E. coli* from Danish pigs and cattle probably reflects the low consumption since 2002, where the use in production animals was restricted; since 2002, the highest fluoroquinolone consumption was seen in poultry, as reflected in the higher resistance level. However, the fluoroquinolone consumption in the broiler production (including rearing) has decreased by 98% from 2004–2008, but increased again in 2009. Fluoroquinolone resistance in *E. coli* from broilers, pigs and cattle has not increased through the past decade. In 2009, one ceftiofur resistant *E. coli* was isolated from broilers. This is to our knowledge the first cephalosporin resistant *E. coli* isolated from broilers in Denmark. Cephalosporins have not been used in the

Table 21. Occurrence of resistance (%) among Escherichia coli *from food animals, food of Danish and imported origin, Denmark*

DANMAP 2009

Substance	Broilers	Cattle	Pigs	Broiler meat		Beef		Pork meat	
	Danish %	Danish %	Danish %	Danish %	Imported %	Danish %	Imported %	Danish %	Imported %
Tetracycline	12	2	35	11	55	3	5	32	48
Chloramphenicol	1	1	5	1	21	0	0	1	8
Florfenicol	1	1	1	0	0	0	0	0	2
Ampicillin	18	2	26	20	55	3	5	29	28
Cephalothin	-	-	-	-	-	-	-	-	-
Ceftiofur	1	0	0	0	4	0	0	1	0
Cefpodoxime	-	-	-	-	-	-	-	-	-
Cefotaxime	0	0	0	0	4	0	0	1	0
Sulfonamide	14	3	33	8	54	3	5	38	34
Trimethoprim	6	0	19	3	38	0	5	25	31
Apramycin	0	0	0	1	2	0	0	0	0
Gentamicin	0	0	0	2	5	0	0	0	0
Neomycin	0	0	6	1	14	0	5	6	0
Spectinomycin	3	0	25	2	28	0	0	16	18
Streptomycin	9	3	43	10	45	3	5	42	35
Ciprofloxacin	12	0	1	4	41	0	0	1	6
Nalidixic acid	11	0	1	4	40	0	0	1	2
Colistin	0	0	0	0	4	0	0	0	0
Number of isolates	152	94	150	143	221	32	39	106	65

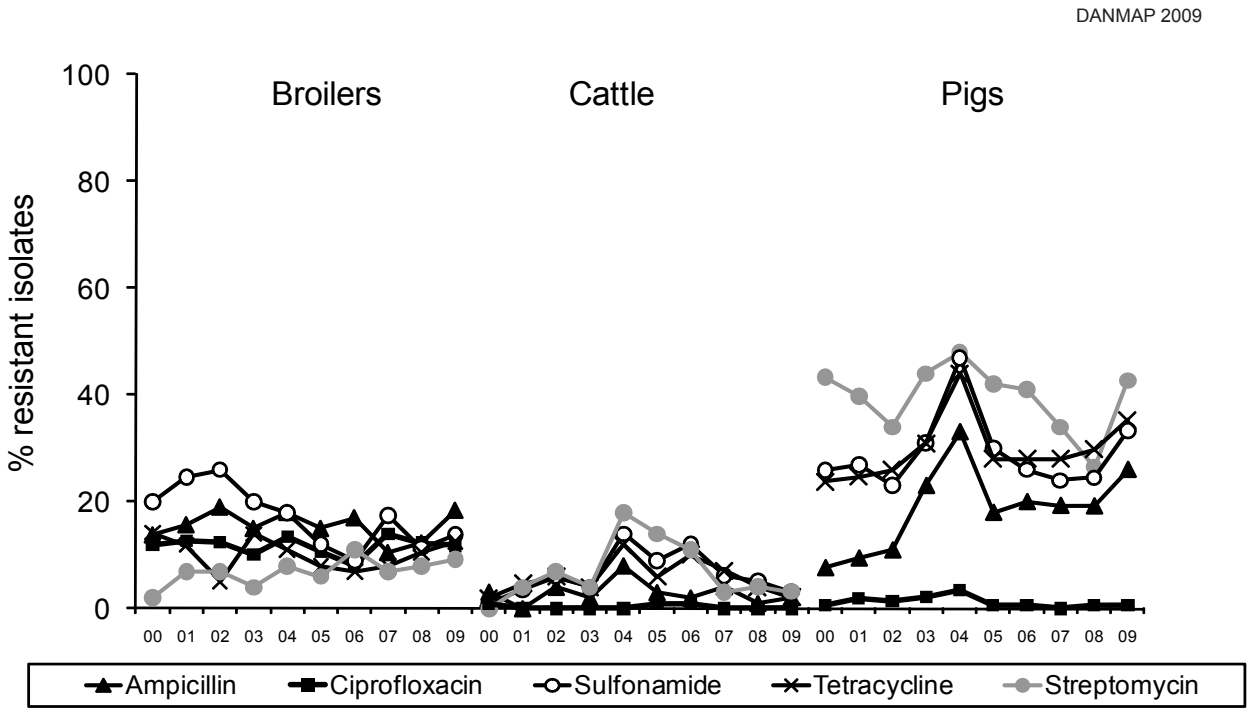

Figure 34. Trends in resistance to selected antimicrobial agents among Escherichia coli *from food animals, Denmark*

broiler production in Denmark in 2001–2009, but may have been used in day-old chicks imported for rearing of parents flocks, or in grand parent floxk abroad. Findings consistent with vertical – not horizontal – transmission of ampicillin- and nalidixic acid-resistant *E. coli* through the broiler production system has been demonstrated [Bortolaia *et al.*, 2010. Vet.Microbiol. P 379-386]. In the same study, cephalosporin resistance was not found in 20 broiler parent farms and 350 broiler farms in 2007, although selective methods were used. Regarding *E. coli* from pigs, the occurrence of ampicillin, chloramphenicol, sulfonamide, streptomycin and spectinomycin resistance increased significantly from 2008 to 2009. For ampicillin, this was part of an increasing trend from 8% in 2000 to 26% in 2009, probably related to an increasing consumption in the same period (Figure). For spectinomycin, a decreasing trend was observed both in resistant *E. coli* and consumption since 2004, while both resistance and consumption increased in 2009. Since 2000, the trends in occurrence of sulfonamide resistant *E. coli* have been similar to the trend in spectinomycin resistant *E. coli,* probably as a consequence of co-selection. For tetracycline, an increasing trend (p=0.058) was seen for tetracycline resistance from 2008 to 2009, as part of a significant increasing trend from 24% in 2000 to 35% in 2009; tetracycline consumption in the pig production has been increasing since 2002, in particular from 2005 to 2007. In 2009 a supplementary investigation of cephalosporin resistant *E. coli* from pigs was performed by use of a selective enrichment method (see focus area).

In cattle, no statistically significant changes in the level of resistance were observed from 2008–2009. (Table 52 in Appendix 1, Figure 34, see also DANMAP 2007). Resistance to tetracycline, ampicillin, and sulfonamide has been decreasing since 2004, with a non-significant increase in ampicillin resistance in 2009. Concurrently, the use of the corresponding antimicrobial groups in calves has been decreasing until 2008. In cows, the use of tetracyclines, sulfonamide, streptomycin and aminopenicillin increased during the same period.

Escherichia coli from food

For Danish meats, *E. coli were* isolated from 53% (106/200) of the pork samples, 21% (32/156) of the beef samples, and 55% (143/259) of the broiler samples. For imported meats, *E. coli* were isolated from 33% (65/196) of the pork samples, 40% (39/98)

of the beef samples and 87% (222/254) of the broiler samples. All *E. coli* isolated from meat were susceptibility tested.
Table 53 in Appendix 1 present the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates collected from broiler meat, pork and beef sampled at wholesale and retail outlets in 2009.

Compared to Danish broiler meat, the level of resistance was significantly higher for 14 of the tested antimicrobial agents in *E. coli* from imported broiler meat, including tetracycline, chloramphenicol, ampicillin, cefoxitime, ceftiofur, colistin, sulfonamide, trimethoprim, neomycin, spectinomycin, streptomycin, ciprofloxacin and nalidixic acid (Table 21).
Cephalosporin resistance was observed among *E. coli* from imported broiler meat (3.6%) where also the highest prevalence of fluoroquinolone resistance was found (41% ciprofloxacin resistance); in Danish broiler meat, no cephalosporin resistance and 4.1% ciprofloxacin resistance was found. In imported pork, two isolates with MIC for nalidixic acid < 32 and for ciprofloxacin > 0.06 were found. The isolates contained transferable fluoroquinolone resistance encoded by *qnrB* and *qnrS*, respectively. The occurrence of resistance in *E. coli* isolates obtained from imported beef was low and not statistically higher compared to isolates from Danish beef.
In *E. coli* from Danish pork, resistance to ampicillin (from 15% to 29%), streptomycin (28% to 42%), and sulfonamide (18% to 38%) has increased significantly from 2004 to 2009. Resistance to ceftiofur was found in one isolate from Danish pork in 2009, and was found for the first time in Danish pork in 2008. In 2009, no statistically significant differences were observed by comparison of resistance in *E. coli* isolates obtained from imported pork and Danish pork. However, the resistance to fluoroquinolone remained low in Danish pork, probably due to the low fluoroquinolone consumption in Danish pigs since 2002; in imported pork, 10% of the *E. coli* isolates were resistant to fluoroquinolone in 2009.
In 2009, a supplementary investigation of cephalosporin resistant *E. coli* from meat was performed by use of a selective enrichment method (see focus area).

Comparison of resistance in *Escherichia coli* from animals and food

Data on the occurrence of resistance in food animals and Danish foods are presented in **Table Icoli5**. For most of the tested antimicrobial agents, the level of resistance in Danish meat reflected the level of resistance in the corresponding animal species with some exceptions. The occurrence of nalidixic acid, sulfonamide and trimethoprim resistance was significantly lower in Danish broiler meat as compared to isolates from broilers. The differences in the level of resistance between broiler meat and broilers might be caused by cross contamination at the slaughter house or due to some resistant isolates being less fit to survive through the food production chain than susceptible isolates.

Textbox 4

European report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food

Continuous monitoring of antimicrobial resistance in different food animal reservoirs is a prerequisite for understanding the dissemination of resistance, for planning targeted interventions and as a guide for authorities and scientists. For that purpose, European legislation on the monitoring of zoonoses and zoonotic agents lays down that European Member States (MSs) are obliged to monitor and report on the antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from animals and food while reporting of resistance data from the indicator organisms (*E. coli* and *Enterococci*) is voluntary.

From 2004 through 2007, 26 MSs and two non-MSs reported antimicrobial susceptibility data on zoonotic and indicator bacteria originating from animals and food. During that period, some countries used inhibitions disk diffusion to test for resistance while others used MIC determination and not all breakpoints were harmonised between countries. In order to be able to compare susceptibility data between countries and over time, all MIC data were reinterpreted using standardised epidemiological cut-off values [EUCAST, Kahlmeter *et al.* 2003]. For inhibition zones there are currently no available harmonised epidemiological cut-off values. Therefore, cut-off values for inhibition zones were set for use in this report in collaboration with the European Union Reference laboratory for Antimicrobial Resistance (EU-RL-AR) where possible and meaningful, based on EUCAST guidelines. Although these reinterpretations of data enables better comparison of data between countries, the monitoring and reporting of data performed by each Member State was not harmonised which might contribute to some differences between countries.

In general, resistance to antimicrobial agents was commonly found among *Salmonella*, *Campylobacter* and the indicator *E. coli* and enterococci isolates from animals and food in the EU. At MS level, the occurrence of antimicrobial resistance remained in most cases relatively stable over time; however, for most of these tested antimicrobial agents, large differences in the occurrence of resistance were observed between countries (Figures 35, 36). There was a tendency that countries reporting high occurrence of resistance in one bacterial species were also among the countries reporting high occurrence in other bacterial species. Since many research studies indicates that the most important risk factor for occurrence of antimicrobial resistance is the consumption of antimicrobial agents, these large differences in resistance observed between countries might indicate that the consumption of antimicrobial agents varies between countries. High occurrence of resistance to ciprofloxacin in *Salmonella* isolates from fowl (*Gallus gallus*) was reported by some MSs and resistance to fluoroquinolone was commonly reported among *C. jejuni* and *C. coli* isolates from animals and broiler meat in several MSs. Denmark restricted the consumption of fluoroquinolones in animals in 2002 because they are critically important in human medicine and compared to other MSs, Denmark were among the countries reporting the lowest occurrence of fluoroquinolone resistance. In addition, Denmark was among the countries reporting the lowest occurrence of resistance within the EU.

When comparing antimicrobial resistance in *Campylobacter jejuni* and *C. coli* from *Gallus gallus* and broiler meat, the same levels of resistance were observed within each country, although the levels of resistance varied between countries (Table 22). This indicates that the level of resistance observed in animal isolates is a good indicator for the levels of resistance humans are exposed to through food.

The full report ”The community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2004-2007” is available at the webpage of the European Food Safety Authority (www.efsa.europa.eu).

Hanne-Dorthe Emborg and Antonio Vieira, on behalf of the National Food Institute, Technical University of Denmark

For further information: Antonio Vieira (antvi@food.dtu.dk)

References:
- **Kahlmeter G. *et al.*, 2003.** European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. Journal of Antimicrobial Chemotherapy, 52, 145-148.
- **EUCAST** (European Committee on Antimicrobial Susceptibility Testing), definitions. Available at: http://www.srga.org/Eucastwt/eucastdefinitions.htm

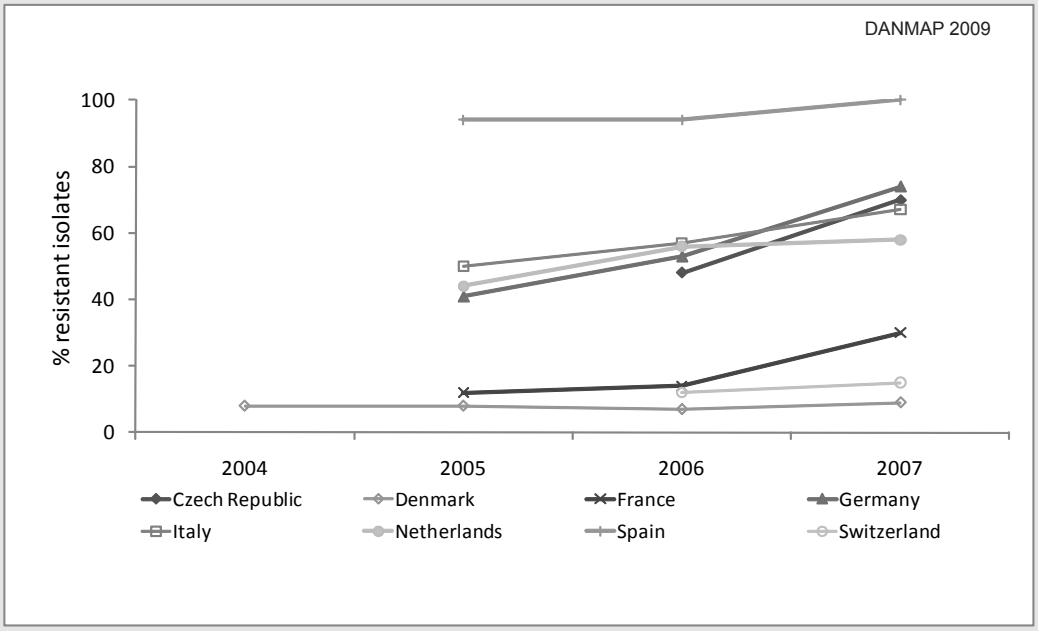

Figure 35. Trends in ciprofloxacin resistance in Campylobacter jejuni *from* Gallus gallus *in reporting Member States, 2004-2007*

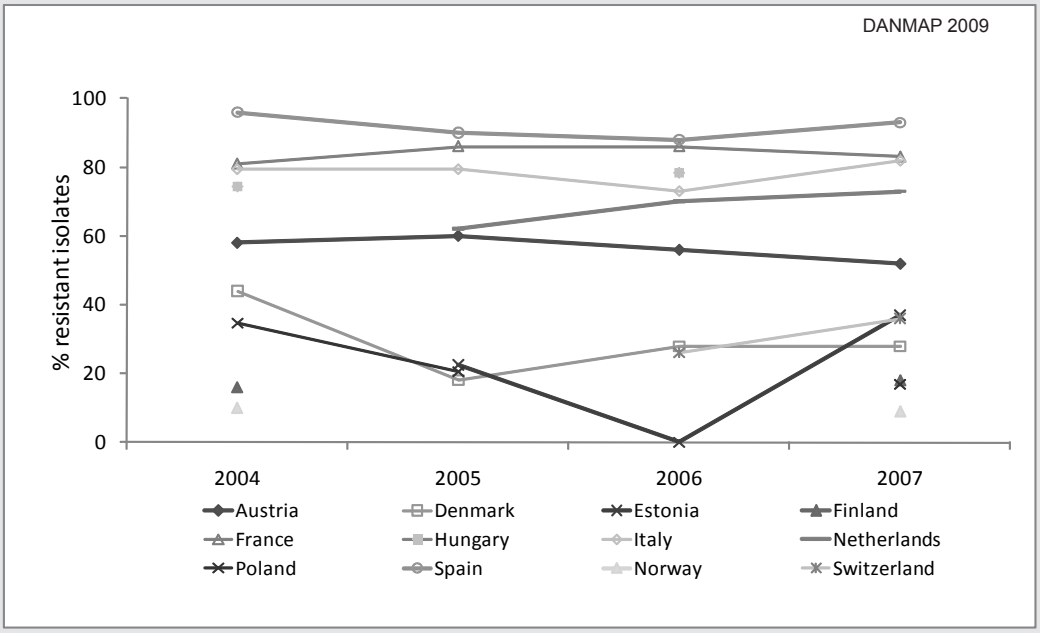

Figure 36. Trends in tetracycline resistance in Escherichia coli *from pigs in reporting Member States, 2004-2007*

Table 22. Comparison of resistance (%) among Campylobacter jejuni *from* Gallus gallus *and broiler meat (*Gallus gallus*) in 2007*

DANMAP 2009

Compound	Austria		Denmark		France		Germany		Norway		Switzerland	
	Galllus gallus	Broiler meat	Galllus gallus	Broiler meat	Galllus gallus	Broiler meat	Galllus gallus	Broiler meat	Galllus gallus	Broiler meat	Galllus gallus	Broiler meat
Tetracycline	27	16	10	10	50	57	54	54	1	0	20	32
Chloramphenicol	0	0	0								0	0
Erythromycin	0	0	1	2	0	3	13	8	0	0	3	0
Gentamicin	0	0	0	0	0	3	7	6	0	0	3	0
Streptomycin	0	0	2	4	4	3			2	0	5	2
Ciprofloxacin	58	66	9	11	30	23	74	64	1	0	15	30
Nalidixic acid	50	66	9	11					1	0	18	30
Number of isolates	26	80	94	114	56	114	100	99	99	29	122	109

Resistance in human clinical bacteria

Escherichia coli

Escherichia coli is part of the normal intestinal flora of both humans and animals but can also cause infections. In humans, *E. coli* can cause a variety of intestinal and extra-intestinal infections such as diarrhoea, urinary tract infections, meningitis, and bloodstream infections. For *E. coli*, this report includes data from 14 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. Results from blood and urine isolates of *E. coli* in hospitals were obtained from all 14 DCM; 12 DCM contributed data on urine isolates in primary health care (Table 23).

E. coli blood isolates obtained from hospitalized patients

The antimicrobial susceptibility of approximately 4000 *E. coli* isolates from blood was reported in 2009. Not all DCM tested for the same antimicrobial agents (Table 23, Figure 37a, b).

The occurrence of resistance to fluoroquinolones increased significantly from 2008 to 2009. Ciprofloxacin resistance increased significantly from 12% to 16% (min. 5%, max. 26% at the individual DCM) and nalidixic acid resistance increased significantly from 13% to 19% (min. 10%, max. 26%) (Figure 37b). The significant increase in occurrence of fluoroquinolone resistance corresponds to the steady increase over the years in the consumption of fluoroquinolones (Table 12, Figure 23). The occurrence of fluoroquinolone resistance was the same as reported to EARSS by many other European countries in 2008 [EARSS 2008, http://www.rivm.nl/earss/result/Monitoring_reports/].

A significant increase was also observed in the resistance to cephalosporins. Cefuroxime (2nd generation cephalosporin) resistance reached 8% (min. 3%, max. 14%), and 7% (min. 2%, max. 14%) of the isolates were resistant to 3rd generation cephalosporin (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) (Table 23). This corresponds to the prevalence of ESBL in *E. coli* blood isolates (7%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area 1). Also, the occurrence of cephalosporin resistance corresponds to the increase over the

Table 23. Resistance (%) to ampicillin, mecillinam, sulfonamide, gentamicin, ciprofloxacin, nalidixic acid, cefuroxime, 3rd generation cephalosporins and carbapenems in Escherichia coli *isolates from humans, Denmark 2009*

DANMAP 2009

Substance	Blood isolates, hospitals a)	Urine isolates, hospitals b)	Urine isolates, primary health care c)
	%	%	%
Ampicillin	44	42 *	42 *
Mecillinam	6 #	7 *	5
Sulfonamide		36 *	38
Gentamicin	5		
Ciprofloxacin	16 *	13 *	11 *
Nalidixic acid	19 *	15 *	14 *
Cefuroxime	8 *	6 *	3 *
3rd generation cephalosporins d)	7 *	6	6
Carbapenems e)	0		
Max. number of isolates tested	4017	43,548	30,909

*) An asterisk indicates a significant increase from 2008 to 2009
#) A number sign indicates a significant decrease from 2008 to 2009
a) All 14 DCM reported data on ampicillin, gentamicin and ciprofloxacin resistance; 13 DCM reported cefuroxime, mecillinam and 3rd generation cephalosporin resistance; 10 DCM reported nalidixic acid resistance; and 8 DCM reported data on carbapenem resistance
b) All 14 DCM reported data on ampicillin resistance, 12 DCM reported mecillinam resistance, 11 DCM reported ciprofloxacin resistance, 10 DCM reported sulfonamide resistance, 9 DCM reported 3rd generation cephalosporin resistance, and 8 DCM reported data on cefuroxime and nalidixic acid resistance. No comparison on 3rd generation cephalosporin resistance in 2008 and 2009 was made, since only one DCM reported data in 2008
c) All 12 contributing DCM reported data on ampicillin and mecillinam resistance, 11 DCM reported sulfonamide resistance, 10 DCM reported ciprofloxacin resistance, 8 DCM reported 3rd generation cephalosporin resistance, 7 DCM reported nalidixic acid resistance, and 6 DCM reported data on cefuroxime resistance. No comparison on 3rd generation cephalosporin resistance in 2008 and 2009 was made, since only one DCM reported data in 2008
d) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime
e) Tested carbapenem was meropenem

years in the consumption of 2nd and 3rd generation cephalosporins (Table 12, Figure 23). The occurrence of 3rd generation cephalosporin resistance in Denmark was above the level reported to EARSS by the other Nordic countries and corresponded to the occurrence reported by several southern European countries in 2008 [EARSS 2008].
In 2009, no *E. coli* isolates from blood were carbapenem resistant.
A significant decrease in the resistance to mecillinam was observed (Table 23, Figure 37b). Among the same 10 DCM reporting data in both 2008 and 2009, resistance decreased significantly from 6% to 4%.

E. coli urine isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 43,000 *E. coli* isolates obtained from hospitalized patients with a urinary tract infection was reported in 2009. Not all DCM tested for the same antimicrobial agents (Table 23). From 2008 to 2009, a significant increase in the occurrence of resistance was detected for ampicillin, mecillinam, sulfonamide, ciprofloxacin, nalidixic acid and cefuroxime (2nd generation cephalosporin) (Table 23, Figure 38a, b). In 2008, only one DCM (Aalborg Hospital) reported data on 3rd generation cephalosporin resistance in all tested isolates making a comparison from 2008 to 2009 uncorrect. The increased occurrence in resistance corresponds to the increasing consumption of penicillins with extended spectrum, fluoroquinolones, and 2nd and 3rd generation cephalosporins seen the last decade (Table 12, Figure 23).
The occurrence of 3rd generation cephalosporin resistance (6%) (Table 23) was higher than the prevalence of ESBL in *E. coli* urine isolates from hospitalized patients (4%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area).

