DANMAP 2006

DANMAP 2006 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark

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This report is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of monitoring of antimicrobial use and antimicrobial resistance in food animals, foods and humans in 2006. The report is produced in collaboration between the National Food Institute, DTU, the National Veterinary Institute, DTU, the Danish Veterinary and Food Administration, the Danish Medicines Agency and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Science, Technology and Innovation and the Ministry of the Interior and Health.

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Introduction

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, foods and humans. The participants in the programme are Statens Serum Institut, the National Veterinary Institute DTU, the National Food Institute DTU, the Danish Veterinary and Food Administration and the Danish Medicines Agency. The objectives of DANMAP are:

- To monitor the consumption of antimicrobial agents in food animals and humans
- To monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin 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.

This report describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs. Trends and comparison to previous years are included. In addition to the monitoring of antimicrobial resistance and consumption of antimicrobial agents the DANMAP programme includes considerable research activities. A few selected summary research reports are presented. Appendix 2 provides a more comprehensive list of DANMAP publications in the international scientific literature.

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List of abbreviations

ADD	Defined Animal Daily Dose
ADD_{kg}	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
DMA	Danish Medicines Agency
DDD	Defined Daily Dose
DVFA	Danish Veterinary and Food Administration
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
SSI	Statens Serum Institut
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

Anatomical Therapeutic Chemical (ATC) classification. This is the 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/).

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

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 compounds are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use, and used mainly in companion animals. The term „antibacterial agents" is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only).

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/).

Defined Animal Daily Dose (ADD and ADDkg). 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, Jacobsen E, Bager F. 2004. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. 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.

Domestically acquired infections. Infections that were reported not to be travel-related and infections with unknown travel history are categorized as domestically acquired, though it is known that many patients with an unknown travel history were in fact infected abroad.

Finishers. Pigs from 30 kilogram live weight to 90-100 kilogram at time of slaughter at 90-100 kilogram live weight.

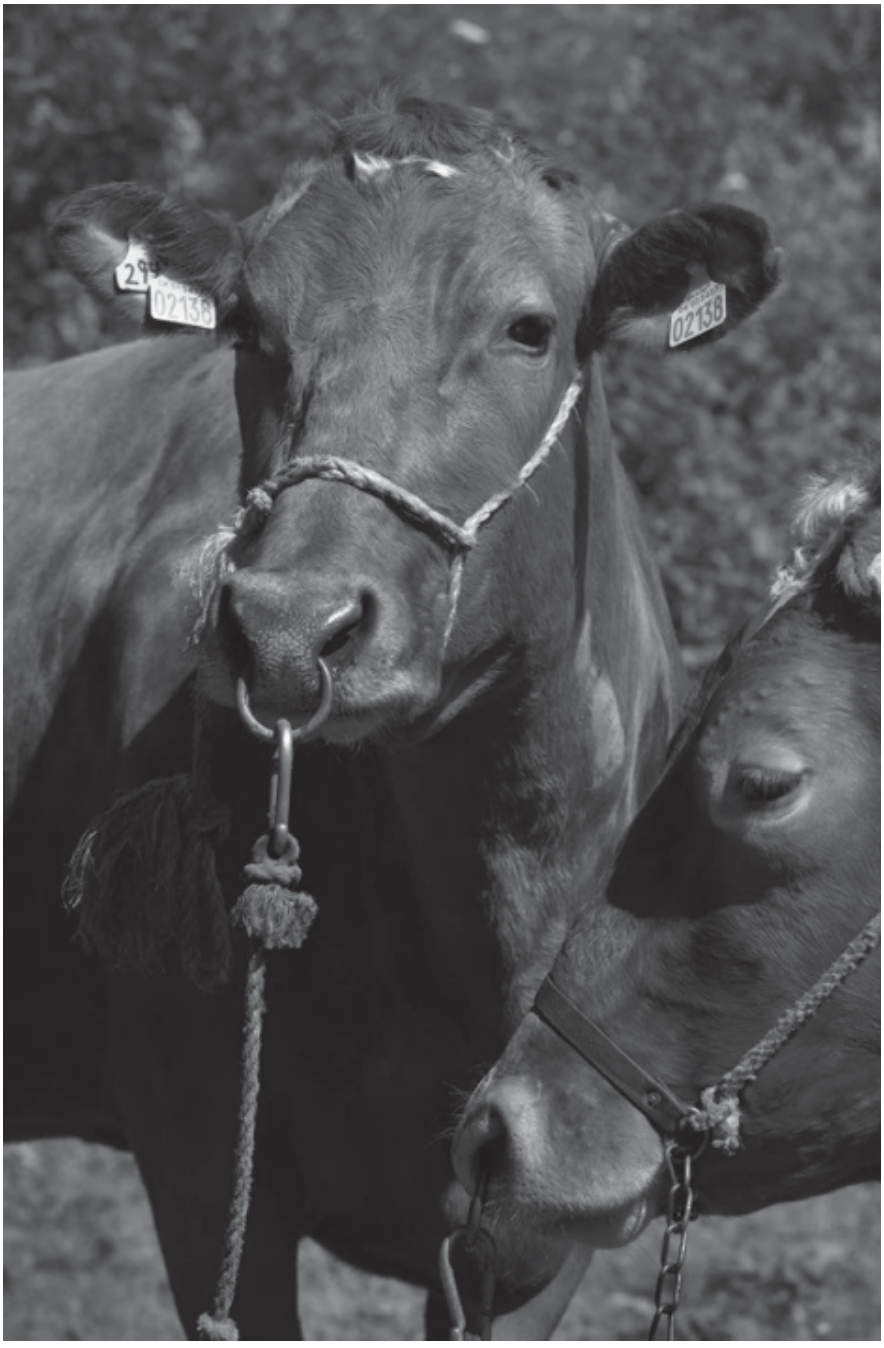
Minimum Inhibitory Concentration (MIC). This is the lowest concentration of antimicrobial in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