E. coli urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 31,000 *E. coli* isolates obtained from patients with a urinary tract infection from primary health care was reported in 2009. Not all DCM tested for the same antimicrobial agents (Table 23). A significant increase in resistance from 2008 to 2009 was detected for ampicillin, cefuroxime (2nd generation cephalosporin), ciprofloxacin and nalidixic acid, whereas the occurrence of resistance to mecillinam and sulfonamide was the same as in 2008. In 2008, only one DCM (Aalborg Hospital) reported data on 3rd generation cephalosporin resistance in all tested isolates making a comparison from 2008 to 2009 uncorrect (Table 23, Figure 39a, b.
The continuously increasing occurrence of fluoroquinolone resistance was cincurrent with the increasing consumption of fluoroquinolones in primary health care (Table 10). The increased consumption of ciprofloxacin in primary health care, most likely explained by reduced price per DDD due to the introduction of generic ciprofloxacin on the Danish market, has been shown to result in an increase in ciprofloxacin resistance in *E. coli* obtained from urine isolates [Jensen US *et al.* 2010. J. Antimicrob. Chemother. 65: 1286-1291].
The occurrence of 3rd generation cephalosporin resistance (6%) (Table 23) was higher than the prevalence of ESBL in *E. coli* urine isolates from general practice patients (2%), as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area).
The high occurrence of resistance to ampicillin (42%) and sulfonamides (38%) in *E. coli* from urine suggests these antimicrobial agents obsolete for the empiric treatment of urinary tract infections. However, the reported resistance levels may be biased due to a significant proportion of urine samples submitted for microbiological diagnosis following failure of empirical treatment.

Recently, a study on resistance in urinary coliform strains isolated from both hospitalized and general practice patients was conducted in the Sønderborg area, the only area not included in DANMAP [Cybulski and Kjaeldgaard. 2010. Int. J. Antimicrob. Agents. 35: 511-518]. This study showed the same trends of increasing resistance to ampicillin, sulfonamide, ciprofloxacin and cefuroxime in urinary *E. coli* as shown by DANMAP. This indicates that the DANMAP results apply for the whole of Denmark. In the Sønderborg study, a significant decrease in resistance to mecillinam in urinary *E. coli* isolates was observed. This is in accordance with the observed significant decrease in mecillinam resistance in *E. coli* blood isolates mentioned above (Table 23, Figure 37b).

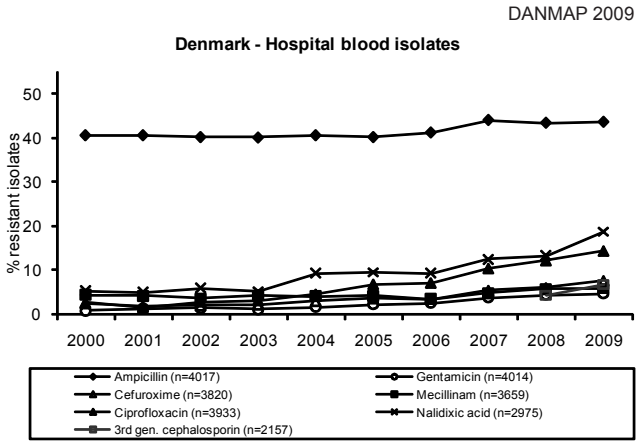

Figure 37a. Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam, ciprofloxacin, nalidixic acid and 3rd generation cephalosporins in Escherichia coli *blood isolates from humans, Denmark*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

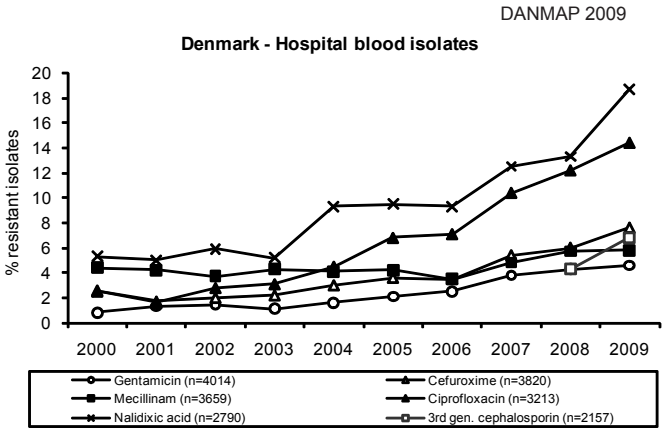

Figure 37b. Resistance (%) to gentamicin, cefuroxime, mecillinam, ciprofloxacin, nalidixic acid and 3rd generation cephalosporins in Escherichia coli *blood isolates from humans, Denmark.*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

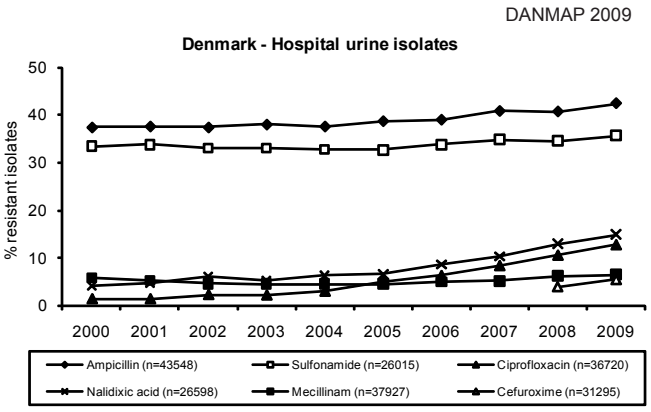

Figure 38a. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid, mecillinam and cefuroxime in Escherichia coli *urine isolates from humans in hospitals, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009.*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

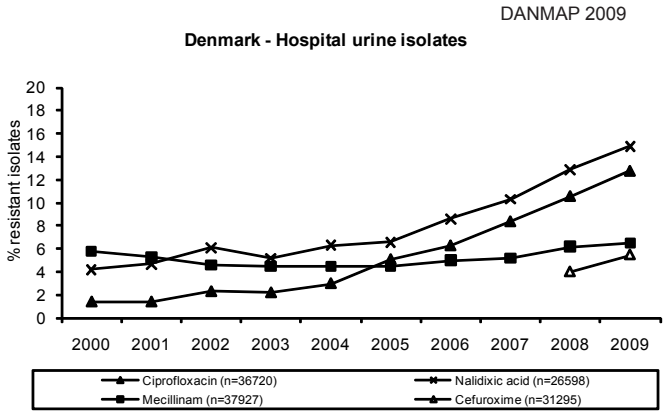

Figure 38b. Resistance (%) to ciprofloxacin, nalidixic acid, mecillinam and cefuroxime in Escherichia coli *urine isolates from humans in hospitals, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009.*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

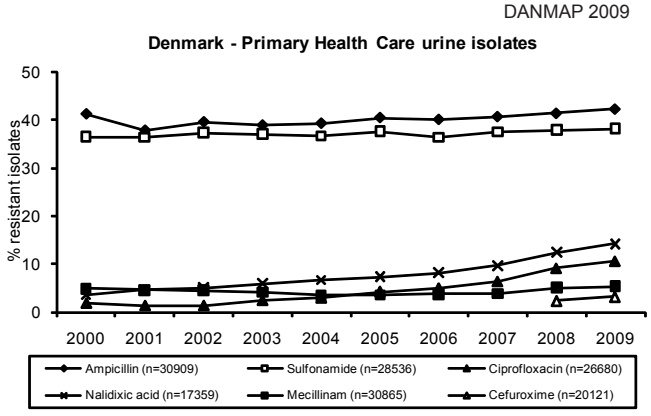

Figure 39a. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid, mecillinam and cefuroxime in Escherichia coli *urine isolates from humans in primary health care, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009.*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

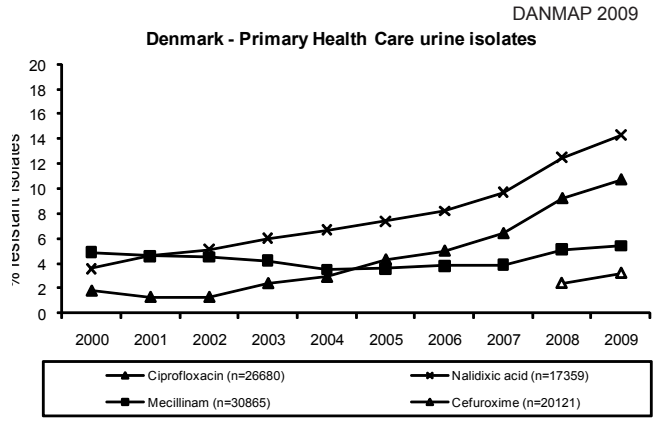

Figure 39b. Resistance (%) to ciprofloxacin, nalidixic acid, mecillinam and cefuroxime in Escherichia coli *urine isolates from humans in primary health care, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009.*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2009

Klebsiella pneumoniae

Klebsiella pneumoniae is part of the intestinal flora in humans but is often the cause of extra-intestinal infections such as urinary tract-, respiratory tract-, wound- and bloodstream infections. Many of these infections are hospital acquired and can be life threatening, especially if the strains are resistant to antimicrobial agents. *K. pneumoniae* is intrinsically resistant to aminopenicillins (e.g. ampicillin). Therefore, infections caused by *K. pneumoniae* are treated with broad spectrum antimicrobial agents such as ciprofloxacin, gentamicin, cephalosporins and carbapenems. Data on antimicrobial resistance in blood and urine isolates of *K. pneumoniae* in hospitals were obtained from 14 DCM (Table 24).

K. pneumoniae blood isolates obtained from hospitalized patients

The antimicrobial susceptibility of approximately 800 *K. pneumoniae* isolates from blood was reported in 2009 (Table 24). Not all DCM tested for the same antimicrobial agents. Until 2007, the occurrence of antimicrobial resistance in *K. pneumoniae* was low and at the same level as in the other Nordic countries. However, since 2007 an increase in resistance has been observed (Figure 40).

The occurrence of 3rd generation cephalosporin resistance in Denmark (12%) (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) (Table 24) was above the level reported to EARSS by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2008 [EARSS 2008]; also, the occurrence of 3rd generation resistance reported from the DCM corresponded to the prevalence of ESBL in 89 *K. pneumoniae* blood isolates from hospitalized patients (15%), as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area 1). Thus, the emergence of 3rd generation cephalosporin resistance among *K. pneumoniae* in Denmark is mostly due to ESBL-producing isolates.

In a study on the spread of 3rd generation cephalosporin resistant *K. pneumoniae* blood isolates in Danish hospitals in 2008, clonal spread of primarily two clones of ESBL-producing isolates within hospitals in the Zealand region was shown [Lester *et al.* 2010. ECCMID. Poster 1251].

The occurrence of fluoroquinolone resistance (ciprofloxacin 18%, nalidixic acid 22%) was the same as reported to EARSS by other European countries in 2008 [EARSS 2008]. In the Eastern part of Denmark (Zealand and Funen), the occurrence of ciprofloxacin resistance (23%) was significantly higher than in the western part (Jutland) (9%).

Aminoglycoside (gentamicin) resistance (9%) was above the level reported to EARSS by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2008 [EARSS 2008].

In 2009, carbapenem (meropenem) resistance was absent in *K. pneumoniae* blood isolates, but the first two *K. pneumoniae* isolates producing carbapenemase (KPC-2) were obtained from colonized patients. These two patients had been travelling to Greece where carbapenem resistance is commonly seen [Hammerum *et al.* Int. J. Antimicrob.Agents. 2010. 35: 610-612].

Multi-resistance in the *K. pneumoniae* isolates was compared among the eight DCM reporting data on 3rd generation cephalosporin, quinolone and gentamicin resistance from 2006–2009. The occurrence of multi-resistant isolates increased significantly from 1% in 2006 to 8% in 2009 (Figure 40). The occurrence of resistance differed among the different DCM, one laboratory reported 16% of the isolates multi-resistant.

The increased occurrence of resistance in *K. pneumoniae* blood isolates corresponds to the increased consumption of broad spectrum antimicrobial agents (e.g. ciprofloxacin, and 2nd and 3rd generation cephalosporins). The emergence of ESBL-producing bacteria has led to an increase in the consumption of carbapenems (Table 12, Figure 23).

Table 24. Resistance (%) to ciprofloxacin, nalidixic acid, gentamicin, cefuroxime, 3rd generation cephalosporins and meropenem in Klebsiella pneumoniae *isolates from blood, Denmark* DANMAP 2009

Substance	2008[a] %	2009[b] %
Gentamicin	7.6	9.0
Ciprofloxacin	16.1	18.1
Nalidixic acid	22.2	21.8
Cefuroxime	15.2	17.1
3rd gen. cephalosporins	9.6	12.0
Meropenem	0.2	0
Max. number of isolates tested	788	886

a) All 14 DCM reported data on gentamicin resistance, 13 DCM reported ciprofloxacin and cefuroxime resistance, 11 DCM reported 3rd gen. cephalosporin resistance, 10 DCM reported nalidixic acid resistance, and 9 DCM reported data on meropenem resistance
b) All 14 DCM reported data on ciprofloxacin and gentamicin resistance, 13 DCM reported cefuroxime resistance, 12 DCM reported 3rd gen. cephalosporin resistance, 10 DCM reported nalidixic acid resistance, and 9 DCM reported data on meropenem resistance

An increase in the total number of isolates per year can also be observed. Among the same eight DCM, a 24% increase in the number of *K. pneumoniae* blood isolates was seen (from 478 isolates in 2006 to 594 isolates in 2009) (Figure 40).

K. pneumoniae urine isolates obtained from hospitalized patients

The antimicrobial susceptibility of approximately 6000 *K. pneumoniae* isolates from hospitalized patients with a urinary tract infection was reported for the first time in 2009. Not all DCM tested for the same antimicrobial agents.

Fluoroquinolone resistance was 22% for nalidixic acid and 17% for ciprofloxacin. However, in the Eastern part of Denmark (Zealand and Funen), the occurrence of ciprofloxacin resistance (21%) was significantly higher than in the western part (Jutland) (10%). Fluoroquinolone resistance in *K. pneumoniae* from urine from hospitalised patients corresponds to the occurrence of resistance detected in isolates from blood.

The occurrence of 3rd generation cephalosporin resistance was 13% (reported as ceftazidime, cefpodoxime or cefotaxime) and corresponded to the prevalence of ESBL in 675 *K. pneumoniae* urine isolates from hospitalized patients (11%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area).

In 2009, carbapenem (meropenem) resistance was present in the *K. pneumoniae* urine isolates from hospitalised patients. The carbapenem resistant isolates were not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the frequency of occurrence could be made since only one DCM reported data on all isolates.

The occurrence of resistance to sulfonamide was 27% (min. 12%, max. 51%), and resistance to mecillinam was 13% (min. 8%, max. 24%).

K. pneumoniae urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 3000 *K. pneumoniae* isolates obtained from patients with a urinary tract infection from primary health care was reported for the first time in 2009. Not all DCM tested for the same antimicrobial agents.

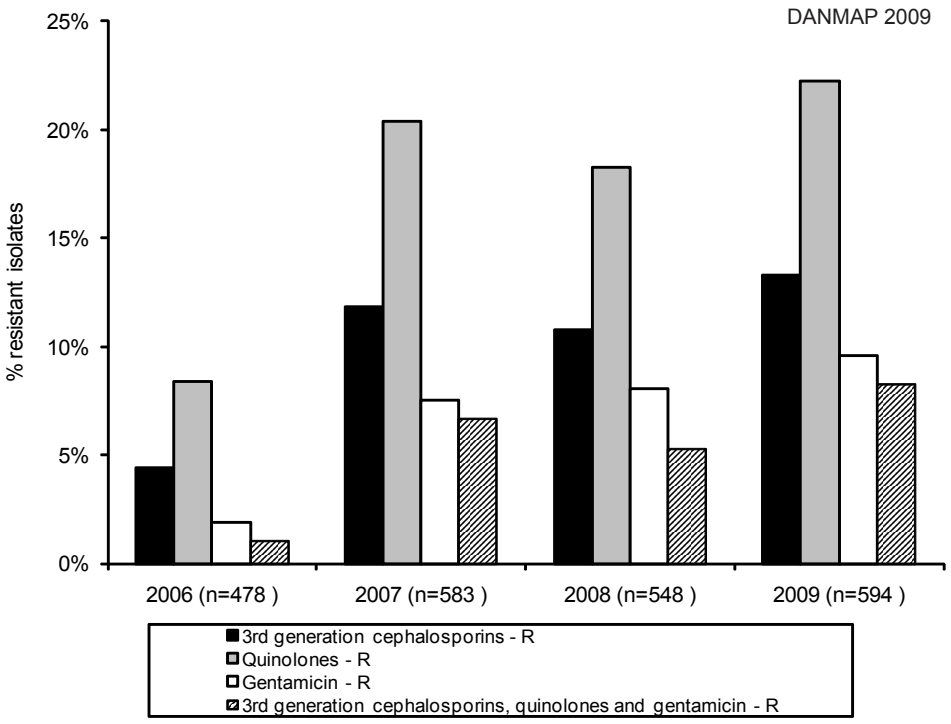

Figure 40. Resistance (%) to 3rd generation cephalosporins, quinolones, gentamicin and multi-resistance (3rd generation cephalosporins, quinolones and gentamicin) in Klebsiella pneumoniae *blood isolates from humans 2006-2009, Denmark*

Data were reported from 8 of 14 departments of clinical microbiology, covering 57.6% of the Danish population

Fluoroquinolone resistance was 20% for nalidixic acid and 13% for ciprofloxacin. However, in the Eastern part of Denmark (Zealand and Funen), the occurrence of ciprofloxacin resistance (16%) was significantly higher than in the western part (Jutland) (8%). Ciprofloxacin resistance in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from both blood and urine from hospitalised patients.
The occurrence of 3rd generation cephalosporin resistance was 8% (reported as cefpodoxime or cefotaxime) and corresponded to the prevalence of ESBL in 385 *K. pneumoniae* urine isolates from general practice patients (7%) as observed in a nation-wide prevalence study of ESBL-producing bacteria performed in October 2009 (See Focus Area). Resistance to 3rd generation cephalosporins in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from both blood and urine from hospitalised patients.

In 2009, carbapenem (meropenem) resistance was observed in one *K. pneumoniae* urine isolate from primary health care. The carbapenem resistant isolate was not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the frequency of occurrence could be made since the DCM reported data on selected isolates only.
The occurrence of resistance to sulfonamide was 30% (min. 22%, max. 43%), and resistance to mecillinam was 15% (min. 11%, max. 33%). Both resistance to sulfonamide and mecillinam was significantly higher in the urine isolates from general practice patients than the urine isolates from hospitalised patients, probably reflecting the usage of sulfonamide and mecillinam in the treatment of urinary tract infections in primary health care.

Pseudomonas aeruginosa

P. 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 colonizer of medical devices (e.g. catheters). *P. aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis and burns. The case fatality rate in these patients is high.

P. aeruginosa blood isolates obtained from hospital patients

For *P. aeruginosa*, this report includes data from 14 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. The antimicrobial susceptibility of approximately 440 *P. aeruginosa* isolates from blood was reported in 2009. Not all DCM tested for the same antimicrobial agents (Table 25). The occurrence of resistance was low for all the tested antimicrobial agents and compared to the other countries reporting to the EARSS report among the lowest.

Table 25. Resistance (%) to ciprofloxacin, gentamicin, 3rd generation cephalosporin, carbapenem and piperacillin-tazobactam in Pseudomonas aeruginosa *isolates obtained from human blood, Denmark* DANMAP 2009

Compound	2007 %	2008 %	2009 %
Ciprofloxacin	5.7	4.5	5.3
Gentamicin	1.2	<1	<1
Ceftazidime	2.4	3.4	3.6
Meropenem	2.3	<1	2.5
Piperacillin/ Tazobactam	3.4	2.3	1.8
Max. number of isolates tested	417	426	440

Streptococcus

Streptococci are part of the normal commensal flora of the mouth, skin, intestine, and upper respiratory tract of humans, but streptococci can also cause infections such as otitis media, tonsillitis, bacterial pneumonia, bacteraemia / sepsis, endocarditis and menigitis.
In this report, data on occurrence of resistance in invasive (from blood or cerebrospinal fluid) streptococcal isolates were obtained from the Neisseria and Streptococcus Reference laboratory covering all the DCMs in Denmark. In Denmark, penicillins and macrolides are often used for treatment of infections caused by streptococci. All invasive non-duplicate *Streptococcus pneumoniae* and group A, B, C and G streptococci were susceptibility tested to erythromycin and penicillin.

Streptococcus pneumoniae

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, otitis media, bacteraemia and meningitis. In 2009, susceptibility testing was performed on 1024 non-duplicate *S. pneumoniae* isolates from invasive infections.
The occurrence of macrolide resistant *S. pneumoniae* decreased significantly from 6.6% in 2008 to 3.6% in 2009 (Figure 41). Macrolide resistance in *S. pneumoniae* isolates from blood and cerebrospinal fluid has been around 6% from 2000 to 2008. The decrease in the number of erythromycin resistant *S. pneumoniae* may be related to the introduction of the pneumococcal conjugated vaccine in the Danish childhood vaccination program in October 2007, but this hypothesis needs to be further tested. In 2009, 35.1% (13 of 37 isolates) of the erythromycin resistant isolates was serotype 14. This was also the dominant erythromycin resistant serotype in the previous years (Table 26).

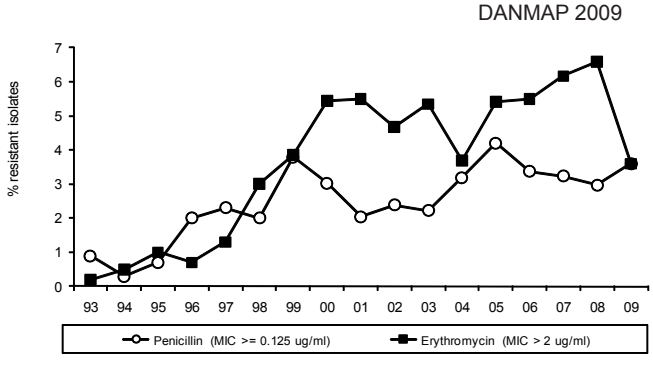

Figure 41. Resistance (%) to penicillin and macrolides in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

The percentage of *S. pneumoniae* invasive isolates being non-susceptible (resistant and intermediate resistant) to penicillin was 3.4% in 2006, 3.2% in 2007, 3.0% in 2008 and 3.6% in 2009 (Figure 41). In 2009, 27% (10 of 37 isolates) of the penicillin non-susceptible isolates was serotype 19A and 16.2% (6 of 37) was serotype 9V. In the previous years, serotype 19A was present in a lower percentage whereas serotype 9V was a dominant type in 2006 and 2007 (Table 26).

The occurrence of resistance to erythromycin and penicillin was similar to the occurrence in other Scandinavian countries but much lower than reported in many of the other European countries reporting to EARSS [EARSS 2007, http://www.rivm.nl/earss/result/Monitoring_reports/].
Using the new penicillin breakpoint (launched January 2008 by CLSI) for susceptibility testing of isolates from patients with invasive disease (except meningitis) treated with intravenous penicillin, three of the 1024 tested isolates (0.3%) in 2009 were non-susceptible intermediate resistant (2 µg/ml<MIC<8 µg/ml) and none were resistant (MIC ≥ 8 µg/ml).

Group A Streptococci

In 2009, 143 invasive GAS (*Streptococcus pyogenes*) isolates were susceptibility tested. As in previous years, no resistance to penicillin in GAS isolates from invasive infections was reported in 2009. Erythromycin resistance was detected in six isolates (4.5%) as compared to two of 136 (1.5%) in 2008 and four of 107 isolates (3.7%) in 2007.

Group B, C and G Streptococci

In 2009, 125 invasive group B streptococci (*Streptococcus agalactiae*) isolates from invasive infections were tested. Erythromycin resistance was detected in 16 isolates (12.8%) compared to 11.4% in 2008 and 8.2% in 2007.

Thirty-seven isolates of invasive group C streptococci were tested in 2009. Three isolates (8.1%) were resistant to erythromycin compared to 4% in 2008 and 5% in 2007.

Nine (4.8%) of the 188 invasive group G streptococci were resistant to erythromycin compared to 10% in 2008 and 8% in 2007.

As in previous years, no resistance to penicillin in group B, C or G isolates from invasive infections was reported in 2009.

Table 26. Serotype distribution (%) of invasive penicillin non-susceptible and erythromycin resistant human isolates of Streptococcus pneumoniae *2006-2009, Denmark*

DANMAP 2009

Serotype	2006			2007			2008			2009		
	NSP[a]	ERP[b]	All tested isolates	NSP[a]	ERP[b]	All tested isolates	PNSP[a]	ERP[b]	All tested isolates	PNSP[a]	ERP[b]	All tested isolates
	%	%	%	%	%	%	%	%	%	%	%	%
14	15.6	68.8	10.9	16.7	69.1	13.0	22.2	60.3	7.9	8.1	35.1	3.1
9V	31.3	6.3	7.0	36.1	8.8	7.7	14.8	3.2	5.5	16.2	8.1	2.7
19F	6.3	4.2	3.1	11.1	4.4	4.0	14.8	4.8	2.7	2.7	5.4	1.9
6B	3.1	8.3	4.3	8.3	4.4	3.4	3.7	1.6	2.5		5.4	2.1
6A/6C	12.5	4.2	4.0	5.6	1.5	3.5	7.4	4.8	4.4	5.4	5.4	4.0
19A	9.4		2.7	8.3	1.5	3.2	11.1	4.8	2.9	27.0	10.8	4.5
11A	3.1		1.6		2.9	1.9		4.8	1.8	2.7	5.4	2.4
33F		4.2	1.7		1.5	1.4		1.6	2.5	2.7	8.1	2.3
23F	3.1		5.2	5.6		5.7		1.6	3.7	5.4		1.7
15A			0.5			0.2	3.7	1.6	0.2	5.4	8.1	0.9
35B			0.3	2.8	1.5	0.6	3.7	1.6	0.3	2.7		0.1
7F			6.8			9.0		1.6	12.5	8.1		13.0
18C	3.1		2.0			2.7	7.4		2.3			1.6
9N			4.0			3.1		3.2	3.8			3.9
4			8.0			8.2			6.4	5.4		6.8
Other serotypes*	12.5	4.2	38.0	5.6	4.4	32.4	14.8	4.8	40.6	8.1	8.1	49.0
Number of isolates	32	48	884	36	68	1079	27	63	937	37	37	1024

*Serotypes with only one non-susceptible or resistant isolate per year and a maximum of two non-susceptible or resistant isolates 2006-2009
a) PNSP: Penicillin non-susceptible *Streptococcus pneumoniae*
b) ERP: Erythromycin resistant *Streptococcus pneumoniae*

Enterococci

Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteremia 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 agents such as penicillin (ampicillin) or a glycopeptide (vancomycin), and an aminoglycoside (gentamicin). For *E. faecalis* and *E. faecium*, this report includes data from 14 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population.

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

In 2009, a maximum of 432 *E. faecium* isolates and 723 *E. faecalis* isolates from blood were tested for antimicrobial susceptibility. Not all laboratories tested for susceptibility to the same antimicrobial agents. From 2002 through 2009, data on *E. faecium* and *E. faecalis* blood isolates covered 11 DCMs yearly. During these years, the number of *E. faecalis* isolates obtained from blood has increased from 405 isolates in 2002 to 612 isolates in 2009 (a 51% increase), whereas the numbers of *E. faecium* isolates have increased from 137 in 2002 to 413 isolates in 2009 (a 201% increase) (Figure 42a). In the period 2001-2009, most of the *E. faecium* isolates were resistant to ampicillin (Figure 42b). From the 14 DCM reporting data on *E. faecium* in 2009, 87% of the tested *E. faecium* isolates were ampicillin resistant, as in 2008. 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 also been observed in hospitals in Denmark during the past years (Figure 23). The antimicrobial pressure in a hospital environment might be a reason for the increasing frequency of *E. faecium* as a cause of bloodstream infections.

Only one of the DCM (Aalborg Hospital) tested all enterococcal blood isolates for high-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates in DCM Aalborg, 34% were HLGR, whereas 56% of the tested *E. faecium* isolates were HLGR in 2009. The occurrence of HLGR was at the same level as reported in 2008 and for *E. faecalis* was similar to the occurrence detected in many countries reporting to EARSS in 2008 (including the UK, Spain and Norway) [EARSS 2008].

Vancomycin resistance was detected in 1.6% of the *E. faecium* (n=6) and less than 1% of the *E. faecalis* (n=1) isolates from bloodstream infections. As described above, most of the *E. faecium* isolates from bloodstream infections were resistant to ampicillin. This may necessitate a change of treatment of enterococcal infections from ampicillin to vancomycin, which in turn will increase the risk of spread of vancomycin resistant enterococci in Danish hospitals. This might already have happened since the consumption of glycopeptides in hospitals has increased from 0.28 DDD/100 occupied bed-days in 1999 to 0.68 DDD/100 occupied bed-days in 2008 and further to 0.93 DDD/100 occupied bed-days in 2009.

Since 2005, SSI has asked all the DCM to send presumeable vancomycin resistant enterococcal isolates from both invasive and non-invasive infections for national surveillance on VRE. In 2009, 18 *vanA E. faecium*, 5 *vanB E. faecium* and 7 *vanB E. faecalis* isolates were received.

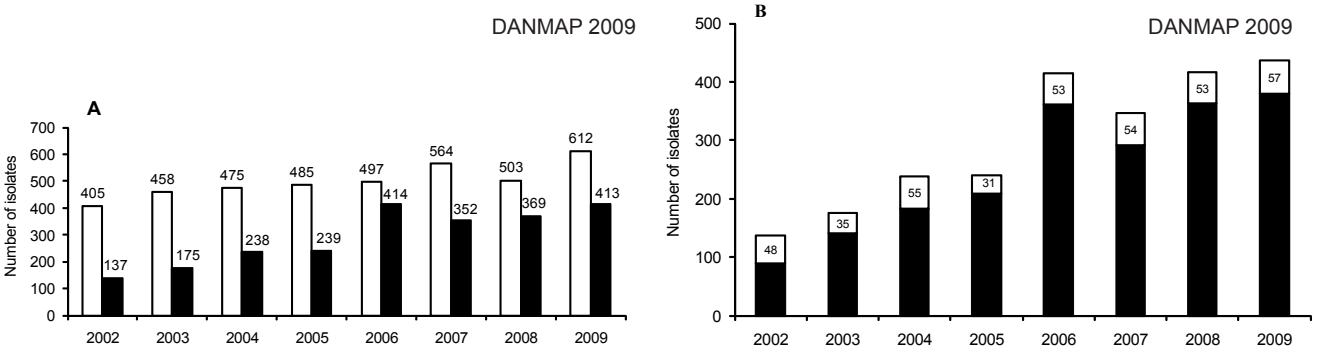

Figure 42a and 42b. Data on enterococcal blood culture isolates from 11 Danish Departments of Clinical Microbiology. (A) The number of enterococcal bacteraemias from 2002 through 2009. The white bars represent E. faecalis *and the black bars represent* E. faecium*. (B) The number of ampicillin resistant and sensitive* E. faecium *blood culture isolates. The black bars represent the ampicillin resistant isolates and the white bars represent the ampicillin sensitive isolates.*

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, *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, arthritis, bacteraemia and endocarditis. Methicillin resistant *S. aureus* (MRSA) has been both laboratory and clinical reportable since November 2006. In recent years *S. aureus*, especially MRSA belonging to clonal complex 398 (CC398), has attracted special attention as this type has been closely connected to livestock animals, especially pigs and has affected people in direct contact with live pigs. All isolates of this type, both human and veterinary with known association to livestock has been resistant to tetracycline and the extensive consumption of tetracyclines in the pig production may serve as a selective factor for this strain type. Other factors such as increased tolerance to heavy metals among the CC398 isolates may also contribute to selection of CC398 (See Textbox 5).

Surveillance of bacteraemia

In 2009, 1466 *S. aureus* bacteraemia cases corresponding to 26.6 per 100,000 inhabitants were reported from all departments of clinical microbiology (DCM) in Denmark. Twenty-three (1.6%) of the cases were caused by MRSA. This is very low compared to most of the other countries participating in the European Antimicrobial Resistance Surveillance System (EARSS). Resistance among *S. aureus* bacteraemia isolates from 2005 - 2009 is presented in Table 27. No significant changes have been observed during this period. Ten (0.7%) of the bacteraemia cases belonged to CC398, the strain type associated to livestock. None of these were MRSA - any association to pig farming is not known. The corresponding numbers were six in 2008 and five in 2007.

Table 27. Occurrence of resistance (%) among isolates from Staphylococcus aureus bacteraemia cases in Denmark 2005 - 2009

DANMAP 2009

Substance	2005	2006	2007	2008	2009
	%	%	%	%	%
Methicillin	1.6	1.4	0.6	1.3	1.6
Penicillin	78	80	78	77	75
Erythromycin	5	5	4	5	7
Clindamycin	4	4	3	4	6
Tetracycline	3	3	2	3	2
Fusidic acid	10	10	9	9	8
Rifampicin	<1	<1	<1	<1	<1
Norfloxacin	3	2	1	2	2
Streptomycin	<1	<1	<1	<1	<1
Kanamycin	2	1	<1	1	1
Mupirocin	0	0	<1	<1	<1
Number of isolates	1428	1329	1345	1344	1466

Surveillance of Methicillin Resistant *S. aureus*

The total number of new MRSA cases[1] was 808 in 2009 (14.7 per 100,000 inhabitants) compared with 851 in 2008. In Figure 43 the numbers of annual cases from 1994 - 2009 are shown with three years moving average. In 2009, two persons were found with two different MRSA strains. At the time of diagnosis, 486 (60%) had infection compared to 447 (52%) in 2008. The proportion of bloodstream infections with MRSA was 1.6% in 2009 (see surveillance of *S. aureus* bacteraemia). The incidence rate of new MRSA cases per year for each DCM in the last three years is shown in Table 28 (Greater Copenhagen is served by three departments of clinical microbiology, and is shown as one). The incidence varied from 26.9 per 100,000 inhabitants in the greater Copenhagen area to 6.2 per 100,000 inhabitants in Esbjerg.

[1] Case = patient found positive for the first time with a specific MRSA strain - regardless whether the patient was infected or colonized

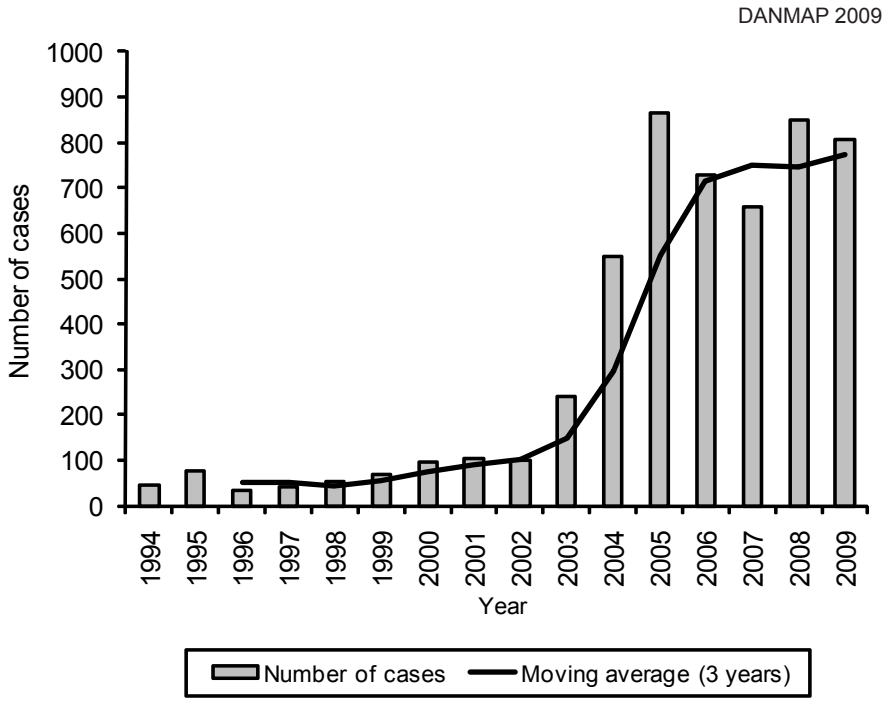

Figure 43. MRSA cases in Denmark 1994 – 2009, with three years moving average

Table 28. Incidence rate of new MRSA cases per 100,000 inhabitants per department of clinical microbiology 2007 - 2009, Denmark

DANMAP 2009

Department of clinical microbiology	2007	2008	2009
Greater Copenhagen*	17.7	23.6	26.9
Hillerød	11.7	21.8	16.5
Statens Serum Institut**	3.9	17.6	17.6
Slagelse	15.3	19.3	15.1
Næstved	8.8	12.1	8.8
Odense	7.1	12.9	8.9
Sønderborg	8.1	11.1	12.8
Esbjerg	14.5	8.8	6.2
Vejle	23.4	20.0	11.6
Herning	2.5	7.7	7.3
Århus	10.1	6.0	10.1
Viborg	8.2	18.5	9.1
Aalborg	8.2	10.7	7.6
Denmark total	12.1	15.5	14.7

* Rigshospitalet (national referral hospital), Hvidovre and Herlev
** Isolates from Roskilde County

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 29. Most of the cases (81%) were acquired in Denmark. The epidemiological classification of MRSA infections 2006 – 2009 is shown in Figure 44. Infections classified as hospital acquired (HA) decreased from 47 cases in 2008 to 30 cases in 2009. Community-acquired infections (CA) are increasing and constitute more than imported (IMP), HA and hospital associated, with community onset (HACO) infections, together (Figure 44). This indicates that the Danish guidelines for prevention of MRSA instituted in 2006 serves its purpose of minimizing health care associated MRSA infections.

Table 29. Epidemiological classification of new MRSA cases 2009, with data from 2008 for comparison

DANMAP 2009

Epidemiologic classification	Exposure	2008		2009	
		No. of cases	No. (%) of cases with infections	No. of cases a)	No. (%) of cases with infections
Acquired outside Denmark (IMP)		137	73 (53)	**156**	**97 (62)**
Detected in Hospitals (HA)		141	47 (33)	**53**	**30 (57)**
Hospital associated, community onset (HACO)	with health care risk	114		**81**	
	with known exposure	17	9 (53)	25	**12 (48)**
	without known exposure	97	76 (78)	56	**37 (66)**
Health care worker		26	5 (19)	**18**	**5 (28)**
Community acquired (CA)	without health care risk	416		**491**	
	with known exposure	171	33 (19)	176	**37 (21)**
	without known exposure	245	197 (80)	315	**265 (84)**
Unclassified		17	6 (35)	**4**	**3 (75)**

a) Epidemiological classification missing for 5 cases

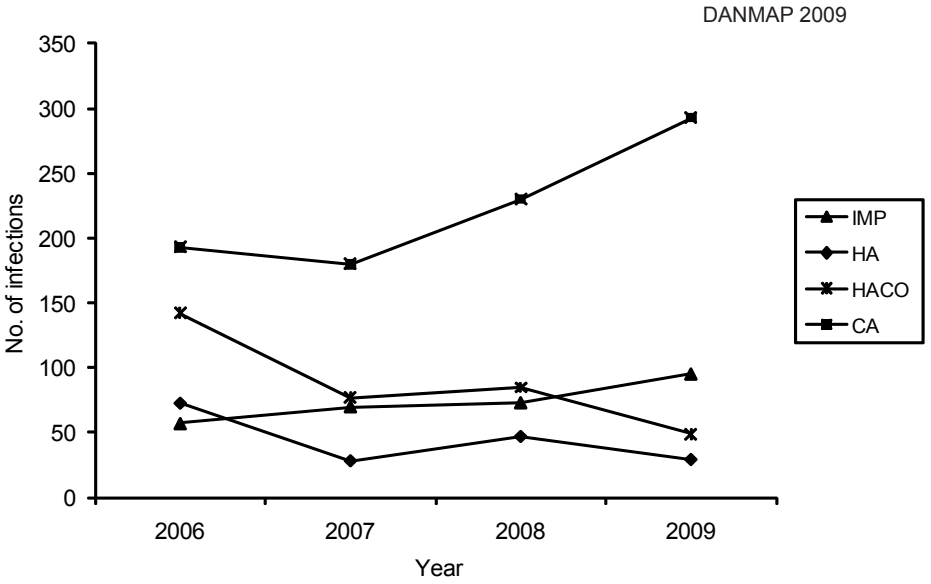

Figure 44. Number of MRSA cases presenting with infection according to epidemiological classification 2006 - 2009

Molecular typing of the MRSA strains

The number of isolates belonging to the 10 dominating *spa* types isolated in 2009 is shown in Table 30. They constituted 55% of the total number of MRSA isolates. Six *spa* types constituted 51% of the 486 clinical infections with MRSA (out of 116 different *spa* types associated with clinical infection). Most prevalent *spa* types causing clinical infections were t008 (n=64), t002 (n=54), t044 (n=49), t024 (n=37), t019 (n=29) and t032 (n=14). Of the 216 strains isolated from asymptomatic carriers, t002 was the most prevalent *spa* type (n=25), followed by t024 (n=20), t008 (n=19) and t034 (n=11). An increase in *spa* type t024 from 45 to 66 cases was observed in 2009. This *spa* type has previously caused outbreaks in nursing homes in the Greater Copenhagen area, but 24 (36%) of the cases in 2009 were classified as community acquired with no known exposure. *spa* types t127 and t189, both involved in outbreaks in neonatal wards in 2008, decreased from 73 and 28 in 2008 to 13 and 9 in 2009, respectively. *spa* type t015, which was among the 10 most prevalent *spa* types in 2008 (n=20), decreased to 10 in 2009.

In 2009, no targeted screening for CC398 types was performed and the number of CC398 *spa* type t034 decreased from 61 in 2008 to 27 in 2009. Thirteen of the 27 t034 cases represented infections **(**Table 30).

Resistance among MRSA isolates

When comparing all MRSA in 2009 with all MRSA isolates in 2008, the occurrence of resistance to erythromycin, clindamycin and rifampicin increased, whereas the occurrence of fusidic acid and streptomycin resistance decreased **(**Table 31**)**. The resistance pattern varied considerably between *spa* types (Table 31)**.** In 2009, 100% of CC398 *spa* type t034 isolates were resistant to tetracycline. In contrast, the majority of t019, a primarily community acquired *spa* type, were susceptible to all tested antimicrobial agents except for beta-lactams. Even though differences in antimicrobial resistance were demonstrated between *spa* types, the success of antimicrobial treatment cannot be predicted based on *spa* type or epidemiological classification.

Table 30. The 10 most prevalent spa *types demonstrated in MRSA cases, Denmark 2009*

DANMAP 2009

spa type	CC group a)	No. of cases	No. causing infections (%)
t008	CC8	90	64 (71)
t002	CC5	87	54 (62)
t024	CC8	66	37 (56)
t044	CC80	61	49 (80)
t019	CC30	43	29 (67)
t034	CC398	27	13 (48)
t032	CC22	24	14 (58)
t223	CC22	16	5 (31)
t005	CC22	15	5 (33)
t127	CC1	13	7 (54)

a) CC = Clonal complex

Table 31. Occurence of resistance (%) in the six most prevalent spa *types demonstrated in MRSA cases, Denmark 2009, compared with all MRSA cases*

DANMAP 2009

spa type	t008	t002	t024	t044	t019	t034	All cases
Clonal complex	CC8	CC5	CC8	CC80	CC30	CC398	
	%	%	%	%	%	%	%
Erythromycin	66	38	89	16	0	56	46
Clindamycin	13	33	83	16	0	67	37
Tetracycline	10	7	0	56	0	100	23
Fusidic acid	13	22	0	89	2	0	16
Rifampicin	0	0	2	0	0	0	3
Norfloxacin	53	39	11	3	0	19	28
Streptomycin	0	0	0	61	2	37	13
Kanamycin	59	25	6	82	2	7	34
Linezolid	0	0	0	0	0	0	0
Mupirocin	0	0	0	0	0	0	2
Number of isolates	90	87	66	61	43	27	808

Textbox 5

Possible association between usage of zinc compounds in pig production and emergence of methicillin resistant *Staphylococcus aureus* CC398 in pigs

Methicillin resistant *Staphylococcus aureus* (MRSA) of clonal complex 398 (CC398) were first identified in 2003 in the Netherlands [Huijsdens *et al.* 2006. Ann Clin Microbiol Antimicrob. 5: 26]. Since then, this clonal complex has been found associated with pigs and other food animal species and as a cause of colonization and infection in humans in several countries in the world. However, the origin of this clonal complex and the factors contributing to its success are yet to be explained.

MRSA and methicillin susceptible *S. aureus* (MSSA) strains isolated from pigs in Denmark were compared and characterized, to find possible factors that could have favoured the selection of MRSA CC398 in the Danish pig farms. A total of 31 MRSA and 60 MSSA isolated from pig farms in Denmark were submitted to *spa* typing and antimicrobial susceptibility testing towards penicillin, tetracycline and erythromycin using broth microdilution for MIC determination. MRSA isolates were additionally subjected to SCC*mec* typing. In addition, all isolates were tested for susceptibility to zinc chloride by agar dilution. All MRSA isolates belonged to *spa* types previously assigned to CC398 (*spa*-types t011, t034, t108), and carried either SCC*mec* type V or IV in one isolate. The MSSA were more diverse and belonged to *spa* types assigned to several clonal complexes (CC5, CC9, CC30, CC97 and CC398). Resistance to tetracycline was observed in all 31 MRSA and all (N=30) MSSA belonging to CC398 and 5 out of 30 non-CC398 MSSA. Penicillin and erythromycin resistance was observed among 100% and 42% of the MRSA and among 80% and 43% of the MSSA of this clonal complex, whereas lower prevalences of resistance were found in other CC-types. Resistance to zinc (MIC 4-12mM) was observed in 74% (N=23/31) of MRSA CC398 whereas all MSSA were susceptible to zinc chloride (MIC 0.5-2mM) (Table 32) [Aarestrup *et al.*, 2010. Vet Microbiol.142: 455-7].

It has previously been suggested that the use of antimicrobial agents and particularly tetracycline, might be related to the emergence of MRSA CC398 [de Neeling *et al*. 2007. Vet Microbiol. 122: 366-72]. Our observations showed that also the MSSA belonging to CC398 displayed tetracycline resistance, indicating that the association between tetracycline resistance and CC398 is not exclusive of MRSA. Therefore, tetracycline might have selected for ST398 in general, but cannot by itself explain why MRSA ST398 has been emerging among production animals.

Interestingly, a very strong association between methicillin resistance and reduced susceptibility to zinc (p<0,001, OR 40,388) was observed. Furthermore, we have identified a gene suspected to cause this resistance (*czrC)* which has been cloned and found to increase the MIC of zinc chloride and cadmium acetate, but not of other metals such as copper sulphate, silver nitrate or sodium arsenate. This gene was found among the zinc resistant MRSA from humans and animals in Denmark [Cavaco *et al*., personal communication] and found located in a type V SCC*mec* cassette in a ST398 isolate from the Netherlands which was subjected to full genome sequencing (accession number AM990992) and also found in other SCC*mec* elements such as type VIII [Zhang *et al*. 2009. Antimicrob. Agents Chemother. 53: 531-540.] which explain the linkage between methicillin resistance and the metal resistance phenotype observed.

Zinc compounds are used very frequently in feed for production animals (especially in pig production) as a feed additive or for control of diarrhoea. Thus, our finding of a strong association between reduced zinc susceptibility and MRSA CC398 could indicate that the use of zinc has contributed to the emergence of MRSA CC398 and/or to its further spread. Future studies on strains from diverse origins and data from experimental and epidemiological studies will help to understand the selection and spread of CC398 and establish adequate control measures.

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Table 32. Susceptibility to zinc chloride, penicillin, erythromycin and tetracycline among mecA positive and mecA negative Staphylococcus aureus *isolated from swine in Denmark*

DANMAP 2009

Isolates	Clonal complex	No. of isolates	Number of isolates with resistance towards (%):			
			Zinc chloride	Penicillin	Erythromycin	Tetracycline
mecA positive	CC398	31	23 (74%)	31 (100%)	13 (42%)	31 (100%)
mecA negative	All	60	0	36 (60%)	15 (25%)	35 (58%)
	CC5	2	0	0	0	0
	CC9	12	0	7 (58%)	1 (8%)	3 (25%)
	CC30	15	0	4 (27%)	1 (7%)	2 (13%)
	CC97	1	0	1 (100%)	0	0
	CC398	30	0	24 (80%)	13 (43%)	30 (100%)

Textbox 6

Surveillance of *Clostridium difficile* 027

In 2009, the Danish National board of Health reinforced the submission of *Clostridium difficile* isolates to Statens Serum Institut (SSI) for further typing in order to intensify the surveillance of the epidemic hypervirulent *C. difficile* PCR ribotype 027 (CD027). In recent years this epidemic variant has been described in North America and in Europe as the cause of outbreaks of increased severity and mortality, longer hospital stay and frequent relapses. CD027 are most often detected in patients who has been treated with antimicrobial agents such as clindamycin, cephalosporins, broad-spectrum penicillins and fluoroquinolones. The emergence of CD027 in Denmark might be related to the increased consumption of these antimicrobial agents.

The criteria used for the selection of the referred isolates were: 1) moxifloxacin resistance, 2) cases with severe clinical manifestations, or 3) cases suspected to be part of an outbreak.
In 2009, SSI received 1847 *C. difficile* isolates in total from 1225 different patients. Genotypic toxin profiling was performed on all isolates, resulting in 728 (59%) different patients infected with *C. difficile* possessing full toxin profile (i.e. genes for toxin A, toxin B and binary toxin). Of these, 596 (82%) were identified with the PCR ribotype 027 (Table 33). Strains harbouring only the genes for toxin A and toxin B - but not for binary toxin - were 431 (35%), and 66 (5%) strains were non-toxigenic.

Table 33. Geographical distribution of Clostridium difficile *PCR ribotype 027 (CD027), Denmark*

DANMAP 2009

Region	*n*
The Capital Region of Denmark	482
The Sealand Region	106
Region of Southern Denmark	4
Central Denmark Region	3
North Denmark Region	1
Denmark	596

Preliminary results indicate that the 30-day mortality of patients infected by strains with full toxin profile (i.e. genes for toxin A, toxin B and binary toxin) was higher - irrespective of the PCR ribotype - compared with patients infected by strains with genes for toxin A and toxin B only, underlining the importance of including other PCR ribotypes than 027 in the national surveillance programme in future.