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.

Sow. Any breeding female that has been served and is on the farm.

Travel associated infections. Infections where travel was reported and therefore these were most likely acquired in a foreign country.

Weaners. Any pig between 7 and 30 kilogram live weight.



Sammendrag

Forbrug af antibiotika
DANMAP giver en samlet fremstilling af anvendelsen af antibiotika til dyr og mennesker. Siden 2001, er alle oplysninger om forbrug af medicin til dyr på besætningsniveau blevet registreres i det landsdækkende register for receptpligtig veterinærmedicin, VetStat. Oplysninger om forbrug af receptpligtig medicin til mennesker er blevet indsamlet af Lægemiddelstyrelsen siden begyndelsen af 1990'erne.

Antibiotikaforbruget til dyr
Det samlede antibiotikaforbrug til dyr steg med 2 % fra 114,1 tons i 2005 til 116,5 tons i 2006. Denne stigning dækker over et 1,2 % fald i antibiotikaforbruget til svin, mens forbruget til fjerkræ og fisk steg med henholdsvis 20 % og 73 %.

Det samlede antibiotikaforbrug til svin faldt med 1,2 % fra 2005 til 2006 og udgjorde 91,4 tons aktivt stof, som svarer til 78 % af det totale veterinære antibiotikaforbrug. I samme periode var svineproduktionen uændret. Makrolider, tetracykliner og pleuromutiliner var også i 2006 de mest anvendte antibiotika til svin. Forbruget af makrolider og pleuromutiliner faldt dog yderligere i 2006 mens forbruget af tetracykliner fortsatte med at stige. Forbruget af fluorokinoloner til svin faldt yderligere til 2,6 kg i 2006 sammenlignet med 94 kg i 2001.

Det samlede antibiotikaforbrug til kvæg blev i 2006 estimeret til 15 tons, som svarer til 13 % af det samlede veterinære antibiotikaforbrug. Dette forbrug var uændret i forhold til 2005. Derimod steg antibiotikaforbruget i fjerkræproduktionen med 20 % fra 2005 til 2006, stigningen skyldes hovedsagelig et stigende forbrug til kalkuner.

Apotekernes salg af antibiotika til brug til pelsdyr steg med 157 % fra 659 kg i 2001 til 1.694 kg i 2006. I den samme periode steg antallet af avlsdyr i pelsdyrproduktionen kun med 17 %. Det samlede antibiotikaforbrug til fiskeopdræt steg med 73 % fra 2.406 kg i 2005 til 4.180 kg i 2006, som hovedsagelig skyldtes øget sygdomsforekomst i forbindelse med høje vandtemperaturer i 2006. Det stigende forbrug var ligeligt fordelt mellem opdræt i ferskvand og opdræt i saltvand, men forbruget af antibiotika per kg fisk produceret var signifikant højere i saltvandsopdrættet (287 mg/kg fisk) sammenlignet med opdræt i ferskvand (64 mg/kg fisk).