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Textbox 7

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

Background: Methicillin resistant *Staphylococcus aureus* (MRSA), especially belonging to the clonal complex CC398, has since 2003 emerged in livestock worldwide. MRSA was isolated in a Danish pig farm for the first time in 2006 and MRSA CC398 was retrospectively found in a Danish patient in 2003. The emergence of MRSA in livestock is probably related to the use of antimicrobial agents most likely antibiotics such as cephalosporins, other beta-lactams and tetracycline, but other factors seem to be of importance as well (see textbox on usage of zinc compounds in the pig production). The aim of this study was to investigate the prevalence of MRSA in pigs at slaughter, and in Danish and imported retail meat.

Materials and Methods: During 2009, nasal swab samples (n=789) were taken from pigs at the slaughterhouse before scalding and 865 meat samples (Danish pork (153), broiler meat (121), and beef (143) as well as imported pork (173), broiler meat (191), and beef (84)) were collected in retail stores and outlets. The samples were randomly selected and only one pig from each farm was included each month (maximum of three from the same farm). The meat samples were collected randomly in all regions of Denmark. MRSA was isolated from one nasal swab or from 25 g of meat after selective enrichment in tryptone soya broth supplemented with 4 mg/L cefoxitin and 75 mg/L aztreonam on brilliance MRSA agar. MRSA was confirmed by PCR and the isolates were *spa* typed by PCR followed by sequencing.

Results: One hundred and one (13%) of the pigs at slaughter were positive for MRSA, representing 100 farms. Among the 101 isolates, 81 isolates were *spa* typed. On the basis of the *spa* types, 93% corresponded to CC398, 5 % corresponded to CC30 and one isolate corresponding to CC1 was found.
From meat samples the highest prevalence of MRSA was found among imported broiler meat (18%), followed by imported pork (7.5%), Danish pork (4.6%) and Danish beef (1.4%). No MRSA was found among Danish broiler meat or imported beef. Fifty-two isolates from meat were *spa* typed. From imported broiler meat 63% corresponded to CC398, 28% corresponded to CC9, one isolate corresponded to CC45 and one of *spa* type t002 and t2576, respectively was found. From Danish beef, Danish pork and imported pork only CC398 was found.

Discussion and conclusions: The prevalence of MRSA in pigs at slaughter was higher than what was found in dust samples from production farms and breeding farms in 2008 in Denmark (3.5% and 0%, respectively) [http://www.efsa.europa.eu/en/scdocs/scdoc/1376.htm]. The most common type was CC398 as found previously in a European study where the prevalence in production farms in Europe was 26.9% (varying from 0% to 51.2%) CC398 counted for 92.5% of the CC types found [http://www.efsa.europa.eu/en/scdocs/scdoc/1376.htm]. In our study, MRSA with *spa* type corresponding to CC30 was detected for the first time in Danish pigs. In a previous study, CC30 was found to be the second most common type (26%) among MSSA from pigs in Denmark [Hasman *et al*. 2010. Vet Microbiol. 141:326-31] the finding of CC30 MRSA may be due to horizontal gene transfer. CC1 has not previously been associated with MRSA in animals. The finding of CC398 in Danish beef (1 sample) is interesting as MRSA CC398 has not previously been reported from Danish cattle and cattle farmers in Denmark. The relevance of these findings should be further investigated. Even though MRSA were present in Danish pigs at slaughter, the most important meat source seemed to be imported broiler meat.
The finding of MRSA in imported broiler meat and also the high prevalence of ESBL (see focus area) may be due to a high consumption of cephalosporins in the broiler production in other countries.
In 2009, 39 persons were newly diagnosed with MRSA CC398 in Denmark. Since 2003, a total of 138 persons have been found positive with MRSA CC398. The far majority of humans found positive for CC398 have had direct or indirect contact to pigs. There has been no sign of meat being a significant route for transmission of MRSA CC398 to humans neither in Denmark nor in other countries.

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Resistance in diagnostic submissions from animals

The DANMAP programme monitors antimicrobial resistance in *Escherichia coli* O149 and *Staphylococcus hyicus* from diagnostic submissions from pigs, and *E. coli* F5 (K99) from diagnostic submissions from cattle. *E.coli* was isolated from faecal samples, typically from pigs or calves with diarrhoea, while *S. hyicus* were isolated from pigs with dermatitis. In 2009, *S.hyicus* were not reported because only 14 isolates were available. Most isolates from diagnostic submissions originate from animals in antimicrobial therapy, or with a history of recent antimicrobial therapy. For this reason a higher frequency of resistance is expected in bacteria from diagnostic submissions compared to bacteria originating from healthy animals sampled at slaughter. In 2009, 49 *E. coli* (O149) were isolated from pigs and 48 *E. coli* F5(K99) strains were isolated from cattle. Table 54 in Appendix 1 presents the distribution of MICs and occurrence of resistance in *E.coli* O149 and *E. coli* F5 (K99) from pigs and cattle, respectively.

Escherichia coli from pigs

Figure 45 presents trends in resistance to selected antimicrobial agents in *E. coli* O149 isolates from pigs from 1999 to 2009. The isolates are mainly from weaning pigs with diarrhoea (>7.5 to 30 kg).

In 2009, 67% of the *E. coli* O149 isolates were resistant to tetracycline, corresponding to the 1999-2000 level (Figure 45). A high level of resistance to tetracycline, sulphonamide and streptomycin continued in 2009, throughout the past decade. The high tetracycline resistance is likely linked to the high consumption of tetracyclines in weaning pigs; the isolates mostly originate from weaning pig diarrhoea. Sulfonamide and streptomycin resistance are not used for weaning pig diarrhoea, and the consumption in weaning pigs has been stable at a low level (see figure V10); however, the resistance to these agents may be co-selected with tetracycline resistance, and thus possibly related to the use of these agents in sow herds. A large proportion (80%) of the tetracycline resistant isolates were resistant to three or more of the tested antimicrobial agents, among which 24% of the isolates were AMP-SUL-STREP-TET, and additional 27% were SUL-TET-STREP resistant.

Ampicillin resistance was stable around 40% during 2005–2007. The level of resistance to ampicillin seemed to reach a maximum in 2004 with 49% of the isolates being resistant. A maximum for consumption of aminopenicillins in pigs also occurred in 2004, with a decrease in consumption from 2004 through 2008. The occurrence of ciprofloxacin resistance decreased (non-significant) to 6% in 2009, as part of a continued significantly decreasing trend since 2007. The occurrence in 2009 was the lowest level since 2003–

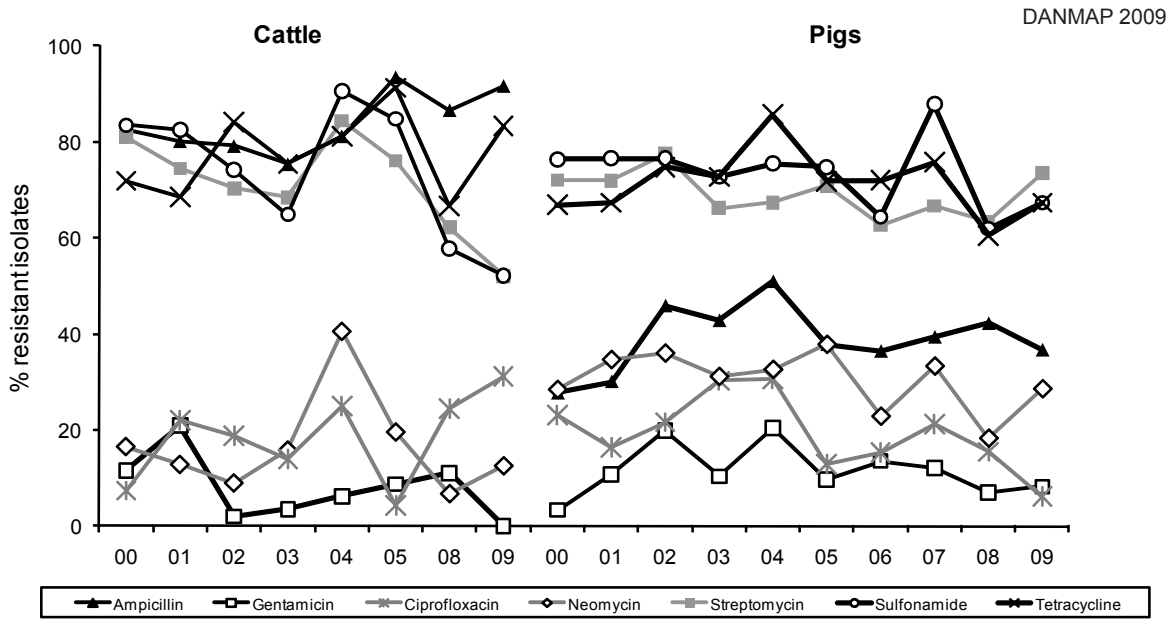

Figure 45. Trends in resistance to selected antimicrobial agents among Escherichia coli *from diagnostic submissions from animals, Denmark*

2004 (29%) when the highest measured level was reached; 2003 was the first year after the restriction of fluoroquinolone use in production animals. In 2009, no resistance to ceftiofur was observed for *E.coli* O149

Escherichia coli from cattle

In cattle, *E.coli* F5 (K99) were almost entirely isolated from diagnostic submissions from calves. No isolates with gentamicin or apramycin resistance where observed in 2009, representing a significant decrease from 11% in 2008 (Figure 45**).** A significant decrease in occurrence of sulphonamide and streptomycin resistance since 2004, continued in 2009. A weak decreasing consumption in these agents in calves was also observed 2005-2009 (Figure 10; Table 35 in Appendix 1). However, as most of the isolates were also resistant to tetracycline and/or other agents, the decrease may also in part be due to reduced co-selection.

In 2005, the most important antimicrobial group used in calves was tetracyclines, and during 2006–2008, it has been the second most used drug (Figure 10). The use of tetracycline, sulphonamides, streptomycin and aminopenicillins in calves increased in 2009, after a decreasing trend during 2005-2008. Resistance to three or more antimicrobials was observed for 73% of the isolates, most frequently combinations of these agents (27% AMP-SUL-STREP-TET and further 29% resistant to three of these agents) often in combination with other types of resistance, and 6% being AMP-CIP-NAL-TET resistant.

In 2009, no *E. coli* F5 (K99) isolates were resistant to ceftiofur, and 1 (2%) isolates were resistant to cefotaxime.

APPENDIX 1
APPENDIX 2
APPENDIX 3

Table 34. Consumption of antimicrobials in pigs given as Animal Daily Doses (ADDs), Denmark DANMAP 2009

ATCvet code	QJ01AA	QJ01BA	QJ01CE	QJ01CA, QJ01CR	QJ01DC, QJ01DD	QJ01E	QJ01FA	QJ01FF	QA07AA	QA07AA10	QJ01MA	QJ01RA	QJ01XX	
Antimicrobial class	Tetracyclines	Amphenicols	Penicillin's, b-lactamase sensitive	Aminopenicillins b)	Cephalosporin's c)	Sulfonamides and trimethoprim	Macrolides	Lincosamides / spectinomycin d)	Aminoglycosides (local GI)	Colistin (local GI)	Fluoroquinolones	Penicillin-streptomycin combinations	Pleuromutilin's	Total
Year	**Sows/piglets (1000's ADD_{200})**													
2001	1,046	0	1,574	719	38	803	763	428	291	1	93	597	533	6,886
2002	1,072	0	1,793	894	60	965	764	555	252	26	51	643	498	7,574
2003	1,104	8	2,039	993	99	1,116	690	568	234	35	23	703	953	8,567
2004	1,135	9	2,256	1,080	113	1,269	719	580	215	35	3	669	1,027	9,110
2005	1,092	10	2,344	1,059	132	1,366	724	567	167	35	4	661	845	9,006
2006	1,232	9	2,371	1,056	149	1,434	780	542	152	35	7	647	955	9,368
2007	1,697	10	2,589	1,184	244	1,568	1,315	615	101	47	6	662	1,300	11,338
2008	1,660	11	2,647	1,195	300	1,635	1,242	558	38	57	0	631	1,842	11,814
2009	1,764	31	2,865	1,404	219	2,033	1,355	535	48	85	0	685	1,726	12,751
	Weaner pigs (1000's ADD_{15})													
2001	36,163	0	2,249	7,158	60	3,446	48,410	13,187	27,324	75	531	1,933	15,230	155,766
2002	31,476	4	2,552	8,308	147	3,987	44,195	16,575	23,752	3,172	188	2,152	18,255	154,763
2003	32,349	112	3,015	10,654	254	4,185	39,308	18,691	22,032	4,377	17	2,211	19,779	156,984
2004	39,194	141	4,144	13,899	263	5,516	49,768	21,189	21,288	4,531	8	3,075	24,984	188,001
2005	45,858	96	4,258	12,115	267	6,192	48,252	18,269	19,633	3,994	5	3,588	26,747	189,272
2006	56,166	48	4,050	10,017	291	4,698	46,666	15,881	19,464	4,212	11	3,513	25,496	190,514
2007	76,701	90	4,472	9,914	407	4,192	54,522	16,203	10,586	5,299	0	3,439	22,655	208,481
2008	83,718	256	4,144	9,730	400	4,559	51,676	16,597	2,857	6,727	0	3,445	30,834	214,943
2009	98,866	149	4,618	11,902	358	4,668	59,205	17,823	2,981	6,862	0	3,782	39,241	250,456
	Finisher pigs (1000's ADD_{50})													
2001	9,223	0	4,149	1,505	16	173	10,840	3,131	262	1	129	296	7,047	36,773
2002	8,936	0	4,630	1,756	36	206	11,027	3,693	220	22	69	351	7,568	38,515
2003	11,492	30	5,249	1,995	56	177	11,605	4,233	192	28	6	423	8,522	44,008
2004	12,689	43	6,502	2,835	60	237	11,599	4,447	124	22	4	380	10,371	49,313
2005	14,074	35	7,488	2,674	62	247	12,033	4,223	236	20	2	368	12,121	53,582
2006	16,231	33	7,702	2,275	50	159	10,316	3,524	213	27	1	297	10,846	51,673
2007	19,320	20	7,917	2,155	54	172	10,362	3,194	109	20	0	226	8,806	52,354
2008	18,824	20	7,544	1,547	53	152	10,006	2,637	5	43	0	158	12,993	53,983
2009	20,000	16	8,195	1,651	39	120	11,823	2,737	13	30	0	129	15,194	59,948
	Age group not given (1000's ADD_{50})													
2001	1,137	0	556	424	9	268	1,471	545	584	0	89	139	806	6,030
2002	800	2	444	296	7	202	929	330	209	22	20	82	630	3,975
2003	768	5	491	305	9	210	951	376	149	39	0	98	676	4,077
2004	915	7	557	289	9	154	1,125	419	170	29	3	69	986	4,731
2005	874	4	563	276	10	184	841	324	85	32	0	85	729	4,007
2006	1,168	2	510	315	11	177	755	279	144	34	0	69	722	4,187
2007	675	1	254	101	11	84	369	186	48	27	0	26	395	2,177
2008	398	1	147	94	9	56	235	90	8	35	0	8	287	1,368
2009	233	0	110	78	10	43	205	56	2	24	0	10	187	958

Data includes sales from pharmacies and feed mills. Consumption in veterinary practice comprises less than 1% of the total consumption in pigs and are not included, except for the use of fluoroquinolones

a) Animal Standard weight is an assumed average weight at treatment, used to calculate numbe of ADD (Animal Daily Doses giving an estimated number of animals treated) from number of ADDkg (mass of animal treated, measured in kg animal bodyweight)

b) Includes a small proportion (< 1‰) of combinations with aminopenicillin and clavulanic acid

c) 3rd and 4th generation cephalosporins

d) Lincosamides and combnations between spectinomycin and lincosamides

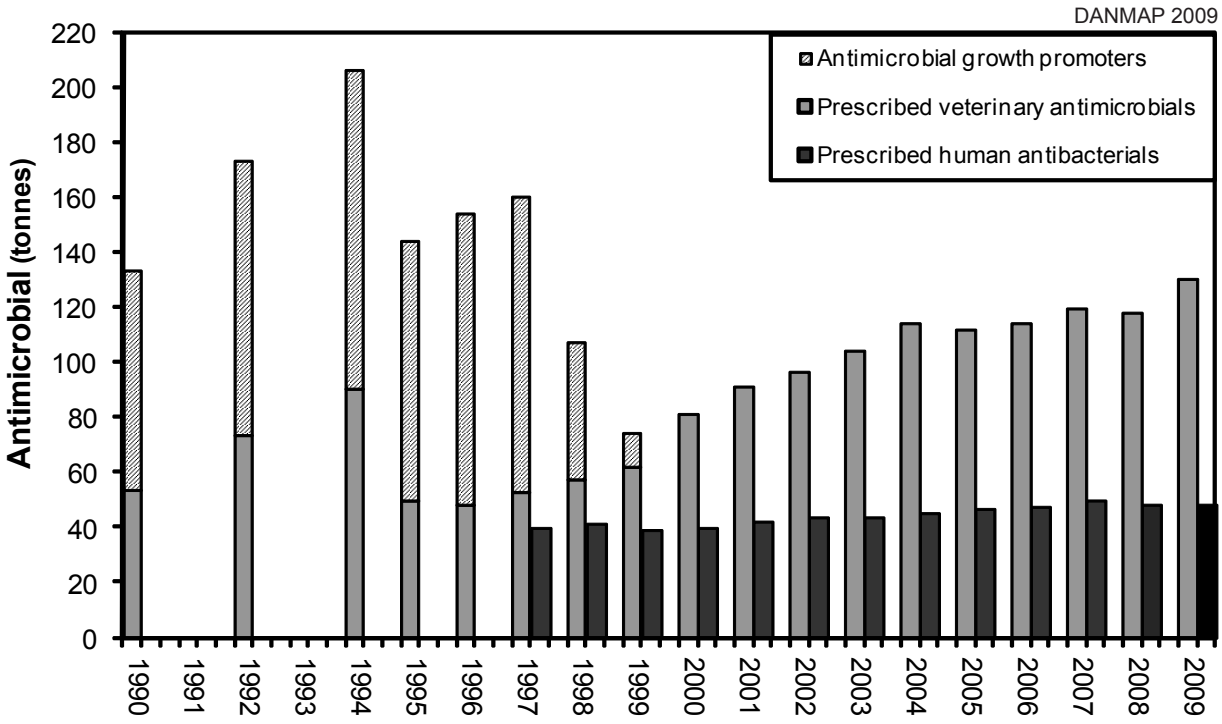

Figure 46. Consumption of prescribed antimicrobials and growth promoters in animal production and prescribed antibacterials in humans, Denmark

Sources: Human therapeutics: The Danish Medicines Agency. Veterinary consumption: 1990–2000, data based on reports from the pharmaceutical industry of total annual sales. (Data 1990–1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996–2000: Danish Medicines Agency and Danish Plant Directorate). 2001–2009: Data from VetStat.

Table 35. Choice of antimicrobialsa) for systemic use in cattle given as Animal Daily Doses (ADDs), Denmark
DANMAP 2009

ATCvet code	QJ01AA	QJ01BA	QJ01CA, QJ01CR	QJ01CE	QJ01DC, QJ01DD	QJ01E	QJ01FA	QJ01FF	QA07AA	QA07AA10	QJ01MA	QJ01RA	
Antimicrobial therapeutic group	Tetracyclines	Amphenicols	Aminopenicillins c)	Penicillin's, β–lactamase sensitive	Cephalosporin's d)	Sulfonamides and trimethoprim	Macrolides	Lincosamides / spectinomycin e)	Aminoglycosides (local GI)	Colistin (local GI)	Fluoroquinolones	Penicillin-streptomycin combinations	Total
Year						**Cows and bulls (1000's ADD_{600})[b)]**							
2005	186	1	58	490	71	65	112	2	19	0	0	22	1,027
2006	193	1	57	498	64	61	116	2	9	0	0	22	1,021
2007	235	1	68	610	79	73	91	2	2	0	0	28	1,189
2008	257	1	80	702	85	75	65	1	1	0	0	34	1,302
2009	279	2	84	804	73	73	53	1	2	0	0	36	1,407
						Calves (1000's ADD_{100})[b)]							
2005	574	61	193	170	33	162	562	19	127	39	2	142	2,083
2006	534	67	145	180	30	141	879	13	108	7	1	136	2,242
2007	561	96	131	183	37	154	881	16	92	8	1	131	2,290
2008	528	129	105	168	30	133	804	13	77	11	0	113	2,111
2009	556	150	102	173	22	166	768	9	95	10	0	117	2,167
						Heifers and steer (1000's ADD_{300})[b)]							
2005	18	0	5	27	3	3	8	1	0	0	0	2	67
2006	19	0	3	26	3	3	9	0	0	0	0	3	67
2007	24	1	6	33	4	3	10	2	0	0	0	4	86
2008	26	1	5	36	4	3	9	2	0	0	0	4	90
2009	26	1	5	37	3	3	6	1	0	0	0	5	88
						Cows and bulls (1000's ADD_{600})[b)]							
2005	7	0	4	5	1	2	6	1	2	0	0	1	29
2006	21	1	13	14	2	4	31	6	5	1	0	2	99
2007	16	0	5	13	2	2	13	2	1	0	0	2	57
2008	2	0	1	3	1	0	2	0	0	0	0	0	10
2009	1	0	0	3	0	0	1	0	0	0	0	0	6

a) Data includes sales from pharmacies and use for cattle in veterinary practice, including sales to the farmer. The consumption in calves is underestimated by up to 5% and consumption in cows is underestimated by up to 17% in individual years, because the use in cattle practice was underestimated by up to 20%. This error was decreasing with time. Therefore, the numbers are not fully representative descriptive for trends over years, but reflects the choice of drug in individual years.
b) Animal Standard weight is an assumed average weight at treatment, used to calculate numbe of ADD (Animal Daily Doses giving an estimated number of animals treated) from number of ADDkg (mass of animal treated, measured in kg animal bodyweight)
c) Includes a small proportion (< 1‰) of combinations with aminopenicillin and clavulanic acid
d) 3rd and 4th generation cephalosporins
e) Lincomycin and lincomycin/spectinomycin combinations

Table 36. Consumption of antibacterial agents for systemic use in primary health care (No. packages/1000 inhabitants/year), Denmark

DANMAP 2009

ATC group a)	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	22.8	22.4	21.7	21.6	22.5	23.8	23.9	24.5	25.0	25.9
J01CA	Penicillins with extended spectrum	103.7	110.9	111.8	111.5	115.3	119.9	119.7	131.3	130.0	130.2
J01CE	Beta-lactamase sensitive penicillins	243.7	251.0	254.4	254.5	253.7	251.1	243.3	253.0	235.9	223.2
J01CF	Beta-lactamase resistant penicillins	24.0	30.1	37.5	41.9	43.0	44.4	44.0	45.8	45.4	45.2
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	1.1	1.2	1.7	2.0	2.5	3.0	4.0	5.8	8.0	12.3
J01D	Cephalosporins and related substances	1.0	1.3	1.4	1.3	1.4	1.6	1.7	1.8	2.1	2.1
J01EA	Trimethoprim and derivatives	7.9	8.2	8.8	9.3	10.2	10.6	10.7	11.5	12.4	10.9
J01EB	Short-acting sulfonamides	47.8	47.8	47.6	47.9	48.3	47.5	45.8	41.0	36.0	34.6
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	1.4	1.4	1.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0
J01FA	Macrolides	97.3	102.2	102.8	99.8	102.7	110.3	101.8	108.6	103.3	99.6
J01FF	Lincosamides	0.4	0.5	0.6	0.6	0.7	1.1	1.4	1.6	2.0	2.5
J01GB	Aminoglycosides	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
J01MA	Fluoroquinolones	9.7	10.6	11.0	13.8	16.2	18.3	19.4	22.9	25.1	25.0
J01XA	Glycopeptides	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2
J01XB	Polymyxins	2.8	2.1	2.0	2.0	2.1	2.0	1.5	0.8	0.8	0.9
J01XC	Steroid antibacterials (fusidic acid)	0.9	0.8	0.8	0.7	0.6	0.7	0.7	0.7	0.8	0.7
J01XE	Nitrofuran derivatives (nitrofurantoin)	10.4	10.4	11.1	11.3	11.7	12.3	12.5	11.9	12.2	12.6
J01XX05	Methenamine	3.5	3.2	3.2	2.6	2.4	2.3	2.0	1.9	2.0	1.9
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01	Antibacterial agents for systemic use (total)	578.5	604.4	618.0	622.3	633.6	649.3	632.6	663.5	641.2	628.0

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 37.Consumption of antibacterial agents for systemic use in primary health care (No. treated patients/1000 inhabitants/year), Denmark

DANMAP 2009

ATC group a)	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	12.0	11.8	11.5	11.4	11.6	12.0	12.3	12.5	12.7	13.0
J01CA	Penicillins with extended spectrum	65.6	69.4	69.2	68.8	70.6	73.0	75.8	82.1	81.3	81.1
J01CE	Beta-lactamase sensitive penicillins	168.9	173.3	173.4	172.6	171.2	170.2	171.3	177.1	164.4	158.8
J01CF	Beta-lactamase resistant penicillins	15.6	19.2	23.9	26.4	27.1	27.8	29.4	29.7	29.9	29.9
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.6	0.7	1.0	1.1	1.3	1.5	2.3	3.6	5.0	8.0
J01D	Cephalosporins and related substances	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5
J01EA	Trimethoprim and derivatives	4.1	4.2	4.5	4.6	5.0	5.4	5.6	5.9	5.9	5.8
J01EB	Short-acting sulfonamides	33.5	33.2	33.0	33.1	33.3	32.7	33.0	29.7	26.3	25.4
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.8	0.8	0.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0
J01FA	Macrolides	65.7	67.7	66.9	64.1	65.9	70.7	67.0	71.4	66.9	64.5
J01FF	Lincosamides	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.6	0.8	1.0
J01GB	Aminoglycosides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01MA	Fluoroquinolones	7.0	7.5	7.7	8.9	10.8	12.2	13.1	15.2	17.1	16.9
J01XA	Glycopeptides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01XB	Polymyxins	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01XC	Steroid antibacterials (fusidic acid)	0.5	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3
J01XE	Nitrofuran derivatives (nitrofurantoin)	5.8	5.7	6.1	6.2	6.4	6.7	7.0	6.5	6.8	7.0
J01XX05	Methenamine	0.6	0.5	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4
J01XX08	Linezolid	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01 b)	Antibacterial agents for systemic use (total)	292.0	300.6	301.5	301.4	302.6	308.0	310.3	320.4	308.2	303.1

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) Total no. of patients treated with an antibiotic is lower than the sum of all antibiotic classes. This is because the Danish Medicines Agency only counts the first treatment for each patient, each year

Table 38. Consumption of antibacterial agents for systemic use in hospitals (DDD/1000 inhabitant-days), Denmark

DANMAP 2009

ATC group a)	Therapeutic group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
J01AA	Tetracyclines	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03
J01CA	Penicillins with extended spectrum	0.35	0.34	0.33	0.33	0.32	0.35	0.35	0.35	0.35	0.35
J01CE	Beta-lactamase sensitive penicillins	0.30	0.32	0.33	0.34	0.33	0.33	0.29	0.28	0.25	0.23
J01CF	Beta-lactamase resistant penicillins	0.16	0.18	0.18	0.18	0.19	0.18	0.18	0.18	0.17	0.17
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.00	0.01	0.01	0.01	0.02	0.03	0.05	0.08	0.10	0.13
J01DB	First-generation cephalosporins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01DC	Second-generation cephalosporins	0.14	0.15	0.17	0.17	0.19	0.22	0.23	0.31	0.33	0.37
J01DD	Third-generation cephalosporins	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03
J01DF	Monobactams	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01DH	Carbapenems	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.05	0.07	0.07
J01EA	Trimethoprim and derivatives	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01EB	Short-acting sulfonamides	0.04	0.04	0.04	0.03	0.03	0.03	0.02	0.01	0.01	0.01
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.05
J01FA	Macrolides	0.10	0.10	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08
J01FF	Lincosamides	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01GB	Aminoglycosides	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04
J01MA	Fluoroquinolones	0.07	0.08	0.10	0.11	0.13	0.16	0.18	0.21	0.24	0.24
J01XA	Glycopeptides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02
J01XB	Polymyxins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XD	Imidazol derivatives	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.06	0.05
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XX	Other antibacterials	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01
J01	Antibacterial agents for systemic use (total)	1.41	1.45	1.51	1.51	1.56	1.67	1.70	1.81	1.87	1.91

a) From the 2009 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 39. Distribution of MICs and occurrence of resistance in Salmonella *Typhimurium from pigs (n=372), Denmark*

DANMAP 2009

Substance	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	39.2	[34.3-44.4]								51.9	8.9			2.7	36.6						
Chloramphenicol	8.3	[5.7-11.6]								0.3	42.2	47.3	1.9	0.3	1.6	6.5					
Florfenicol	4.3	[2.5-6.9]								1.1	79.6	12.9	2.2	3.8		0.5					
Ampicillin	40.6	[35.6-45.8]							37.1	19.6	2.7	0.3			40.3						
Ceftiofur	0	[0-1.0]						39.5	55.6	4.8											
Cefotaxime	0	[0-1.0]				90.1	9.4	0.5													
Sulfonamide	50.5	[45.3-55.7]													48.9	0.3	0.3				50.5
Trimethoprim	9.4	[6.6-12.8]							90.6						9.4						
Apramycin	2.2	[0.9-4.2]									94.4	3.5			2.2						
Gentamicin	2.2	[0.9-4.2]						91.7	5.4	0.8			1.3	0.8							
Neomycin	4.0	[2.3-6.6]								93.8	2.2			0.3	3.8						
Spectinomycin	12.9	[9.7-16.7]											2.4	71.5	13.2	0.3	2.2	10.5			
Streptomycin	46.0	[40.8-51.2]										44.6	9.4	1.6	2.7	9.9	31.7				
Ciprofloxacin	0.3	[0.01-1.5]	25.3	68.8	5.6		0.3														
Nalidixic acid	0.3	[0.01-1.5]									71.5	26.1	2.2			0.3					
Colistin	0	[0-1.0]							99.5	0.5											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 40. Distribution of MICs and occurrence of resistance in Salmonella *Enteritidis from imported broiler meat (n=30), Denmark*

DANMAP 2009

Substance	% Resistant	95% Confidence interval	Distribution (%) of MICs															
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	0	[0-11.6]								80.0	20.0									
Chloramphenicol	0	[0-11.6]									73.3	26.7								
Florfenicol	0	[0-11.6]								3.3	96.7									
Ampicillin	0	[0-11.6]							23.3	73.3	3.3									
Ceftiofur	0	[0-11.6]						20.0	80.0											
Cefotaxime	0	[0-11.6]				93.3	6.7													
Sulfonamide	0	[0-11.6]													93.3	6.7				
Trimethoprim	0	[0-11.6]							100											
Apramycin	0	[0-11.6]									100									
Gentamicin	0	[0-11.6]						93.3	6.7											
Neomycin	0	[0-11.6]								100										
Spectinomycin	0	[0-11.6]											56.7	43.3						
Streptomycin	6.7	[0.8-22.1]										93.3			6.7					
Ciprofloxacin	46.7	[28.3-65.7]	3.3		50.0		13.3	13.3	20.0											
Nalidixic acid	46.7	[28.3-65.7]									46.7	6.7				46.7				
Colistin	0	[0-11.6]							73.3	26.7										

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 41. Distribution of MICs and occurrence of resistance in Salmonella *Typhimurium from imported turkey meat (n=21) and pork (Danish n=74; imported n=37), Denmark*

DANMAP 2009

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Turkey meat	Imported	90.5	[69.6-98.8]								9.5				19.0	71.4					
	Pork	Danish	43.2	[31.8-55.3]								44.6	12.2			4.1	39.2					
		Imported	54.1	[36.9-70.5]								43.2	2.7			24.3	29.7					
Chloramphenicol	Turkey meat	Imported	19.0	[5.4-41.9]									19.0	61.9				19.0				
	Pork	Danish	13.5	[6.7-23.5]								1.4	37.8	45.9	1.4	1.4	2.7	9.5				
		Imported	24.3	[11.8-41.2]									13.5	62.2				24.3				
Florfenicol	Turkey meat	Imported	19.0	[5.4-41.9]									71.4	9.5		19.0						
	Pork	Danish	5.4	[1.5-13.3]									79.7	10.8	4.1	2.7	1.4	1.4				
		Imported	24.3	[11.8-41.2]									73.0	2.7		21.6	2.7					
Ampicillin	Turkey meat	Imported	76.2	[52.8-91.8]							4.8	19.0					76.2					
	Pork	Danish	32.4	[22.0-44.3]							36.5	31.1					32.4					
		Imported	83.8	[68.0-93.8]							8.1	8.1					83.8					
Ceftiofur	Turkey meat	Imported	0	[0-16.1]						28.6	71.4											
	Pork	Danish	0	[0-4.9]						43.2	56.8											
		Imported	0	[0-9.5]						27.0	73.0											
Cefotaxime	Turkey meat	Imported	0	[0-16.1]				100														
	Pork	Danish	0	[0-4.9]				98.6	1.4													
		Imported	0	[0-9.5]				91.9	8.1													
Sulfonamide	Turkey meat	Imported	81.0	[58.1-94.6]													19.0				81.0	
	Pork	Danish	48.6	[36.9-60.6]													50.0	1.4			48.6	
		Imported	75.7	[58.8-88.2]													24.3				75.7	
Trimethoprim	Turkey meat	Imported	28.6	[11.3-52.2]							71.4						28.6					
	Pork	Danish	8.1	[3.0-16.8]							90.5	1.4					8.1					
		Imported	5.4	[0.7-18.2]							94.6						5.4					
Apramycin	Turkey meat	Imported	0	[0-16.1]									90.5	9.5								
	Pork	Danish	0	[0-4.9]									95.9	2.7	1.4							
		Imported	0	[0-9.5]									89.2	8.1	2.7							
Gentamicin	Turkey meat	Imported	0	[0-16.1]						95.2	4.8											
	Pork	Danish	0	[0-4.9]						91.9	8.1											
		Imported	0	[0-9.5]						97.3	2.7											
Neomycin	Turkey meat	Imported	0	[0-16.1]								100										
	Pork	Danish	12.2	[5.7-21.8]								85.1	2.7				12.2					
		Imported	0	[0-9.5]								97.3	2.7									

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Spectinomycin	Turkey meat	Imported	23.8	[8.2-47.2]												66.7	9.5			23.8			
	Pork	Danish	16.2	[8.7-26.6]											1.4	63.5	18.9		1.4	14.9			
		Imported	27.0	[13.8-44.1]												54.1	18.9			27.0			
Streptomycin	Turkey meat	Imported	57.1	[34.0-78.2]										28.6	14.3		14.3		42.9				
	Pork	Danish	48.6	[36.9-60.6]										45.9	5.4		5.4	12.2	31.1				
		Imported	56.8	[39.5-72.9]										27.0	16.2	10.8	16.2	10.8	18.9				
Ciprofloxacin	Turkey meat	Imported	0	[0-16.1]	9.5	90.5																	
	Pork	Danish	0	[0-4.9]	31.1	66.2	2.7																
		Imported	21.6	[9.8-38.2]	8.1	70.3			2.7	18.9													
Nalidixic acid	Turkey meat	Imported	0	[0-16.1]									61.9	38.1									
	Pork	Danish	0	[0-4.9]									78.4	21.6									
		Imported	21.6	[9.8-38.2]									51.4	27.0				21.6					
Colistin	Turkey meat	Imported	0	[0-16.1]							100												
	Pork	Danish	0	[0-4.9]							98.6	1.4											
		Imported	0	[0-9.5]							97.3	2.7											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration.
MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 42. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from human cases, acquired domestically with (n=173) a) and without (n=55) b) outbreaks included, reported as associated with travel abroad (n=121) or with an unknown origin (n=58), Denmark*

DANMAP 2009

Substance	Origin a) b)	% Resistant [95% Confidence interval]	Distribution (%) of MICs																
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Domestically acquired a)	0 [0-2.1]								100.0										
	Domestically acquired b)	0 [0-6.5]								100.0										
	Travel abroad reported	9.9 [5.2-16.7]								90.1					9.9					
	Unknown origin	1.7 [0.04-9.2]								96.6	1.7				1.7					
Chloramphenicol	Domestically acquired a)	0 [0-2.1]									67.6	32.4								
	Domestically acquired b)	0 [0-6.5]									72.7	27.3								
	Travel abroad reported	0 [0-3.0]									83.5	15.7	0.8							
	Unknown origin	0 [0-6.2]									77.6	20.7	1.7							
Florfenicol	Domestically acquired a)	0 [0-2.1]								0.6	97.7	1.2	0.6							
	Domestically acquired b)	0 [0-6.5]									96.4	3.6								
	Travel abroad reported	0 [0-3.0]								2.5	94.2	1.7	1.7							
	Unknown origin	0 [0-6.2]								0.0	98.3	1.7								
Ampicillin	Domestically acquired a)	0.6 [0.01-3.2]							75.7	23.1	0.6				0.6					
	Domestically acquired b)	1.8 [0.05-9.9]							72.7	23.6	1.8				1.8					
	Travel abroad reported	14.0 [8.4-21.5]							65.3	20.7					14.0					
	Unknown origin	1.7 [0.04-9.2]							69.0	27.6	1.7				1.7					
Ceftiofur	Domestically acquired a)	0 [0-2.1]						82.1	17.9											
	Domestically acquired b)	0 [0-6.5]						83.6	16.4											
	Travel abroad reported	0.8 [0.02-4.5]						75.2	24.0			0.8								
	Unknown origin	0 [0-6.2]						63.8	36.2											
Cefotaxime	Domestically acquired a)	0 [0-2.1]				99.4	0.6													
	Domestically acquired b)	0 [0-6.5]				100.0														
	Travel abroad reported	0.8 [0.02-4.5]				97.5	1.7					0.8								
	Unknown origin	0 [0-6.2]				96.6	3.4													
Sulfonamide	Domestically acquired a)	0 [0-2.1]													98.8	1.2				
	Domestically acquired b)	0 [0-6.5]																		
	Travel abroad reported	2.5 [0.5-7.1]													96.7	0.8				2.5
	Unknown origin	1.7 [0.04-9.2]													96.6	1.7				1.7
Trimethoprim	Domestically acquired a)	0.6 [0.01-3.2]							98.8	0.6					0.6					
	Domestically acquired b)	1.8 [0.05-9.7]							96.4	1.8					1.8					
	Travel abroad reported	2.5 [0.5-7.1]							96.7	0.8					2.5					
	Unknown origin	1.7 [0.04-9.2]							98.3						1.7					
Apramycin	Domestically acquired a)	0 [0-2.1]									99.4	0.6								
	Domestically acquired b)	0 [0-6.5]									98.2	1.8								
	Travel abroad reported	0 [0-3.0]									99.2	0.8								
	Unknown origin	0 [0-6.2]									98.3	1.7								
Gentamicin	Domestically acquired a)	0 [0-2.1]						99.4	0.6											
	Domestically acquired b)	0 [0-6.5]						100.0												

Substance	Origin a) b)	% Resistant [95% Confidence interval]	Distribution (%) of MICs																
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
	Travel abroad reported	0.8 [0-4.5]						99.2					0.8							
	Unknown origin	0 [0-6.2]						100												
Neomycin	Domestically acquired a)	0 [0-2.1]								100										
	Domestically acquired b)	0 [0-6.5]								100.0										
	Travel abroad reported	0 [0-3.0]								100										
	Unknown origin	0 [0-6.2]								100										
Spectinomycin	Domestically acquired a)	0 [0-2.1]											42.8	55.5	1.7					
	Domestically acquired b)	0 [0-6.5]											43.6	50.9	3.0					
	Travel abroad reported	0.8 [0.02-4.5]											66.1	33.1			0.8			
	Unknown origin	0 [0-6.2]											56.9	43.1						
Streptomycin	Domestically acquired a)	0 [0-21]										100								
	Domestically acquired b)	0 [0-6.5]										100.0								
	Travel abroad reported	0.8 [0.02-4.5]										99.2	1.0			0.8				
	Unknown origin	0 [0-6.2]										100.0								
Ciprofloxacin	Domestically acquired a)	3.5 [1,3-7,4]	41.0	55.5			3.5													
	Domestically acquired b)	10.9 [4.1-22.2]	30.9	58.2			10.9													
	Travel abroad reported	18.2 [12,4-27,1]	30.6	48.8	2.5	5.0	12.4	0.8												
	Unknown origin	15.5 [7,3-27,4]	32.8	50.0	1.7	8.6	5.2		1.7											

Vertical solid lines indicate EUCAST epidemiological cut-off values and vertical dotted lines indicate EUCAST clinical breakpoints or if not present, CLSI clinical breakpoints. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.
a) In 2009, 118 of the 173 domestically acquired isolates were part of outbreaks. These isolates were excluded from this category.
b) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'

Table 43. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from human cases, acquired domestically with (n=560) a) and without (n=154) b) outbreaks included, reported as associated with travel abroad (n=58) or with an unknown origin (n=121), Denmark* DANMAP 2009

Substance	Origin a) b)	% Resistant [95% Confidence interval]		Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Domestically acquired a)	9.6	[7.4-12.5]								88.9	1.3	0.2		1.6	8.0					
	Domestically acquired b)	27.3	[20.4-35]								72.7				5.8	21.4					
	Travel abroad reported	37.9	[25.5-51.6]								62.1			1.7	5.2	31.0					
	Unknown origin	35.5	[27-44.8]								64.5				9.1	26.4					
Chloramphenicol	Domestically acquired a)	5.7	[4.0-8.1]								1.3	64.3	28.2	0.5	0.4	2.7	2.7				
	Domestically acquired b)	11.7	[7.1-17.8]								3.9	50.6	33.8			1.9	9.7				
	Travel abroad reported	15.5	[7.3-27.4]								3.4	46.6	34.5		5.2	5.2	5.2				
	Unknown origin	11.6	[6.5-18.7]								0.8	45.5	41.3	0.8		4.1	7.4				
Florfenicol	Domestically acquired a)	2.5	[1.4-4.3]								8.2	83.6	4.3	1.4	0.9	0.7	0.9				
	Domestically acquired b)	9.1	[5.1-14.8]								5.8	79.9	3.9	1.3	3.2	2.6	3.2				
	Travel abroad reported	3.4	[0.4-11.9]								5.2	81.0	5.2	5.2	3.4						
	Unknown origin	9.1	[4.6-15.7]								8.3	72.7	7.4	2.5	6.6	0.8	1.7				
Ampicillin	Domestically acquired a)	10.2	[7.9-13.1]							81.6	7.1	0.9			0.2	10.0					
	Domestically acquired b)	31.8	[24.6-39.8]							58.4	9.7					31.8					
	Travel abroad reported	31.0	[19.5-44.5]							53.4	15.5					31.0					
	Unknown origin	33.1	[24.8-42.2]							52.9	13.2	0.8				33.1					
Ceftiofur	Domestically acquired a)	0.2	[0-1.2]						80.4	18.9	0.5			0.2							
	Domestically acquired b)	0.6	[0.02-3.6]						72.7	26.6				0.6							
	Travel abroad reported	0	[0-6.2]						70.7	29.3											
	Unknown origin	0.8	[0.02-4.6]						75.2	23.1	0.8	0.8									
Cefotaxime	Domestically acquired a)	0.2	[0-1.2]				97.7	2.1					0.2								
	Domestically acquired b)	0.6	[0.02-3.6]				96.8	2.6					0.6								
	Travel abroad reported	0	[0-6.2]				100.0														
	Unknown origin	0.8	[0-4.5]				93.4	4.1	1.7			0.8									
Sulfonamide	Domestically acquired a)	15.5	[12.7-18.9]													83.2	1.3				15.5
	Domestically acquired b)	32.5	[25.2-40.5]													67.5					32.5
	Travel abroad reported	34.5	[22.5-48.1]													65.5					34.5
	Unknown origin	41.3	[32.4-50.6]													56.2	2.5				41.3
Trimethoprim	Domestically acquired a)	6.4	[4.6-8.9]							93.0	0.5	0.2				6.3					
	Domestically acquired b)	7.8	[4.1-13.2]							90.9	1.3	0.6				7.1					
	Travel abroad reported	6.9	[1.9-16.7]							93.1						6.9					
	Unknown origin	6.6	[2.9-12.6]							92.6	0.8				0.8	5.8					
Apramycin	Domestically acquired a)	0	[0-0.7]									98.2	1.6	0.5							
	Domestically acquired b)	0	[0.02-3.6]									98.1	1.3	0.6							
	Travel abroad reported	0	[0-6.2]									98.3	1.7								
	Unknown origin	0	[0-3.0]									96.7	3.3								
Gentamicin	Domestically acquired a)	0.5	[0.1-1.7]						97.9	1.3	0.4				0.5						
	Domestically acquired b)	1.9	[0.4-5.6]						97.4	0.6					1.9						
	Travel abroad reported	1.7	[0-9.2]						98.3						1.7						
	Unknown origin	1.7	[0.2-5.8]						95.0	2.5	0.8				1.7						

Substance	Origin a) b)	% Resistant [95% Confidence interval]	Distribution (%) of MICs																
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Neomycin	Domestically acquired a)	1.3 [0.-2.7]								98.0	0.7			0.2	1.1					
	Domestically acquired b)	4.5 [1.8-9.1]								94.8	0.6			0.6	3.9					
	Travel abroad reported	5.2 [1.1-14.4]								94.8				1.7	3.4					
	Unknown origin	2.5 [0.5-7.1]								97.5					2.5					
Spectinomycin	Domestically acquired a)	6.3 [4.5-8.7]											0.2	89.6	3.9	0.5	0.2	5.5		
	Domestically acquired b)	13.0 [8.1-19.3]											0.6	83.1	3.2	1.3	0.6	11.0		
	Travel abroad reported	17.2 [8.6-29.4]											3.4	75.9	3.4	3.4		13.8		
	Unknown origin	14.9 [9.1-22.5]											1.7	78.5	5.0	0.8		14.0		
Streptomycin	Domestically acquired a)	12.0 [9.5-15]										79.8	8.2	2.3	1.6	1.6	6.4			
	Domestically acquired b)	27.9 [21-35.7]										67.5	4.5	4.5	5.8	4.5	13.0			
	Travel abroad reported	27.6 [16.7-40.9]										67.2	5.2		8.6	10.3	8.6			
	Unknown origin	35.5 [27-44.8]										62.0	2.5	0.8	9.1	7.4	18.2			
Ciprofloxacin	Domestically acquired a)	1.4 [0.7-2.9]	25.7	71.1	1.8	0.4	0.9		0.2											
	Domestically acquired b)	3.9 [1.4-8.3]	32.5	60.4	3.2		3.2		0.6											
	Travel abroad reported	13.8 [6.1-25.4]	34.5	51.7		1.7	6.9	5.2												
	Unknown origin	7.4 [3.5-13.7]	19.8	70.2	2.5	0.8	4.1	0.8	0.8			0.8								
Nalidixic acid	Domestically acquired a)	0.9 [0.3-2.2]									95.5	3.2	0.4			0.9				
	Domestically acquired b)	3.2 [1.1-7.4]									90.9	5.2	0.6			3.2				
	Travel abroad reported	8.6 [2.9-19.0]									82.8	6.9	1.7			8.6				
	Unknown origin	6.6 [2.9-12.6]									86.8	5.8	0.8			6.6				
Colistin	Domestically acquired a)	0.2 [0-1.2]							99.3	0.5		0.2								
	Domestically acquired b)	0.6 [0.02-3.6]							98.1	1.3		0.6								
	Travel abroad reported	0 [0-6.2]																		
	Unknown origin	0 [0-3.0]							98.3	1.7										

Vertical solid lines indicate EUCAST epidemiological cut-off values and vertical dotted lines indicate EUCAST clinical breakpoints or if not present, CLSI clinical breakpoints. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.
a) In 2009, 406 of the 560 domestically acquired isolates were part of outbreaks. These isolates were excluded from this category
b) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection. Isolates from patients which had not reported travel to the GP or was not interviewed by phone was characterized as 'unknown origin'

Table 44. Distribution of MICs and occurrence of resistance in Campylobacter coli *from pigs (n=113), Denmark*

DANMAP 2009

Substance	% Resistant	95% Confidence interval	Distribution (%) of MICs												
			0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	8.8	[4.3-15.7]			46.9	25.7	15.0	3.5		0.9	0.9	7.1			
Chloramphenicol	0	[0-3.2]						14.2	50.4	30.1	5.3				
Erythromycin	12.4	[6.9-19.9]				27.4	22.1	29.2	8.0	0.9			12.4		
Gentamicin	0	[0-3.2]		15.0	44.2	38.1	2.7								
Streptomycin	47.8	[38.3-57.4]					44.2	8.0		1.8	1.8	44.2			
Ciprofloxacin	12.4	[6.9-19.9]	24.8	34.5	24.8	3.5			1.8	10.6					
Nalidixic acid	12.4	[6.9-19.9]						1.8	12.4	46.9	19.5	7.1	0.9	11.5	

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 45. Distribution of MICs and occurrence of resistance in Campylobacter jejuni *from broilers (n=75) and cattle (n=87), Denmark*

DANMAP 2009

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs												
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	Broilers	12.0	[5.6-21.6]			38.7	42.7	5.3	1.3				12.0			
	Cattle	2.3	[0.3-8.1]			81.6	16.1						2.3			
Chloramphenicol	Broilers	0	[0-4.8]						6.7	86.7	5.3	1.3				
	Cattle	0	[0-4.2]						65.5	34.5						
Erythromycin	Broilers	0	[0-4.8]				1.3	14.7	80.0	4.0						
	Cattle	0	[0-4.2]				43.7	41.4	13.8	1.1						
Gentamicin	Broilers	0	[0-4.8]		10.7	72.0	17.3									
	Cattle	0	[0-4.2]		27.6	60.9	11.5									
Streptomycin	Broilers	1.3	[0.03-7.2]					94.7	4.0				1.3			
	Cattle	4.6	[1.3-11.4]					92.0	3.4			1.1	3.4			
Ciprofloxacin	Broilers	13.3	[6.6-23.2]	8.0	48.0	28.0	2.7				13.3					
	Cattle	19.5	[11.8-29.4]	13.8	62.1	3.4	1.1				19.5					
Nalidixic acid	Broilers	13.3	[6.6-23.2]						4.0	49.3	33.3			1.3	12.0	
	Cattle	19.5	[11.8-29.4]						3.4	63.2	12.6	1.1			19.5	