I 2006 udgjorde cephalosporinforbruget til svin 0,1 % af det samlede antibiotikaforbrug til svin. Fra 2001 til 2006 steg cephalosporinforbruget til svin med 300 % fra 24 kg i 2001 til 98 kg i 2006. Cephalosporiner betragtes som vigtige antibiotika til behandling af syge mennesker. Samtidig med det stigende cephalosporinforbrug til svin bliver der hyppigere isoleret ESBL-producerende patogene *E. coli* og *Salmonella* Typhimurium (se nedenfor). Det er almindeligt, at intramammaria der indeholder cephalosporin anvendes til behandling af køer med yverbetændelse. Både i 2005 og i 2006 blev der brugt 367 kg antibiotika som intramammaria, heraf var de 25 % eller 91 kg cephalosporiner. Forbruget af cephalosporiner til kæledyr steg fra 313 kg i 2005 til 354 kg i 2006. Både i 2005 og 2006 svarede det til 55 % af det samlede veterinære cephalosporinforbrug.

Antibiotikaforbruget til mennesker
Fra 2005 til 2006 steg forbruget af antibiotika til behandling af mennesker med 3,1 %, til 33,5 millioner DDD eller 16,9 DDD/1.000 indbygger-dage.

I primærsektoren steg det totale forbrug af antibiotika med 3,1 %. Fordelingen mellem de enkelte antibiotikaklasser forblev uændret, og mere end 75 % af forbruget bestod af beta-laktamasefølsomme penicilliner, penicilliner med udvidet spektrum samt makrolider. Forbruget af penicilliner kombineret med beta-laktamase inhibitorer og fluorokinoloner steg yderligere i 2006. Det stigende fluorokinolonforbrug skyldes sandsynligvis den lave pris samt lav resistensforekomst i forhold til andre antibiotika. Det øgede forbrug af fluorokinoloner har allerede resulteret i en øget forekomst af fluorokinolon resistens i *E. coli* isoleret fra urinvejsinfektioner. Dette henleder opmærksomheden på risikoen ved det stigende fluorokinolonforbrug.

Antibiotikaforbruget forsatte med at stige på de danske sygehuse i 2006. I 2006 blev forbruget på sygehusene estimeret til 649 DDD/1000 sengedage, svarende til en gennemsnitlig stigning på 54 % fra 1997 til 2006. Stigningen er derimod kun på 12 % i den samme periode, når forbruget bliver opgjort i DDD/1.000 udskrevne patienter. Forskellen imellem de to estimater for hospitalsforbrug kan forklares ved et vedblivende fald i antallet af sengedage, mens der samtidig ses en forsat stigning i antallet af udskrevne patienter. Imidlertid er der også markante ændringer i forbrugsmønstret. Den tidligere påpegede trend, med stigende forbrug af penicilliner kombineret med beta-laktamase hæmmere, cephalosporiner, fluorokinoloner og carbapenemer, på

bekostning af beta-laktamasefølsomme penicilliner, penicilliner med udvidet spektrum, aminoglykosider og makrolider, fortsatte som tidligere beskrevet.
I 2006 udgjorde cephalosporiner, fluorokinoloner, carbapenemer 28,8 % af totalforbruget på danske sygehuse, sammenlignet med 15,4 % i 1997. Nye data viser, at det øgede forbrug af nye bredspektrede antibiotika allerede har resulteret i en øget resistensforekomst på hospitalerne (se nedenfor).

Resistens i zoonotiske bakterier
Resistensforekomsten blandt *S.* Typhimurium isolater fra dyr (svin, kvæg og fjerkræ) var uændret fra 2005 til 2006 bortset fra tetracyklin- og sulfonamid resistens, som steg blandt isolater fra kvæg. Derimod har der fra 1999 til 2006 været en stigning i forekomsten af tetracyklin-, chloramphenicol-, sulfonamide- og ampicillin resistens i *S*. Typhimurium isolater fra svin. I den samme periode er andelen af fuldt følsomme *S*. Typhimurium isolater faldet stødt. Resistensstigningen er observeret samtidig med et stigende forbrug af både tetracykliner, sulfonamider og bredspektrede penicilliner til behandling af svin. Derudover blev den første ESBL-producerende *Salmonella* Typhimurium isoleret fra et raskt svin i 2006.

Sammenlignes resistensforekomsten blandt *S*. Typhimurium isolater fra dansk svinekød med *S*. Typhimurium isolater fra importeret svinekød, var resistensforekomsten i det importerede svinekød højere for 5 af de testede antibiotika. Blandt *S*. Typhimurium isolater fra importeret kalkunkød blev der ligeledes observeret høj forekomst af resistens overfor adskillige antibiotika.