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 46. Distribution of MICs and occurrence of resistance in Campylobacter jejuni *from broiler meat (Danish n=26; imported n=62), Denmark*

DANMAP 2009

Substance	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs											
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	Danish	3.8	[0.1-19.6]			84.6	11.5					3.8			
	Imported	51.6	[38.6-64.5]			38.7	1.6	3.2	4.8		1.6	50.0			
Chloramphenicol	Danish	0	[0-13.2]						76.9	19.2	3.8				
	Imported	0	[0-5.8]						33.9	46.8	9.7	9.7			
Erythromycin	Danish	0	[0-13.2]				38.5	46.2	15.4						
	Imported	0	[0-5.8]				17.7	50.0	32.3						
Gentamicin	Danish	0	[0-13.2]		30.8	61.5	7.7								
	Imported	0	[0-5.8]		35.5	51.6	12.9								
Streptomycin	Danish	0	[0-13.2]					100							
	Imported	0	[0-5.8]					100							
Ciprofloxacin	Danish	0	[0-13.2]	15.4	57.7	26.9									
	Imported	56.5	[43.3-69.0]	8.1	22.6	6.5	6.5			56.5					
Nalidixic acid	Danish	0	[0-13.2]						11.5	46.2	42.3				
	Imported	56.5	[43.3-69.0]						3.2	25.8	12.9	1.6	1.6	54.8	

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 47. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *from human cases categorized as acquired domestically (n=62) or reported as associated with travel abroad (n=31), Denmark*

DANMAP 2009

Substance	Origin	%	95% Confidence interval	Distribution (%) of MICs												
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	Domestically acquired	11.3	[4.7-21.9]				67.7	16.1	4.8					11.3		
	Travel abroad reported	38.7	[21.8-57.8]				45.2	9.7	6.5					38.7		
Chloramphenicol	Domestically acquired	0	[0-5.8]							79.0	17.7	3.2				
	Travel abroad reported	0	[0-11.2]							35.5	58.1	6.5				
Erythromycin	Domestically acquired	0	[0-5.8]					33.9	59.7	6.5						
	Travel abroad reported	0	[0-11.2]					22.6	58.1	12.9	6.5					
Gentamicin	Domestically acquired	0	[0-5.8]			33.9	62.9	3.2	0.5							
	Travel abroad reported	3.2	[0.1-16.7]			22.6	64.5	9.7			3.2					
Streptomycin	Domestically acquired	1.6	[0.04-8.7]						96.8	1.6		1.6				
	Travel abroad reported	6.5	[0.8-21.4]						93.5				3.2	3.2		
Ciprofloxacin	Domestically acquired	24.2	[14.2-36.7]		37.1	33.9	4.8				1.6	22.6				
	Travel abroad reported	61.3	[42.2-78.2]		12.9	19.4	3.2	3.2				61.3				
Nalidixic acid	Domestically acquired	24.2	[14.2-36.7]							9.7	59.7	6.5				24.2
	Travel abroad reported	61.3	[42.2-78.2]								35.5	3.2		3.2		58.1

Vertical solid lines indicate EUCAST epidemiological cut-off values and vertical dotted lines indicate EUCAST clinical breakpoints or if not present, CLSI clinical breakpoints. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

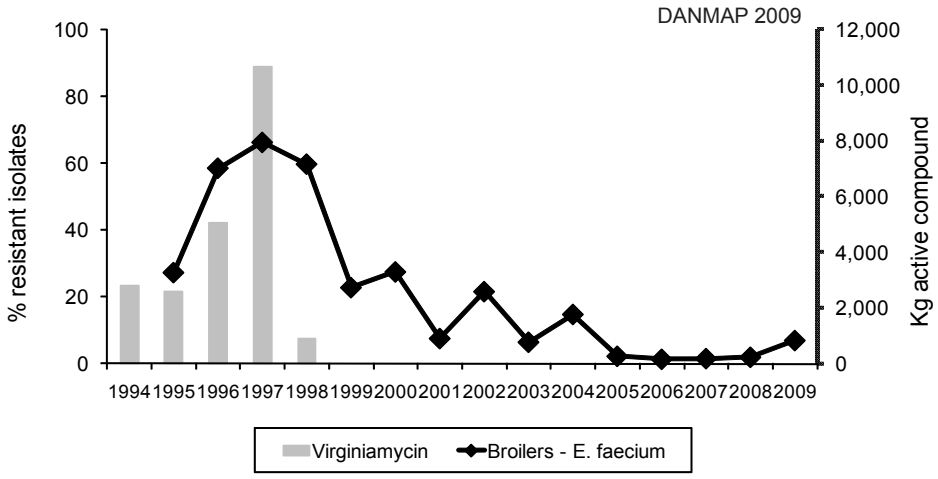

Figure 47. Trend in occurrence of resistance to streptogramins among Enterococcus faecium *from broilers and the consumption of virginiamycin, Denmark, 1994-2009*

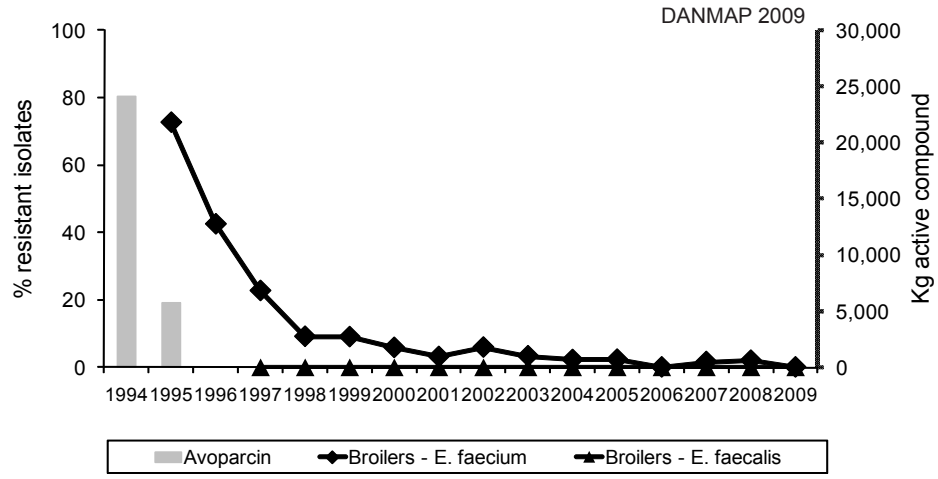

Figure 48. Trend in occurrence of resistance to avoparcin among Enterococcus faecium *and* Enterococcus faecalis *from broilers and the consumption of avoparcin, Denmark, 1994-2009*

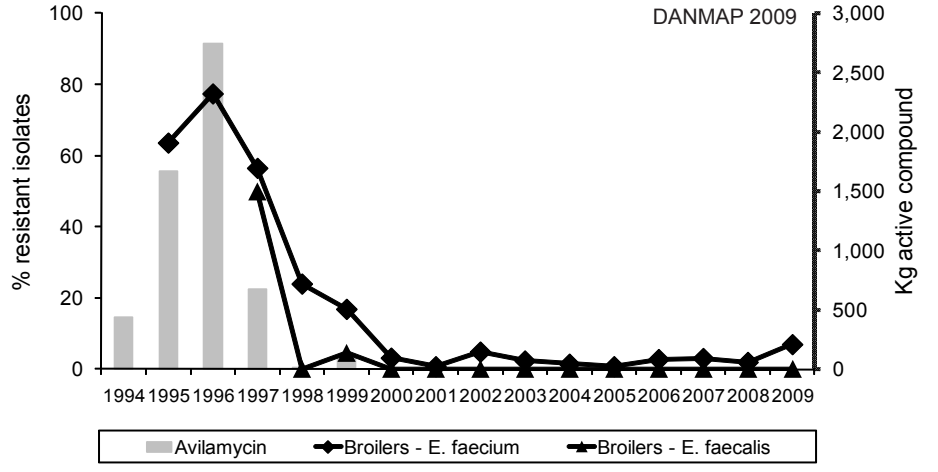

Figure 49. Trend in occurrence of resistance to avilamycin among Enterococcus faecium *and* Enteroccus faecalis *from broilers and the consumption of avilamycin, Denmark 1994-2009*

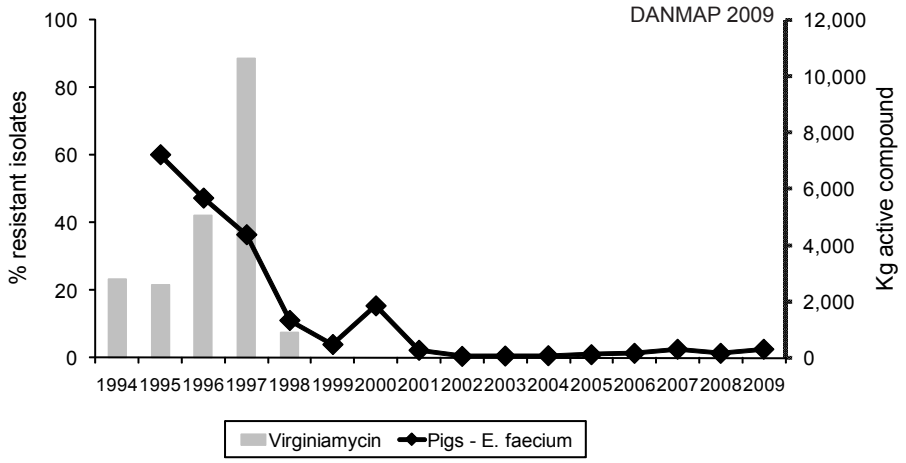

Figure 50. Trend in occurrence of resistance to streptogramins among Enterococcus faecium *from pigs and the comsumption of virginiamycin, Denmark, 1994-2009*

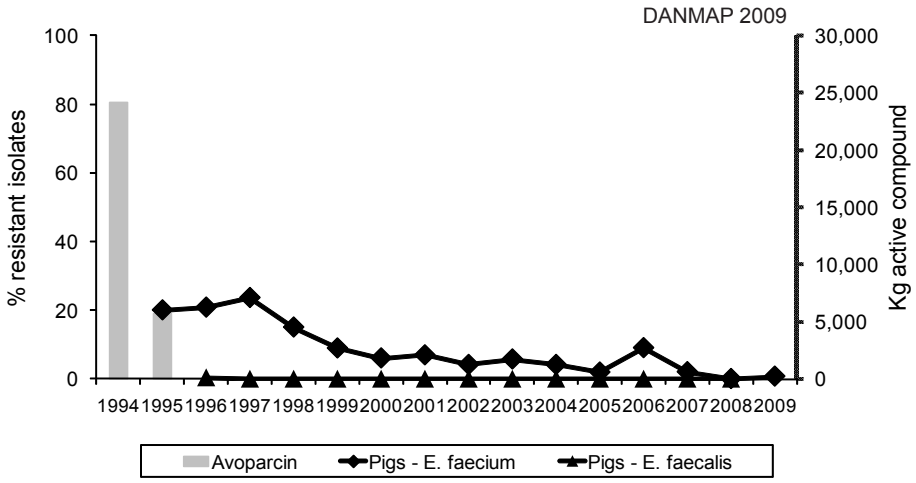

Figure 51. Trend in occurrence of resistance to avoparcin among Enterococcus faecium *and* Enterococcus faecalis *from pigs and the consumption of avoparcin, Denmark, 1994-2009*

Table 48. Distribution of MICs and occurrence of resistance in Enterococcus faecium *from broilers (n=43) and pigs (n=151), Denmark*

DANMAP 2009

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broilers	16.3	[6.8-30.7]							83.7					4.7	11.6							
	Pigs	66.2	[58.1-73.7]							33.1	0.7				3.3	62.9							
Tigecycline	Pigs	0	[0-2.4]		2.0	62.9	33.1	2.0															
Chloramphenicol	Broilers	0	[0-8.2]									37.2	58.1	2.3	2.3								
	Pigs	0	[0-2.4]								2.0	28.5	67.5	1.3	0.7								
Penicillin	Broilers	14.0	[5.3-27.9]								34.9	18.6	20.9	11.6	9.3	4.7							
	Pigs	35.8	[28.1-44.0]								15.2	13.2	7.3	28.5	35.1	0.7							
Ampicillin	Broilers	14.0	[5.3-27.9]								79.1	7.0	11.6			2.3							
	Pigs	29.8	[22.6-37.8]								35.8	34.4	29.1		0.7								
Erythromycin	Broilers	23.3	[11.8-38.6]						30.2	18.6	25.6	2.3				23.3							
	Pigs	35.8	[28.1-44.0]						9.3	2.0	30.5	22.5				35.8							
Gentamicin	Broilers	0	[0-8.2]											95.3	4.7								
	Pigs	0	[0-2.4]											96.7	3.3								
Kanamycin	Broilers	2.3	[0.06-12.3]														32.6	41.9	23.3			2.3	
	Pigs	30.5	[23.2-38.5]														17.9	40.4	9.3	2.0		30.5	
Streptomycin	Broilers	4.7	[0.6-15.8]													95.3						4.7	
	Pigs	48.3	[40.1-56.6]													51.0	0.7	1.3	1.3	7.9	18.5	19.2	
Vancomycin	Broilers	0	[0-8.2]							83.7	16.3												
	Pigs	0.7	[0.02-3.6]							88.7	6.6	4.0				0.7							
Quinupristin/ dalfopristin	Broilers	7.0	[1.5-19.1]						23.3	32.6	25.6	11.6	7.0										
	Pigs	2.6	[0.7-6.6]						8.6	6.6	51.0	31.1	2.6										
Avilamycin	Broilers	7.0	[1.5-19.1]									83.7	7.0	2.3	2.3	4.7							
	Pigs	0	[0-2.4]									98.7	1.3										
Salinomycin	Broilers	62.8	[46.7-77.0]								23.3	14.0	55.8	4.7	2.3								
	Pigs	0	[0-2.4]								99.3	0.7											
Linezolid	Broilers	0	[0-8.2]							7.0	90.7	2.3											
	Pigs	0	[0-2.4]							9.9	88.1	2.0											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 49. Distribution of MICs and occurrence of resistance in Enterococcus faecalis *from broilers (n=19) and pigs (n=133), Denmark*

DANMAP 2009

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broilers	52.6	[28.9-75.6]							47.4					26.3	26.3							
	Pigs	88.0	[81.2-93.0]							12.0					7.5	80.5							
Tigecycline	Pigs	0	[0-2.7]	0.8	0.8	9.0	66.2	23.3															
Chloramphenicol	Broilers	0	[0-17.6]										100										
	Pigs	19.5	[13.2-27.3]									17.3	62.4		0.8	7.5	12.0						
Penicillin	Broilers	0	[0-17.6]								31.6	47.4	15.8	5.3									
	Pigs	0	[0-2.7]								14.3	84.2	1.5										
Ampicillin	Broilers	0	[0-17.6]								100												
	Pigs	0	[0-2.7]								100												
Erythromycin	Broilers	47.4	[24.4-71.1]						10.5	15.8	15.8	10.5	15.8	5.3	5.3	21.1							
	Pigs	48.9	[40.1-57.7]						35.3	12.8	3.0					48.9							
Gentamicin	Broilers	0	[0-17.6]											94.7	5.3								
	Pigs	19.5	[13.2-27.3]											79.7	0.8			3.0	6.8	6.0	3.8		
Kanamycin	Broilers	5.3	[0.1-26.0]														68.4	10.5	15.8			5.3	
	Pigs	30.8	[23.1-39.4]														69.2				0.8	30.1	
Streptomycin	Broilers	21.1	[6.1-45.6]													52.6	15.8	5.3	5.3			21.1	
	Pigs	37.6	[29.3-46.4]													14.3	48.1			0.8	1.5	35.3	
Vancomycin	Broilers	0	[0-17.6]							42.1	57.9												
	Pigs	0	[0-2.7]							22.6	63.2	14.3											
Avilamycin	Broilers	0	[0-17.6]									100											
	Pigs	0	[0-2.7]									100											
Salinomycin	Broilers	10.5	[1.3-33.1]								42.1	47.4	10.5										
	Pigs	0	[0-2.7]								100												
Linezolid	Broilers	0	[0-17.6]							26.3	73.7												
	Pigs	0	[0-2.7]							30.1	69.9												

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 50 Distribution of MICs and occurrence of resistance in Enterococcus faecium *from broiler meat (Danish n=98; imported n=90), imported beef (n=15) and pork (Danish n=17; imported n=22), Denmark*

DANMAP 2009

Substance	Food type	Origin	% resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broiler meat	Danish	14.3	[8.0-22.8]							85.7				1.0	2.0	11.2							
		Imported	52.2	[41.4-62.9]							47.8					2.2	50.0							
	Beef	Imported	6.7	[0.2-31.9]							93.3						6.7							
	Pork	Danish	17.6	[3.8-43.4]							82.4						17.6							
		Imported	13.6	[2.9-34.9]							86.4						13.6							
Tigecycline	Broiler meat	Danish	0	[0-3.7]		1.0	77.6	20.4	1.0															
		Imported	0	[0-4.0]			54.4	42.2	3.3															
	Beef	Imported	0	[0-21.8]		6.7	73.3	13.3	6.7															
	Pork	Danish	0	[0-19.5]		17.6	70.6	11.8																
		Imported	0	[0-15.4]			81.8	13.6	4.5															
Chloramphenicol	Broiler meat	Danish	0	[0-3.7]								1.0	25.5	69.4	3.1	1.0								
		Imported	0	[0-4.0]									16.7	73.3	7.8	2.2								
	Beef	Imported	0	[0-21.8]									26.7	73.3										
	Pork	Danish	0	[0-19.5]									41.2	47.1	5.9	5.9								
		Imported	0	[0-15.4]								4.5	27.3	68.2										
Penicillin	Broiler meat	Danish	2.0	[0.2-7.2]								69.4	18.4	6.1	4.1	1.0	1.0							
		Imported	23.3	[15.1-33.4]								24.4	30.0	7.8	14.4	7.8	15.6							
	Beef	Imported	6.7	[0.2-31.9]								60.0	33.3				6.7							
	Pork	Danish	5.9	[0.1-28.7]								23.5	58.8	5.9	5.9	5.9								
		Imported	9.1	[1.1-29.2]								45.5	36.4	4.5	4.5	4.5	4.5							
Ampicillin	Broiler meat	Danish	1.0	[0.03-5.6]								93.9	5.1	1.0										
		Imported	24.4	[16.0-34.6]								62.2	13.3	8.9	1.1	2.2	12.2							
	Beef	Imported	6.7	[0.2-31.9]								93.3					6.7							
	Pork	Danish	5.9	[0.1-28.7]								88.2	5.9	5.9										
		Imported	9.1	[1.1-29.2]								86.4	4.5	4.5			4.5							
Erythromycin	Broiler meat	Danish	16.3	[9.6-25.2]						14.3	9.2	18.4	41.8	7.1	1.0	2.0	6.1							
		Imported	61.1	[50.3-71.2]						14.4	7.8	8.9	7.8	5.6	3.3	1.1	51.1							
	Beef	Imported	13.3	[1.7-40.5]						20.0		33.3	33.3	6.7			6.7							
	Pork	Danish	35.3	[14.2-61.7]						5.9	23.5	11.8	23.5	17.6	5.9		11.8							
		Imported	31.8	[13.9-54.9]						4.5	4.5	40.9	18.2	22.7			9.1							
Gentamicin	Broiler meat	Danish	0	[0-3.7]											99.0	1.0								
		Imported	0	[0-4.0]											100									
	Beef	Imported	0	[0-21.8]											100									
	Pork	Danish	0	[0-19.5]											100									
		Imported	0	[0-15.4]											95.5	4.5								
Kanamycin	Broiler meat	Danish	0	[0-3.7]														46.9	34.7	14.3	4.1			
		Imported	13.3	[7.1-22.1]														10.0	45.6	23.3	7.8	1.1	12.2	
	Beef	Imported	6.7	[0.2-31.9]														26.7	46.7	13.3	6.7		6.7	
	Pork	Danish	11.8	[1.5-36.4]														41.2	23.5	23.5		5.9	5.9	
		Imported	9.1	[1.1-29.2]														40.9	27.3	22.7			9.1	
Streptomycin	Broiler meat	Danish	1.0	[0.03-5.6]													98.0	1.0			1.0			
		Imported	34.4	[24.7-45.2]													64.4	1.1		2.2	6.7	8.9	16.7	
	Beef	Imported	6.7	[0.2-31.9]													93.3						6.7	
	Pork	Danish	11.8	[1.5-36.4]													88.2				5.9	5.9		
		Imported	9.1	[1.1-29.2]													90.9					4.5	4.5	

Substance	Food type	Origin	% resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Vancomycin	Broiler meat	Danish	0	[0-3.7]							50.0	43.9	6.1											
		Imported	1.1	[0.03-6.0]							75.6	13.3	10.0				1.1							
	Beef	Imported	0	[0-21.8]							73.3	20.0	6.7											
	Pork	Danish	0	[0-19.5]							94.1	5.9												
		Imported	0	[0-15.4]							100													
Quinupristin/ dalfopristin	Broiler meat	Danish	3.1	[0.6-8.7]					1.0	50.0	6.1	34.7	5.1	2.0	1.0									
		Imported	7.8	[3.2-15.4]						23.3	12.2	36.7	20.0	6.7	1.1									
	Beef	Imported	0	[0-21.8]						46.7	13.3	40.0												
	Pork	Danish	5.9	[0.1-28.7]						29.4		58.8	5.9	5.9										
		Imported	4.5	[0.1-22.8]						22.7	4.5	63.6	4.5	4.5										
Avilamycin	Broiler meat	Danish	1.0	[0.03-5.6]									95.9	1.0	2.0	1.0								
		Imported	3.3	[0.7-9.4]									93.3	2.2	1.1	2.2	1.1							
	Beef	Imported	0	[0-21.8]									93.3		6.7									
	Pork	Danish	0	[0-19.5]									94.1	5.9										
		Imported	4.5	[0.1-22.8]									95.5				4.5							
Salinomycin	Broiler meat	Danish	37.8	[28.2-48.1]								14.3	48.0	37.8										
		Imported	14.4	[7.9-23.4]								26.7	58.9	14.4										
	Beef	Imported	0	[0-21.8]								100												
	Pork	Danish	0	[0-19.5]								100												
		Imported	4.5	[0.1-22.8]								95.5		4.5										
Linezolid	Broiler meat	Danish	0	[0-3.7]							2.0	93.9	4.1											
		Imported	0	[0-4.0]							12.2	84.4	3.3											
	Beef	Imported	0	[0-21.8]								100												
	Pork	Danish	0	[0-19.5]								100												
		Imported	0	[0-15.4]							9.1	86.4	4.5											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 51. Distribution of MICs and occurrence of resistance in Enterococcus faecalis *from broiler meat (Danish n=39; imported n=88), beef (Danish n=28; imported n=33) and pork (Danish n=96; imported n=109), Denmark*

DANMAP 2009

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broiler meat	Danish	25.6	[13.0-42.1]							69.2	5.1				7.7	17.9							
		Imported	58.0	[47.0-68.4]							40.9	1.1				20.5	37.5							
	Beef	Danish	17.9	[6.1-36.9]							82.1						17.9							
		Imported	15.2	[5.1-31.9]							81.8	3.0					15.2							
	Pork	Danish	19.8	[12.4-29.2]							79.2	1.0				1.0	18.8							
		Imported	48.6	[38.9-58.4]							50.5	0.9				5.5	43.1							
Tigecycline	Broiler meat	Danish	0	[0-9.0]			17.9	74.4	7.7															
		Imported	0	[0-4.1]		1.1	26.1	59.1	13.6															
	Beef	Danish	0	[0-12.3]		3.6	42.9	53.6																
		Imported	0	[0-10.6]			36.4	60.6	3.0															
	Pork	Danish	0	[0-3.8]	1.0	1.0	29.2	65.6	3.1															
		Imported	0	[0-3.3]		3.7	26.6	58.7	11.0															
Chloramphenicol	Broiler meat	Danish	2.6	[0.06-13.5]									15.4	82.1			2.6							
		Imported	9.1	[4.0-17.1]									15.9	73.9	1.1		9.1							
	Beef	Danish	3.6	[0.09-18.3]									32.1	64.3			3.6							
		Imported	0	[0-10.6]									27.3	72.7										
	Pork	Danish	5.2	[1.7-11.7]									11.5	83.3			5.2							
		Imported	0.9	[0.02-5.0]									33.9	65.1				0.9						
Penicillin	Broiler meat	Danish	0	[0-9.0]								25.6	74.4											
		Imported	0	[0-4.1]								30.7	68.2	1.1										
	Beef	Danish	0	[0-12.3]								39.3	60.7											
		Imported	0	[0-10.6]								45.5	54.5											
	Pork	Danish	0	[0-3.8]								28.1	70.8	1.0										
		Imported	0	[0-3.3]								52.3	45.9	1.8										
Ampicillin	Broiler meat	Danish	0	[0-9.0]								97.4	2.6											
		Imported	0	[0-4.1]								98.9	1.1											
	Beef	Danish	0	[0-12.3]								92.9	7.1											
		Imported	0	[0-10.6]								100												
	Pork	Danish	0	[0-3.8]								99.0	1.0											
		Imported	0	[0-3.3]								95.4	4.6											
Erythromycin	Broiler meat	Danish	25.6	[13.0-42.1]						41.0	20.5	12.8			2.6		23.1							
		Imported	50.0	[39.1-60.9]						34.1	11.4	4.5		1.1	5.7	1.1	42.0							
	Beef	Danish	7.1	[0.9-23.5]						57.1	28.6	7.1					7.1							
		Imported	3.0	[0.08-15.8]						36.4	45.5	15.2					3.0							
	Pork	Danish	12.5	[6.6-20.8]						40.6	27.1	19.8			2.1		10.4							
		Imported	8.3	[3.8-15.1]						45.0	39.4	6.4	0.9				8.3							
Gentamicin	Broiler meat	Danish	0	[0-9.0]											100									
		Imported	3.4	[0.7-9.6]											95.5	1.1					1.1	2.3		
	Beef	Danish	0	[0-12.3]											100									
		Imported	0	[0-10.6]											97.0	3.0								
	Pork	Danish	3.1	[0.6-8.9]											95.8	1.0					1.0	2.1		
		Imported	1.8	[0.2-6.5]											98.2						0.9	0.9		