Blandt *S*. Enteritidis isolater fra mennesker med infektioner erhvervet i udlandet var resistensforekomsten overfor ciprofloxacin og nalidixinsyre højere end i isolater fra infektioner erhvervet i Danmark.

Resistensforekomsten var uændret i *Campylobacter jejuni* isolater fra fjerkræ og i *Campylobacter coli* fra svin fra 2005 til 2006. Blandt *C. jejuni* isolater fra mennesker med infektioner erhvervet i udlandet var resistensforekomsten overfor nalidixinsyre og tetracyklin højere, end i isolater fra infektioner erhvervet i Danmark.

Resistens i indikator bakterier
Resistensforekomsten var uændret for både indikator *E. coli* og enterokokker isoleret fra svin, kvæg og fjerkræ fra 2005 til 2006. De eneste undtagelser var et signifikant fald i sulfonamid resistens i *E. coli* fra fjerkræ og en signifikant stigning i avilamycin resistens hos *Enterococcus faecium* fra fjerkræ. Generelt var resistensforekomsten signifikant højere i *Enterococcus faecalis*, *E. faecium* og *E. coli* isolater fra importeret fjerkrækød sammenlignet med isolater fra dansk fjerkrækød.

De første fund af vancomycin resistente *E. faecalis* isoleret fra kød blev rapporteret i DANMAP 2006. De vancomycin resistente *E. faecalis* isolater blev påvist i kalkunkød importeret fra Tyskland i 2005 og 2006. I løbet af de 11 år DANMAP har eksisteret, har der ikke før været rapporteret vancomycin resistente *E. faecalis* fra kød eller dyr. Fra 2005 til 2006, var der ingen signifikante ændringer i resistensforekomsten hos enterokokker og *E. coli* isolater fra raske personer. Resistens niveauet i *E. faecium, E. faecalis* og *E. coli* fra raske mennesker og dansk fjerkrækød var generelt på samme niveau, hvorimod resistens niveauet generelt var højere i importeret kalkunkød og importeret fjerkrækød sammenlignet med isolater fra raske personer.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker
Antallet af methicillin resistente *S. aureus* (MRSA) isolater faldt fra 851 i 2005 til 706 i 2006 (tallet inkluderer både patienter med infektion samt personer der er asymptomatiske bærere; et isolat per person). Hovedparten af faldet skyldtes en reduktion i antallet af tilfælde som følge af det langvarige udbrud med en MRSA ST22 stamme i Vejle Amt, der nu synes under kontrol. I resten af landet var der en lille stigning fra 540 til 561 nye tilfælde.
I 64 % af tilfældene var der infektion på diagnosetidspunktet. I 82 % af infektiøse tilfælde erhvervet i Danmark startede infektionen udenfor hospitalerne (community onset). Hud- og bløddelsinfektioner dominerede på diagnosetidspunktet (72 % af alle infektioner), men MRSA forårsagede også alvorlige infektioner herunder bakteriæmi. I 2006 blev der konstateret 19 tilfælde af MRSA bakteriæmi (1,4 % af alle *S. aureus* bakteriæmier), hvilket er sammenligneligt med de 23 (1,5 % af alle *S. aureus* bakteriæmier) der blev fundet i 2005.

Blandt *Streptococcus pneumoniae* og *Streptococcus pyogenes* (Gruppe A streptokokker) var der i 2006 fortsat lav resistens overfor penicillin og makrolid.

Ciprofloxacin resistens blandt *E. coli* isoleret fra urin steg signifikant såvel i primærsektoren som på hospita-

lerne fra 2005 til 2006. Således var 5,0 % af isolaterne fra primærsektoren og 6,3 % af isolaterne fra hospitalerne resistente i 2006. Stigningen i ciprofloxacin resistens skete sideløbende med et fortsat øget forbrug af fluorokinoloner (primært ciprofloxacin) gennem de seneste år; både i primærsektoren og på hospitalerne. Resistens overfor mecillinam og sulfonamider i *E. coli* isoleret fra urin på hospitalerne steg signifikant til henholdsvis 33,8 % og 5,0 % i 2006. Sulfonamid resistens i *E. coli* isoleret fra urin i primærsektoren faldt signifikant fra 37,6 % i 2005 til 36,4 % i 2006, modsat de tidligere år, hvor der har været en signifikant stigning. Gentamicin resistens blandt *E. coli* isolater fra blod steg signifikant fra 1,7 % i 2004 til 2,5 % i 2006. Denne stigning i resistens overfor gentamicin skete uden tilsvarende stigning i forbruget af gentamicin. Blandt de mest almindelige bakterier isoleret fra kliniske prøver fra danske patienter, var resistensniveauet fortsat lavt. På trods heraf antyder stigningerne i antibiotikaresistens, der er blevet observeret i de seneste år, at resistensniveauet er under forandring, og dette understreger vigtigheden af en tæt overvågning af antibiotikaresistens, både i primærsektoren og på hospitalerne.