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Kanamycin	Broiler meat	Danish	2.6	[0.06-13.5]														97.4					2.6	
		Imported	20.5	[12.6-30.4]														78.4		1.1			20.5	
	Beef	Danish	7.1	[0.9-23.5]														92.9					7.1	
		Imported	0	[0-10.6]														100						
	Pork	Danish	4.2	[1.1-10.3]														94.8	1.0				4.2	
		Imported	5.5	[2.0-11.6]														93.6	0.9				5.5	
Streptomycin	Broiler meat	Danish	12.8	[4.3-27.4]													15.4	71.8					12.8	
		Imported	28.4	[19.3-39.0]													17.0	54.5				1.1	27.3	
	Beef	Danish	7.1	[0.9-23.5]													42.9	50.0					7.1	
		Imported	3.0	[0.08-15.8]													45.5	45.5	6.1				3.0	
	Pork	Danish	4.2	[1.1-10.3]													49.0	45.8	1.0				4.2	
		Imported	3.7	[1.0-9.1]													59.6	35.8	0.9			0.9	2.8	
Vancomycin	Broiler meat	Danish	0	[0-9.0]							28.2	61.5	10.3											
		Imported	0	[0-4.1]							21.6	60.2	18.2											
	Beef	Danish	0	[0-12.3]							32.1	67.9												
		Imported	0	[0-10.6]							24.2	75.8												
	Pork	Danish	0	[0-3.8]							40.6	54.2	5.2											
		Imported	0	[0-3.3]							31.2	62.4	6.4											
Avilamycin	Broiler meat	Danish	0	[0-9.0]									100											
		Imported	0	[0-4.1]									97.7	2.3										
	Beef	Danish	0	[0-12.3]									100											
		Imported	0	[0-10.6]									100											
	Pork	Danish	0	[0-3.8]									100											
		Imported	0	[0-3.3]									100											
Salinomycin	Broiler meat	Danish	0	[0-9.0]								82.1	17.9											
		Imported	0	[0-4.1]								92.0	8.0											
	Beef	Danish	0	[0-12.3]								100												
		Imported	0	[0-10.6]								100												
	Pork	Danish	0	[0-3.8]								100												
		Imported	0	[0-3.3]								100												
Linezolid	Broiler meat	Danish	0	[0-9.0]							17.9	79.5	2.6											
		Imported	0	[0-4.1]							27.3	70.5	2.3											
	Beef	Danish	0	[0-12.3]							14.3	82.1	3.6											
		Imported	0	[0-10.6]							18.2	81.8												
	Pork	Danish	0	[0-3.8]							3.1	96.9												
		Imported	0	[0-3.3]							17.4	81.7	0.9											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 52. Distribution of MICs and occurrence of resistance in Escherichia coli *from broilers (n=152), cattle (n=94) and pigs (n=150), Denmark*

DANMAP 2009

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	12.5	[7.7-18.8]								87.5			0.7	2.6	9.2						
	Cattle	2.1	[0.3-7.5]								83.0	14.9			1.1	1.1						
	Pigs	35.3	[27.7-43.5]								62.7	2.0		1.3	1.3	32.7						
Chloramphenicol	Broilers	0.7	[0.02-3.6]								9.9	63.8	25.7				0.7					
	Cattle	1.1	[0.03-5.8]								2.1	40.4	56.4				1.1					
	Pigs	4.7	[1.9-9.4]								2.0	52.7	40.0	0.7	2.0	0.7	2.0					
Florfenicol	Broilers	0.7	[0.02-3.6]								11.8	65.8	21.7				0.7					
	Cattle	1.1	[0.03-5.8]								2.1	43.6	50.0	3.2			1.1					
	Pigs	0.7	[0.02-3.7]								2.0	53.3	41.3	2.7			0.7					
Ampicillin	Broilers	18.4	[12.6-25.5]							11.2	42.1	27.6	0.7		0.7	17.8						
	Cattle	2.1	[0.3-7.5]							2.1	25.5	61.7	8.5			2.1						
	Pigs	26.0	[19.2-33.8]							6.0	24.0	38.0	6.0			26.0						
Ceftiofur	Broilers	1.3	[0.2-4.7]						96.1	2.6	1.3											
	Cattle	0	[0-3.8]						100													
	Pigs	0	[0-2.4]						99.3	0.7												
Cefotaxime	Broilers	0	[0-2.4]				96.7	3.3														
	Cattle	0	[0-3.8]				98.9	1.1														
	Pigs	0	[0-2.4]				99.3	0.7														
Sulfonamide	Broilers	13.8	[8.8-20.3]													85.5		0.7			13.8	
	Cattle	3.2	[0.7-9.0]													96.8					3.2	
	Pigs	33.3	[25.9-41.5]													66.7					33.3	
Trimethoprim	Broilers	5.9	[2.7-10.9]							94.1		0.7	0.7			4.6						
	Cattle	0	[0-3.8]							100												
	Pigs	18.7	[12.8-25.8]							81.3						18.7						
Apramycin	Broilers	0	[0-2.4]									68.4	31.6									
	Cattle	0	[0-3.8]									92.6	5.3	2.1								
	Pigs	0	[0-2.4]									80.7	17.3	2.0								
Gentamicin	Broilers	0	[0-2.4]						71.7	27.6	0.7											
	Cattle	0	[0-3.8]						88.3	8.5	3.2											
	Pigs	0	[0-2.4]						84.0	14.7	1.3											

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Neomycin	Broilers	0	[0-2.4]								99.3	0.7										
	Cattle	0	[0-3.8]								95.7	3.2	1.1									
	Pigs	6.0	[2.8-11.1]								89.3	4.0	0.7	0.7	2.7	2.7						
Spectinomycin	Broilers	2.6	[0.7-6.6]											75.0	21.1	1.3	1.3	1.3				
	Cattle	0	[0-3.8]											93.6	5.3	1.1						
	Pigs	24.7	[18.0-32.4]											59.3	11.3	4.7	4.7	12.7	7.3			
Streptomycin	Broilers	9.2	[5.1-15.0]										84.9	5.9	2.0	3.3	2.0	2.0				
	Cattle	3.2	[0.7-9.0]										94.7	2.1	1.1			2.1				
	Pigs	42.7	[34.6-51.0]										52.7	4.7	5.3	16.0	9.3	12.0				
Ciprofloxacin	Broilers	11.8	[7.2-18.1]	77.0	11.2		3.9		7.2	0.7												
	Cattle	0	[0-3.8]	71.3	28.7																	
	Pigs	0.7	[0.02-3.7]	76.0	23.3					0.7												
Nalidixic acid	Broilers	10.5	[6.1-16.5]									88.8	0.7		1.3	3.9	5.3					
	Cattle	0	[0-3.8]									95.7	4.3									
	Pigs	0.7	[0.02-3.7]									97.3	1.3	0.7			0.7					
Colistin	Broilers	0	[0-2.4]							98.0	2.0											
	Cattle	0	[0-3.8]							100												
	Pigs	0	[0-2.4]							100												

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 53. Distribution of MICs and occurrence of resistance in Escherichia coli *from broiler meat (Danish n=143; imported n=221), beef (Danish n=32; imported n=39) and pork (Danish n=106; imported n=65), Denmark* DANMAP 2009

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broiler meat	Danish	11.2	[6.5-17.5]								79.7	9.1			0.7	10.5					
		Imported	54.8	[47.9-61.4]								41.2	4.1			1.8	52.9					
	Beef	Danish	3.1	[0.08-16.2]								81.3	15.6				3.1					
		Imported	5.1	[0.6-17.3]								84.6	10.3				5.1					
	Pork	Danish	32.1	[23.3-41.8]								63.2	4.7			1.9	30.2					
		Imported	47.7	[35.1-60.5]								50.8	1.5		1.5	1.5	44.6					
Chloramphenicol	Broiler meat	Danish	0.7	[0.02-3.8]								2.1	40.6	56.6				0.7				
		Imported	20.8	[15.7-26.8]								0.9	26.7	49.8	1.8	7.2	4.1	9.5				
	Beef	Danish	0	[0-10.9]									18.8	81.3								
		Imported	0	[0-9.0]									15.4	84.6								
	Pork	Danish	0.9	[0.02-5.1]								6.6	34.0	58.5		0.9						
		Imported	7.7	[2.5-17.0]								3.1	40.0	49.2		3.1	1.5	3.1				
Florfenicol	Broiler meat	Danish	0	[0-2.5]								1.4	43.4	55.2								
		Imported	0.5	[0.01-2.5]								1.8	35.7	51.6	10.4			0.5				
	Beef	Danish	0	[0-10.9]									21.9	75.0	3.1							
		Imported	0	[0-9.0]									15.4	82.1	2.6							
	Pork	Danish	0	[0-3.4]								6.6	37.7	54.7	0.9							
		Imported	1.5	[0.04-8.3]								3.1	50.8	43.1	1.5	1.5						
Ampicillin	Broiler meat	Danish	19.6	[13.4-27.0]							4.9	33.6	39.9	2.1			19.6					
		Imported	54.8	[47.9-61.4]								14.0	28.5	2.7			54.8					
	Beef	Danish	3.1	[0.08-16.2]								18.8	78.1		3.1							
		Imported	5.1	[0.6-17.3]							2.6	15.4	71.8	5.1			5.1					
	Pork	Danish	29.2	[20.8-38.9]							2.8	23.6	42.5	1.9		0.9	28.3					
		Imported	27.7	[17.3-40.2]								38.5	29.2	4.6		3.1	24.6					
Ceftiofur	Broiler meat	Danish	0	[0-2.5]						98.6	1.4											
		Imported	3.6	[1.6-7.0]						94.6	1.8		0.5	1.8	1.4							
	Beef	Danish	0	[0-10.9]						100												
		Imported	0	[0-9.0]						97.4	2.6											
	Pork	Danish	0.9	[0.02-5.1]						99.1					0.9							
		Imported	0	[0-5.5]						98.5	1.5											
Cefotaxime	Broiler meat	Danish	0	[0-2.5]				97.9	2.1													
		Imported	4.1	[1.9-7.6]				93.7	2.3			0.5	0.5	3.2								
	Beef	Danish	0	[0-10.9]				96.9	3.1													
		Imported	0	[0-9.0]				100														
	Pork	Danish	0.9	[0.02-5.1]				97.2	1.9					0.9								
		Imported	0	[0-5.5]				98.5	1.5													
Sulfonamide	Broiler meat	Danish	7.7	[3.9-13.3]													92.3				7.7	
		Imported	53.8	[47.0-60.6]													46.2				53.8	
	Beef	Danish	3.1	[0.08-16.2]													96.9				3.1	
		Imported	5.1	[0.6-17.3]													94.9				5.1	
	Pork	Danish	37.7	[28.5-47.7]													62.3				37.7	
		Imported	33.8	[22.6-46.6]													66.2				33.8	
Trimethoprim	Broiler meat	Danish	3.5	[1.1-8.0]							96.5						3.5					
		Imported	38.0	[31.6-44.8]							61.1	0.9					38.0					
	Beef	Danish	0	[0-10.9]							100											
		Imported	5.1	[0.6-17.3]							94.9						5.1					
	Pork	Danish	25.5	[17.5-34.9]							73.6	0.9					25.5					
		Imported	30.8	[19.9-43.4]							69.2						30.8					

Substance	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Apramycin	Broiler meat	Danish	1.4	[0.2-5.0]									74.1	23.1	1.4		1.4						
		Imported	1.8	[0.5-4.6]									70.1	24.9	3.2		1.8						
	Beef	Danish	0	[0-10.9]									53.1	43.8	3.1								
		Imported	0	[0-9.0]									79.5	20.5									
	Pork	Danish	0	[0-3.4]									83.0	17.0									
		Imported	0	[0-5.5]									80.0	20.0									
Gentamicin	Broiler meat	Danish	2.1	[0.4-6.0]						75.5	18.9	3.5		0.7	0.7	0.7							
		Imported	4.5	[2.2-8.2]						64.7	26.7	4.1		1.4	0.5	2.7							
	Beef	Danish	0	[0-10.9]						71.9	28.1												
		Imported	0	[0-9.0]						71.8	25.6	2.6											
	Pork	Danish	0	[0-3.4]						78.3	18.9	2.8											
		Imported	0	[0-5.5]						76.9	21.5	1.5											
Neomycin	Broiler meat	Danish	1.4	[0.2-5.0]								94.4	4.2			0.7	0.7						
		Imported	14.0	[9.7-19.3]								81.9	3.6	0.5		4.1	10.0						
	Beef	Danish	0	[0-10.9]								100											
		Imported	5.1	[0.6-17.3]								84.6	10.3			5.1							
	Pork	Danish	5.7	[2.1-11.9]								89.6	2.8	1.9		1.9	3.8						
		Imported	0	[0-5.5]								90.8	9.2										
Spectinomycin	Broiler meat	Danish	2.1	[0.4-6.0]											83.2	14.0	0.7	1.4	0.7				
		Imported	28.1	[22.2-34.5]											47.5	18.1	6.3	3.6	10.9	13.6			
	Beef	Danish	0	[0-10.9]											90.6	9.4							
		Imported	0	[0-9.0]											84.6	12.8	2.6						
	Pork	Danish	16.0	[9.6-24.4]											70.8	9.4	3.8	2.8	9.4	3.8			
		Imported	18.5	[9.9-30.0]											58.5	18.5	4.6	4.6	4.6	9.2			
Streptomycin	Broiler meat	Danish	10.5	[6.0-16.7]										84.6	4.9	2.1	1.4	4.2	2.8				
		Imported	45.2	[38.6-52.1]										43.4	11.3	5.4	8.6	8.6	22.6				
	Beef	Danish	3.1	[0.08-16.2]										93.8	3.1		3.1						
		Imported	5.1	[0.6-17.3]										87.2	7.7				5.1				
	Pork	Danish	42.5	[32.9-52.4]										51.9	5.7	3.8	4.7	16.0	17.9				
		Imported	35.4	[23.9-48.2]										56.9	7.7	3.1	9.2	12.3	10.8				
Ciprofloxacin	Broiler meat	Danish	4.2	[1.6-8.9]	54.5	41.3			3.5		0.7												
		Imported	41.2	[34.6-48.0]	27.6	31.2	0.5	4.1	13.6	10.9	2.3	1.4	0.9	7.7									
	Beef	Danish	0	[0-10.9]	50.0	50.0																	
		Imported	0	[0-9.0]	51.3	48.7																	
	Pork	Danish	0.9	[0.02-5.1]	55.7	43.4			0.9														
		Imported	6.2	[1.7-15.0]	70.8	23.1	1.5		1.5	1.5				1.5									
Nalidixic acid	Broiler meat	Danish	4.2	[1.6-8.9]									93.7	2.1				4.2					
		Imported	40.3	[33.7-47.1]									58.4	0.9	0.5	0.9	3.6	35.7					
	Beef	Danish	0	[0-10.9]									96.9	3.1									
		Imported	0	[0-9.0]									100										
	Pork	Danish	0.9	[0.02-5.1]									98.1	0.9				0.9					
		Imported	1.5	[0.04-8.3]									92.3	4.6	1.5			1.5					
Colistin	Broiler meat	Danish	0	[0-2.5]							99.3	0.7											
		Imported	3.6	[1.6-7.0]							94.6	1.8	3.6										
	Beef	Danish	0	[0-10.9]							100												
		Imported	0	[0-9.0]							100												
	Pork	Danish	0	[0-3.4]							100												
		Imported	0	[0-5.5]							100												

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table 54. Distribution of MICs and occurrence of resistance in Escherichia coli *from cattle (n=48) and pigs (n=48), Denmark*

DANMAP 2009

Substance	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Cattle	83.3	[69.8-92.5]								16.7					83.3						
	Pigs	66.7	[51.6-79.6]								33.3				6.3	60.4						
Chloramphenicol	Cattle	16.7	[7.5-30.2]									10.4	70.8	2.1		2.1	14.6					
	Pigs	14.6	[6.1-27.8]									72.9	12.5		8.3	4.2	2.1					
Florfenicol	Cattle	2.1	[0.05-11.1]									18.8	72.9	6.3			2.1					
	Pigs	0	[0-7.4]								2.1	72.9	20.8	4.2								
Ampicillin	Cattle	91.7	[80.0-97.7]								4.2	4.2				91.7						
	Pigs	35.4	[22.2-50.5]							6.3	50.0	8.3				35.4						
Ceftiofur	Cattle	0	[0-7.4]						91.7	8.3												
	Pigs	0	[0-7.4]						100													
Cefotaxime	Cattle	2.1	[0.05-11.1]				95.8	2.1	2.1													
	Pigs	0	[0-7.4]				100															
Sulfonamide	Cattle	52.1	[37.2-66.7]													47.9					52.1	
	Pigs	66.7	[51.6-79.6]													33.3				2.1	64.6	
Trimethoprim	Cattle	33.3	[20.4-48.4]							66.7						33.3						
	Pigs	45.8	[31.4-60.8]							54.2						45.8						
Apramycin	Cattle	0	[0-7.4]									75.0	22.9	2.1								
	Pigs	6.2	[1.3-17.2]									83.3	10.4			6.3						
Gentamicin	Cattle	0	[0-7.4]						66.7	33.3												
	Pigs	6.2	[1.3-17.2]						77.1	16.7					6.3							
Neomycin	Cattle	12.5	[4.7-25.2]								75.0	10.4	2.1			12.5						
	Pigs	29.2	[17.0-44.1]								66.7	2.1	2.1	2.1		27.1						
Spectinomycin	Cattle	16.7	[7.5-30.2]											50.0	29.2	4.2	2.1	10.4	4.2			
	Pigs	56.2	[41.2-70.5]											29.2	4.2	10.4	8.3	2.1	45.8			
Streptomycin	Cattle	52.1	[37.2-66.7]										43.8	4.2	10.4	16.7	16.7	8.3				
	Pigs	75.0	[60.4-86.4]										20.8	4.2	12.5	12.5	14.6	35.4				
Ciprofloxacin	Cattle	31.2	[18.7-46.3]	54.2	14.6			25.0	4.2				2.1									
	Pigs	6.2	[1.3-17.2]	79.2	14.6			4.2	2.1													
Nalidixic acid	Cattle	31.2	[18.7-46.3]									68.8					31.3					
	Pigs	6.2	[1.3-17.2]									91.7	2.1				6.3					
Colistin	Cattle	0	[0-7.4]							100												
	Pigs	0	[0-7.4]							97.9	2.1											

Vertical solid lines indicate EUCAST epidemiological cut-off values. Exceptions and further details can be found in appendix 2.
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Materials and Methods

Demographics

Hospitals in Denmark

The reported number of hospitals in each Region of Denmark corresponds to the number of administratively distinct public hospitals, which do not specialise in psychiatric care (somatic hospitals) and report data to the Danish Medicines Agency and the National Board of Health. This number is lower than the actual number of geographically distinct hospitals in Denmark due to grouping of the hospitals under the same administration and name.

Certain categories of hospitals were excluded. This year, data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

Data on consumption of antimicrobials

Consumption of antimicrobial agents in animals

Consumption data presented in this report were obtained from the national monitoring program, VetStat since 2001. Prior to 2001, data were based on overall sales figures from the pharmaceutical industry (see Table 5).
In Denmark, all therapeutic drugs are prescription-only and Vet Stat collects data on all medicines prescribed by veterinarians for use in animals. In addition, data on consumption of coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters are collected by VetStat. Data on coccidiostatics were reported until 2004, but due to problems in data transfer, data were not reported in 2005 and 2006. Data on coccidiostatics for 2007–2009 will be presented in later reports after validation of data.

Until 2007, antimicrobial agents could only be purchased at the pharmacy or in medicated feed from the feed mills. The pharmacy either sells the medicines to vetenarians for use in practice or for re-sale to farmers, or sells directly to the animal holder on presentation of a prescription. By law, the profit that veterinarians can make on the sale of medicines is very limited and there is no economic encouragement for the veterinarian to sell drugs.

From April 2nd 2007, the monopoly of the pharmacy was suspended and private companies (two in 2009) can now on certain conditions (identical to the pharmacies) sell veterinary specialities for production animals on prescription. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration cost related to sale of large quantities to the veterinarians. In 2009, the animal owners and veterinarians purchased the antimicrobial agents equally from the pharmacies (49%), and the veterinary drug trading companies (49%), while only 2.4% was purchased from the feed mills. The veterinarians purchase the veterinary medicines at the pharmacies, and in 2009, they used or distributed 11 % of the antimicrobial agents.

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 the use of all prescription medicines in production animals on a monthly basis. For most veterinarians, the registration of data is linked to the writing of invoices. The amount of drugs reported by the veterinary practitioners is validated against pharmacy data on the total sales of therapeutic drugs for use in practice. The electronic registration of the sales at the pharmacies is linked to the billing process, ensuring a high data quality regarding amounts an 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 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 medicine, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in Defined Animal Daily Doses (ADD). The ADD system is a national veterinary equivalent to the international Defined Daily Doses (DDD) system applied in the human field (www.whocc.no). See further on the ADD system in Textbox 1.

The consumption is compared with production in kg meat or number of animals produced. Due to an increasing number of pigs exported around 30 kg, involving 24% of pigs produced in 2009, 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)= $(ADD_s *(N_f/N_w)+ ADD_w *(N_f/N_w) + ADD_f) / N_f$,

where ADD_s = Amounts of antimicrobial used in sow herds, measured in ADD_{kg} ; ADD_w = Amounts of antimicrobial used in weaning pigs herds, measured in ADD_{kg} ; ADD_f = Amounts of antimicrobial used in finisher pigs, measured in ADD_{kg} ; $N_{w=}$ Number of pigs produced to 30 kg bodyweight, including pigs exported at 15-50 kg (mostly at 30 kg); $N_{f=}$ Number of pigs produced to slaughter, whether exported domestically or exported.

Antimicrobial agents used in humans and animals are presented in Table 4.

Consumption of antimicrobial agents in humans

Consumption data presented in this report were obtained from the Danish Medicines Agency (DMA) (http://www.laegemiddelstyrelsen.dk). The DMA has the legal responsibility for monitoring the consumption of all human medicinal products. This is carried out by monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies, to the DMA. Data from the primary health care sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

In Denmark, all antimicrobial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDD), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and place (pharmacy, hospital pharmacy, institution) of the transaction, and information regarding reimbursement of cost, if applicable. Information on the indication for the prescription is not yet available. The data are transferred monthly to the DMA in an electronic format.

The present report includes data on the consumption of antibacterial agents for systemic use, or group J01 of the 2009 update of ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as a number of DDDs per 1000 inhabitants and per day (DDD/1000 inhabitant-days). Consumption in primary health care is also reported as a number of packages per 1000 inhabitants. Consumption of antibacterial agents in hospitals is expressed as a number of DDDs per 1000 inhabitants and per day (DDD/1000 inhabitant-days) to compare with primary health care and as a number of DDDs per 100 occupied bed-days and per day (DDD/100 occupied bed-days). Since antimicrobial consumption expressed as DDD/100 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DDD/100 discharged patients. Data on the number of occupied bed-days (or patient-days) and number of discharges in each hospital were obtained from the National Board of Health (http://www.sundhedsdata.dk).

Collection of bacterial isolates

Animals

Animal isolates included in this DANMAP report are *Escherischia coli, Enterococcus faecium, Enterococcus faecalis, Campylobacter coli* and *Campylobacter jejuni* collected from healthy production animals at slaughter; *E. coli* O149 and *E. coli* F5 (K99) collected from diagnostic submissions; and finally, *Salmonella* Typhimurium isolates collected from sub-clinical infections as well as from cases of clinical salmonellosis:

***Campylobacter*, indicator *E. coli* and Enterococci.**
Samples from healthy pigs, cattle and broilers are collected at slaughter for the DANMAP programme by meat inspection staff or company personnel and sent for examination to the National Food Institute, DTU or the National Veterinary Institute, DTU (for broilers). The slaughter plants included in the DANMAP programme account for 88-95% of the total number of animals slaughtered in Denmark per year. The number of samples taken at the slaughter plant is proportional to the number of animals slaughtered at each plant per year. Each sample represents one herd or flock.

For broilers, samples are collected weekly throughout the year (approximately 400 samples per year) representing all broiler houses in Denmark. For cattle and pigs, samples are collected once a month from January through November (approximately 80 and 30 samples per month from pigs and cattle, respectively). Accordingly, the bacterial isolates 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 populations. An overview of the number of samples analysed, the number of isolates obtained and the number of MIC-determinations performed for pigs, cattle and broilers is presented in Table 55. Only one isolate per herd (of each bacterial species) is finally included in the DANMAP report. Samples from cattle are not analyzed for enterococci due to increasingly low findings in previous years. For *Campylobacter*, the isolation rate of *C. coli* from cattle and broilers and of *C. jejuni* from pigs is low, and MIC-determinations are not performed because of the low number of isolates.

Isolates from diagnostic submissions. Isolates from diagnostic submissions are collected for the DANMAP programme at both the National Food Institute, DTU and at the Laboratory of Swine Diseases, Danish Meat Association, Kjellerup. Both *E. coli* O149 from diarrhoeic pigs and *E. coli* F5 (K99) from diarrhoeic cattle are included, with no more than one isolate representing each herd. *Staphylococcus hyicus* isolates from skin infections in pigs were also collected, but not reported due to a low number of isolates in 2009.

Salmonella. The National Food Institute, DTU is the national reference laboratory for *Salmonella* in animals, feeding stuffs and food and receives all such isolates for typing. Among all *Salmonella* isolates serotyped at the National Food Institute and at the National Veterinary Institute, DTU, one isolate per serotype per farm is selected for the DANMAP report.

The majority of the *Salmonella* isolates from pigs (95% in 2009) originates from the Danish *Salmonella* surveillance programmes: The results of a serosurveillance at the slaughterhouses and in all breeding herds appoint risk herds to be further examined by analysing pen-faecal samples 1) from finisher herds at level 2 and level 3 farms (i.e. farms with high level of *S*. Typhimurium antibodies in serum samples taken at slaughter), 2) from related (supplying) sow herds, and finally 3) from breeding and multiplier herds with high serum levels in three monthly samples. In 2009, 977 herds were appointed as risk herds from the serosurveillance and *S.* Typhimurium was isolated from 351 of these herds. In addition, *Salmonella* in samples from pig herds investigated due to clinical disease (not necessarily salmonellosis) were included (21 isolates in 2009).

For broilers, all flocks are sampled due to the *Salmonella* surveillance programme including flocks intended for export before slaughter. Samples are collected 2-3 weeks before slaughter. Since 2008, an additional AM (ante mortem)-testing of broiler flocks was introduced 7-10 days prior to slaughter. In 2009, 3707 flocks (7081 samples) were analysed, of which 33 were positive for *Salmonella*. In 2009, no *S*. Enteritidis and only a few *S*. Typhimurium isolates were observed.

Table 55. Number of samples analysed and number of isolates obtained and MIC-tested from healthy production animals at slaughter.

DANMAP 2009

		E. coli	*E. faecium*	*E. faecalis*	*C. jejuni*	*C. coli*
Pigs	No. of samples analysed	284	772	772	160	160
	No. of isolates obtained	279	169	136	23	137
	No. of isolates MIC-tested/reported	150	151	133	0	113
Cattle	No. of samples analysed	161	0	0	188	188
	No. of isolates obtained	156	0	0	107	20
	No. of isolates MIC-tested/reported	94	0	0	87	0
Broilers	No. of samples analysed	398	398	398	398	398
	No. of isolates obtained	257	57	20	93	12
	No. of isolates MIC-tested/reported	152	43	19	75	0

Data in this table should not be used for reportation of prevalences of the bacterial specie.

For cattle, a total of 390 herds were examined based on clinical indication, 29 *Salmonella* were isolated including 16 *S*. Dublin and 8 *S*. Typhimurium isolates. Due to the low number of *S.* Typhimurium isolates from broilers and cattle, these are not presented in this report.

Further details on the sampling procedures in the *Salmonella* surveillance programmes are described in the Annual Report on Zoonoses in Denmark, 2009.

Food

***Campylobacter*, indicator *E. coli* and Enterococci.** The food isolates originated from food samples collected at wholesale and retail outlets by the Regional Veterinary and Food Control Authorities (RFCA) in all regions of Denmark during the course of routine inspection carried out by the authorities, or on specific request from the Danish Veterinary and Food Administration (DVFA) for the DANMAP programme. The collected material consists of both Danish and imported foods. The food samples are collected according to the guidelines for microbiological examination of foods from the DVFA [Vejledning nr. 9613 af 20. Dec. 2002 om offentlig mikrobiologisk kontrol af fødevarer].

***Salmonella*.** The *Salmonella* isolates from Danish pork and beef originate from the *Salmonella* surveillance programme, comprising swab samples of pork and beef carcasses taken at the slaughterhouses after cooling. In 2009, a total of 24,385 samples where pooled into 4,991 tested samples. Due to loss of sensitivity from pooling, this method corresponds to the testing of 14,377 single samples (Annual report on Zoonoses in Denmark, 2001). *Salmonella* was isolated from 157 pooled samples, (est. prevalence =1.1%), of which 36% were *S*. Typhimurium (n=57). From own control at the slaughterhouses, 19 isolates were submitted to the National Food Institute, DTU, susceptibility tested and included in the DANMAP report.
From beef, a total of 1,606 pooled samples were tested, corresponding to an estimated 4,438 single samples. *Salmonella* was isolated from 13 beef samples, of which only one was *S*. Typhimurium.

Salmonella isolates from Danish broiler meat, imported poultry meat and other imported fresh meats originate from the case-by-case risk assessment programme; for each tested batch of meat, 12 pooled samples (each 1-60 single samples) are tested for *Salmonella.* In 2009, 100 batches of Danish broiler meat were sampled, with no positive batches. For imported broiler meat, 736 batches were tested and 30 batches were positive for *Salmonella*. For imported turkey, 62 batches of 342 batches were positive for *Salmonella*. For imported pork, 37 of 301 tested batches were positive. For imported beef, only 5 batches were positive of 125 batches and the estimated prevalence in positive batches was 5.7%. As the sampling is risk based, the findings are not indicative of the prevalence at retail.

Humans

***Salmonella enterica* serovars Typhimurium and Enteritidis and *Campylobacter jejuni*.** Antimicrobial susceptibility was performed on a sample of human faecal isolates submitted to Statens Serum Institut (SSI). *Campylobacter* spp. isolates were submitted from clinical microbiological laboratories covering three geographical regions: Northern Jutland, Funen and Roskilde/Køge. Information on travel history was obtained for these patients. Exact figures of the proportion tested and the sampling strategy for the different species can be found in the corresponding chapters of this report.

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

Invasive *Streptococcus pneumoniae*, *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. All blood and spinal fluid isolates nationwide are sent to SSI for determination or confirmation of susceptibility testing and typing.

***Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, non-invasive *Streptococcus pneumoniae*, non-invasive *Streptococcus pyogenes*, invasive *E. faecium* and invasive *E. faecalis*.** Data were provided on all isolates recorded from either blood samples (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. faecium* and *E. faecalis*), urine samples (*E. coli*, *Klebsiella pneumoniae*) or all non-invasive samples (*S. pyogenes* and *Streptococcus pneumoniae*) submitted for susceptibility testing to the participating DCM at Statens Serum Institut or the following hospitals: Rigshospitalet, Hvidovre, Herlev, Hillerød, Slagelse, Næstved, Odense, Esbjerg, Vejle, Herning, Århus, Viborg and Aalborg.

In 2009, no samples were collected from healthy humans.

Isolation and identification of bacteria

Animals

***Salmonella* spp.** Examination of samples was done by non-selective pre-enrichment of 25 g material in a 1:10 dilution with buffered peptone water (BPW) and incubated 16-20 hours at 37°C. A plate with Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium was inoculated with 0.1 ml of BPW deposited as 3 drops. After incubation overnight at 41.5°C material from MSRV swarming zones were inoculated onto Brilliant Green Agar (samples from cattle and pigs) or Rambach Agar (samples from poultry). Overnight incubation at 37°C was followed by serotyping of suspect colonies by slide agglutination. For cattle samples, in addition 1.0 ml of the BPW suspension was incubated in 9 ml selenite cystein broth overnight at 41.5°C before inoculation on MSRV agar.

***Campylobacter* spp.** Samples from pigs and poultry were examined by direct inoculation on selective agar (mCCD) followed by incubation in micro-aerophilic atmosphere for 1-2 days at 41.5°C. For cattle, selective enrichment in Preston broth at a ratio of 1:10 incubated in microaerophilic atmosphere for 24 h at 41.5°C was performed followed by inoculation of 10µl of the enrichment broth to mCCD agar. *Campylobacter* suspect colonies were verified by microscopy and oxidase activity. Species-identification was performed by catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. All isolates of *C. jejuni* and *C. coli* were stored (at -80°C).

***Escherichia coli* from healthy animals (indicator *E. coli*).** The material was inoculated directly onto Drigalski agar and incubated at 37°C overnight. For cattle and pigs, yellow colonies were inoculated onto CHROM Orientation agar and red colonies were identified as *E. coli* after incubation at 37°C overnight. For poultry, yellow colonies were identified by catalase and oxidase activity, indole, citrate, methyl red and Voges-Proskauer reaction.

Enterococci. For pigs, one drop of faecal material suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz-Bartley agar and incubated for 2 days at 42°C. Three colonies with morphology typical of *E. faecalis* and *E. faecium* were sub-cultivated on blood agar. Colonies were identified by the following criteria: Colour, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. For broilers, cloacal swabs were incubated overnight at 42°C in Enterococcus Selective Broth. Cultures were inoculated on Slanetz-Bartley agar and incubated for 48 h at 37°C followed by the same identification criteria as for pigs. All isolates of *E. faecium* and *E. faecalis* were stored (at -80°C). Like in previous years, no samples from cattle were investigated for enterococci.

Pathogens. The diagnostic submissions were examined according to the standard procedures at the participating laboratories.

Food

***Salmonella* spp.** was isolated according to the guidelines for microbiological examination of foods from the DVFA [NMKL No. 71, 5th ed., 1999]. Sero- and phage-typing was performed at the National Food Institute, DTU.

***Campylobacter* spp.** was isolated according to the guidelines for microbiological examination of foods from the DVFA [NMKL No. 119, 3rd ed., 2007]. Identification was performed at the Regional Veterinary and Food Control Authorities (RFCA) by microscopy, oxidase activity, catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. All isolates of *C. jejuni*, *C. coli* and *C. lari* were stored (at -80°C), and sent to the National Food Institute, DTU, for MIC-testing of *C. jejuni* and *C. coli*.

Indicator *E. coli* was isolated by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulfphate-broth, which was incubated overnight at 44°C, and subsequently streaked onto violet red bile agar and incubated for 24h at 44°C by RFCA. Presumptive *E. coli* were further identified by CHROM Orientation agar and sent to the National Food Institute, DTU for MIC-testing. All isolates were stored (at -80°C.)

Enterococci were isolated by adding 5 g of the sample to 45 ml of azide dextrose broth, which was incubated overnight at 44°C and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours, colonies typical of *E. faecium* and *E. faecalis* were sent to the National Food Institute, DTU for further identification by the following criteria: Colour of material, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. All isolates of *E. faecium* and *E. faecalis* were stored (at -80°C.)

Humans

***Salmonella* spp.** isolates were serotyped according to the Kauffman-White Scheme.

***Campylobacter* spp**. Species identification was performed using a species specific PCR assay [Klena JD *et al.*, J. Clin. Microbiol. 2004; 42: 5549-5557].

***Staphylococcus aureus*.** Sequencing of the *S. aureus* specific *spa* gene was used both for species conformation and typing purposes. Any *spa* negative isolates were confirmed as *S. aureus* by coagulase test. The *spa* typing [Harmsen *et al*. 2003. J. Clin. Microbiol. 41: 5442-5448] and additional typing by multi locus sequence typing (MLST) was performed [Enright *et al*. 2000. J. Clin. Microbiol. 38: 1008-1015] and annotated using eBURST v.3 software (www.mlst.net). Based on the *spa* and MLST typing, each isolate was assigned to a clonal complex (CC). For MRSA isolates, presence of the *mecA* methicillin resistance gene was confirmed by PCR [Larsen *et al.* 2008. Clin. Microbiol. Infect. 14: 611-614].

Susceptibility testing

MIC-testing

Antimicrobial susceptibility testing of *Salmonella* spp., *Campylobacter* spp., indicator *E. coli*, *Enterococcus* spp. and the veterinary pathogens was performed as microbroth dilution MIC with the Sensititre system (Trek Diagnostic Systems Ltd., UK). Inoculation and incubation procedures were in accordance with the CLSI guidelines. The following quality control strains were used for internal control: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.

An overview of the interpretation of MIC-values is presented in Table 56. Since 2007, data were interpreted using EUCAST epidemiological cut-off values, and if not available, EUCAST or CLSI clinical breakpoints were applied. Exceptions and further details are described in Table 56. Data from previous years presented in this DANMAP report are not corrected for the change in interpretation (e.g. all data are presented with use of the interpretation applied for the year in question). All MIC-distributions for human isolates are presented with both epidemiological cut-off values and clinical breakpoints in order to visualize the impact of changing the interpretation criteria.

All isolates from animals and foods were susceptibility tested at the National Food Institute, DTU, except for broilers, where the testing was performed at the National Veterinary Institute, DTU. The *Salmonella* spp. and *Campylobacter* spp. of human origin were susceptibility tested at the SSI.

From 1998–2007, a performance test for susceptibility testing was carried out almost once a year to ascertain the quality and comparability of susceptibility testing in the laboratories providing MIC-data. Today, the laboratories are accreditated by DANAK (the Danish national body for accreditation) or awaiting an accreditation, ensuring MIC-data of high quality.

One isolate per bacterial species per herd, per food sample, or per patient was tested for antimicrobial susceptibility. For animal isolates in excess numbers (indicator *E.coli*, enterococci and *Campylobacter* spp. from healthy production animals), a random selection of 100 or 150 isolates was appointed for susceptibility testing. Due to a relatively low number of isolates, *C. jejuni* from pigs and *C. coli* from cattle and broilers were not susceptibility tested.

Staphylococcus aureus from humans

Susceptibility testing was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco, Denmark) on Danish Blood Agar (SSI Diagnostika, Denmark) towards penicillin, cefoxitin, streptomycin, kanamycin, erythromycin, clindamycin (only when an isolate was resistant to erythromycin), tetracycline, fusidic acid, rifampicin, norfloxacin, mupirocin and linezolid. A cefoxitin 60 µg tablet was used for screening for methicillin susceptibility. Isolates with an inhibition zone <29 mm were further tested for the presence of the *mec*A gene by PCR. In addition, MRSA isolates were screened for susceptibility towards glycopeptides by spot test on Brain-Heart infusion (BHI) agar (Becton Dickinson, Germany) with teicoplanin (5 mg/L) and confirmed using Etest® (AB Biodisk, Sweden) on BHI with inoculum of McFarland 2.0. In case of MIC ≥8 mg/L for vancomycin and teicoplanin or an MIC ≥12 mg/L for teicoplanin, population analysis profile against vancomycin was performed [**Wootton *et al.* 2001.** J. Antimicrob. Chemother. 47: 399-403].

Invasive *Streptococcus pneumonia* from humans

Screening for penicillin-resistant *S. pneumoniae* was performed using a 1 µg oxacillin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark),

Table 56. Interpretation used for MIC-determination on bacterial isolates from animals, food and humans.

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Antimicrobial agent	*Salmonella*		*E. coli*		*E. faecium*		*E. faecalis*		*C. jejuni*		*C. coli*	
	Epid cut-off µg/ml	Clin break µg/ml	Epid cut-off µg/ml	Clin break µg/ml	Epid cut-off µg/ml	Clin break µg/ml	Epid cut-off µg/ml	Clin break µg/ml	Epid cut-off µg/ml	Clin break µg/ml	Epid cut-off µg/ml	Clin break µg/ml
Ampicillin	>4	>8	>8	>8	>4	>8	>4	>8				
Apramycin	>16		>16									
Avilamycin					>16		>8					
Cefotaxime	>0.5	>2	>0.25	>2								
Cefoxitin												
Ceftiofur	>2		>1									
Chloramphenicol	>16	>16*	>16	>16*	>32	>16*	>32	>16*	>16		>16	
Ciprofloxacin	>0.06	>1	>0.03	>1					>1	>1	>1	>1
Colistin	>2	>2	>2	>2								
Erythromycin					>4	>4*	>4	>4*	>4	>4	>16	>16*
Florfenicol	>16		>16									
Gentamicin	>2	>4	>2	>4	>32	>512*	>32	>512*	>1		>2	
Kanamycin					>1,024		>1,024					
Linezolid					>4	>4	>4	>4				
Nalidixic acid	>16	>16*	>16	>16*					>16		>32	
Neomycin	>4		>8									
Penicillin					>16	>8*	>16	>8*				
Quinupristin/ dalfopristin *a)*					>4 *b)*	>4						
Salinomycin					>4		>4					
Spectinomycin	>64		>64									
Streptomycin	>16		>16		>128		>512		>2		>4	
Sulfonamide	>256 b)	>256*	>256 b)	>256*								
Tetracycline	>8	>8*	>8	>8*	>4	>8*	>4	>8*	>2	>8*	>2	>8*
Tiamulin												
Tigecycline					>0.25	>0.5	>0.25	>0.5				
Trimethoprim	>2	>4	>2	>4								
Vancomycin					>4	>4	>4	>4				

Epidemiological cut-off values and clinical breakpoints recommended by EUCAST are marked in grey.
EUCAST epidemiological cut-off values were used for interpretation. If not available, interpretation criteria were applied by DANMAP.
a) Trade name synercid. EUCAST epid. cut-off value (>1) was not applied according to investigations presented in DANMAP 2006.
b) CLSI clinical breakpoint was applied.
* CLSI clinical breakpoint (presented if EUCAST clinical breakpoints are not available).

and for erythromycin-resistant *S. pneumoniae* using a 78 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika). The breakpoints used are those defined by the CLSI. Penicillin and erythromycin MICs are determined using the Etest (AB Biodisk, Solna, Sweden) on Danish Blood Agar (Resistensplade, SSI Diagnostika) incubated at 36°C, 5% CO_2. The breakpoints used are those defined by Etest.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans

Screening for penicillin-resistant streptococci was performed using a 1 µg oxacillin disk (Oxoid, Greve, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant streptococci using a 78 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika). Erythromycin resistant streptococci are tested with 15 µg erythromycin disk (Oxoid) and 15 µg clindamycin disk (Oxoid, Greve, Denmark) on Danish Blood Agar (Resistensplade, SSI Diagnostika). Erythromycin MICs are determined using the Etest (AB Biodisk, Solna, Sweden) on Danish Blood Agar (Resistensplade, SSI Diagnostika) incubated at 36°C, 5% CO_2. The breakpoints used are those defined by the CLSI. Resistant isolates are defined as both fully and intermediary resistant isolates.

E.coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, non-invasive *Streptococcus pneumoniae*, non-invasive *Streptococcus pyogenes*, invasive *E. faecium* and *E. faecalis* from humans

In 2008, the DCM at Statens Serum Institut, the hospitals in Næstved, Odense and Viborg, and Rigshospitalet, which is the national referral hospital,

used the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by A/S Rosco.
However, the DCM at Odense Hospital used Neo-Sensitabs® on Müeller-Hinton II agar (SSI Diagnostica) when testing urine isolates and Columbia agar with 4.5% NaCl (SSI Diagnostika) for oxacillin-susceptibility of staphylococci. The DCM at Vejle Hospital used the Neo-Sensitabs® on Müeller-Hinton II agar (SSI Diagnostica) and the breakpoints defined for this medium by A/S Rosco. The DCM at Esbjerg Hospital used the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Müeller-Hinton II agar (SSI Diagnostika) when testing *E. coli*. The DCM at Aalborg Hospital also used the Neo-Sensitabs® on Mueller-Hinton II agar (SSI Diagnostika) in combination with the tablet diffusion method (A/S Rosco) and the breakpoints defined by the Swedish Reference Group for Antibiotics (SRGA). The only material exception from SRGA was that the wildtype population of *E. coli* was deemed susceptible for ampicillin (and not intermediary susceptible).

In 2008, the DCM at Hillerød, Hvidovre, Herlev, Herning and Århus Hospitals used the disk diffusion method (Oxoid, Basingstoke, UK) on Iso-Sensitest (ISA) medium (Oxoid). The DCM at Slagelse Hospital used the same disks on Iso-Sensitest (ISA) medium with or without 5% horse blood (Oxoid) according to test material and bacterial species. All laboratories performing the disk diffusion method used the breakpoints defined by the Swedish Reference Group for Antibiotics (Available from: URL: http://www.srga.org/). However, the DCM at Århus Hospital changed to EUCAST breakpoints for urine samples continuously during 2009 and for enterobacteria in blood per November 2009.

All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

Quinupristin/dalfopristin breakpoint

The epidemiological cut-off value suggested by EUCAST for quinupristin/dalfopristin when testing *E. faecium* is >1 µg/ml. In DANMAP, *E. faecium* isolates with MICs >4 µg/ml are reported resistant to quinupristin/dalfopristin due to an evaluation study presented in the DANMAP 2006 report, page 49-50.

Data handling

Animal isolates

The results from the primary examination of samples from slaughterhouses and primary production for the bacteria of interest – positive as well as negative findings – and of the susceptibility testing were stored in an Oracle Database 8i Enterprise Edition® at the National Food Institute, DTU. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant epidemiological cut-off value. Each isolate was identified by the bacterial species, including subtype as applicable and by the date of sampling and the species of animal. Information on the farm of origin was also recorded. All handling and evaluation of results was carried out using SAS®Software, SAS Enterprise Guide 3.0.

Food isolates

Results from the analysis of food samples were reported via the database administrated by the Danish Veterinary and Food Administration, except for the data on Salmonella, which were reported to and extracted from the laboratory database at the National Food Institute, DTU. For each bacterial isolate, information is available on the food type, bacterial species, date and place of sampling, date of examination of the sample, country of slaugther, the RFCA that collected and processed the sample, and an identification number, which makes it possible to obtain further information about the isolate from the Authority. Furthermore, more detailed information about the country of origin was recorded whenever possible.

Human isolates

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

Staphylococcus aureus. For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). In this database, patients were only registered the first time they were diagnosed with MRSA regardless of whether it was colonisation or infection. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation),

imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalization with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset). MRSA cases with community onset were further classified according to risk factors during the previous 12 months as either health care associated with community onset (HACO) or community acquired (CA). Health care associated risk factors included prior hospitalisations or stay in long-term care facilities within 12 months prior to MRSA isolation and being a health care worker. Community risk factors included known MRSA positive household members or other close contacts. Non-Danish origin defined as the person or one of the parents being born outside Denmark were investigated through the Danish civil registry.

***Streptococcus pneumoniae, Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci.** Data on susceptibility testing of isolates are stored as MICs in a Microsoft® Access database at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed with Microsoft® Excel.

***Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, non-invasive *Streptococcus pneumoniae*, non-invasive *Streptococcus pyogenes*, invasive *E. faecium* and invasive *E. faecalis*.** Fourteen DCM provided data on resistance levels in *E. coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, non-invasive *Streptococcus pneumoniae*, non-invasive *Streptococcus pyogenes*, 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, Slagelse, and Aalborg Hospitals.
- - MADS (DCM, Skejby Hospital, Århus, Denmark) for the DCM at Rigshospitalet and Næstved, Odense, Esbjerg, Vejle, Herning, Århus (Skejby) and Viborg Hospitals.
- - SafirLIS Microbiology (Profdoc Lab AB, Borlänge, Sweden) for the DCM at Hillerød Hospital.

For the former Roskilde County, resistance data on *E. coli* from blood samples were obtained from the DCM at SSI, and resistance data on *E. coli* from hospital urine samples from the chemical laboratory at Roskilde Hospital.
Generally, resistance data were excluded if susceptibility to a certain antimicrobial agent was tested on only a selected number of isolates.

Calculation of confidence limits and differences between proportions

Estimation of exact 95% (two-sided) confidence intervals for proportions were based on binomial probability distributions as described in Armitage & Berry (2001), Statistical Methods in Medical Research, 4th ed. 2001, Oxford: Blackwell Scientific Publications. Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 3.0 or StatCalc in EpiInfo™ v. 6. Fishers exact test (2-tailed) was applied when appropriate. P-values were reported to the first significant figure except P-values smaller than 0.0001, these were reported as $P<0.0001$.

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