Summary

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. The Danish Medicine Agency has contributed with data to this report and the earlier DANMAP reports. In animals, data on all medicines prescribed by veterinarians for use in animals have been registered at farm level by the VetStat programme since 2001.

Antimicrobial consumption in animals

The overall consumption of antimicrobial agents in production animals increased by 2%, from 114.1 tonnes in 2005 to 116.5 tonnes in 2006. This increased consumption covers a 1.2% decrease in antimicrobial consumption in pig while in poultry and aquaculture the consumption increased by 20% and 73%, respectively.

From 2005 to 2006, antimicrobial consumption in pigs decreased by 1.2% to 91.4 tonnes active compound (78% of the total antimicrobial consumption in animals), in the same period the production of pigs remained unchanged. Macrolides, tetracyclines and pleuromutilins remained the most commonly used antimicrobial agents in pigs. A further decrease in macrolide and pleuromutilin consumption was observed in 2006 compared to 2004 while the consumption of tetracycline continued to rise in 2006. The consumption of fluoroquinolones in pigs further decreased to 2.6 kg in 2006, as compared to 94 kg in 2001.

In 2006, the antimicrobial consumption in cattle was estimated at 15 tonnes, or 13% of the total consumption in production animals, which is approximately the same level of consumption as in 2005. Antimicrobial consumption in poultry increased by 20% from 2005 to 2006. Most of this increase was due to increased consumption in turkeys.

The registered sales from pharmacies of antimicrobials for use in fur animals have increased by 157% from 659 kg in 2001 to 1,694 kg in 2006. In the same period the number of fur breeding animals has increased by 17%. The consumption of antimicrobials in aquaculture increased by 73% to 4,180 kg antimicrobials in 2006 compared to 2,406 kg in 2005. The main reason for this increase was the relative high water temperatures during most of 2006. The increased consumption was equally distributed between freshwater and marine aquaculture. However, the antimicrobial consumption per kg fish produced was significantly higher in marine aquaculture (287 mg/kg fish) compared to freshwater aquaculture (64 mg/kg fish).

Consumption of cephalosporins in pigs comprised 0.1% of the total antimicrobial comsumption in pigs in 2006. However, the consumption of cephalosporins in pigs increased by 300% from 24 kg in 2001 to 98 kg in 2006. Cephalosporins are like fluoroquinolones regarded as import antimicrobials agents in human medicine. Concomitant with the observed increase in cephalosporin consumption in pigs, isolation of ESBL-producing pathogenic *Escherichia coli* and *Salmonella* Typhimurium is observed more often (see below). In cattle, cephalosporins are commonly used as intramammaries. In both 2005 and 2006, a total of 367 kg antimicrobials were used as intramammaries of which 25% or 91 kg was cephalosporins. In pet animals, the consumption of cephalosporins increased further to 354 kg compared to 313 kg in 2005, corresponding to 55% of the total veterinary use of cephalosporins in both 2005 and 2006.

Antimicrobial consumption in humans

From 2005 to 2006, the overall consumption of antibacterial agents for systemic use in humans in Denmark increased by 3.1% to 33.5 million DDDs or 16.9 DDD/1,000 inhabitant-days.

In the primary health care sector, consumption of antibacterial agents increased by 3.1% in 2006, without significant changes in the distribution of the antibacterial agents used. Consumption of beta-lactamase sensitive penicillins, penicillins with extended spectrum and macrolides, represented almost 75% of the total consumption. Consumption of combinations of penicillins including beta-lactamase inhibitors and consumption of fluoroquinolones increased further in 2006. For fluoroquinolones this was likely due to their low price and low resistance as compared to other antibacterial agents. The increase in consumption of fluoroquinolones has already resulted in increased resistance to fluoroquinolones in *E. coli* isolates from urinary tract infections highlighting the risk of a continued increase in fluoroquinolone consumption.

In Danish hospitals, consumption of antibacterial agents continued to increase. From 1997 to 2006, average hospital consumption increased by 54% to an estimated 649 DDD/1,000 bed-days, whereas this increase was only 12% for the same period when presented as DDD/1,000 discharged patients. The difference between the two estimates of hospital consumption could be explained by the continuous decrease in the number of registered bed-days and, conversely, by the continuous increase in the number of registered discharges in Denmark. In 2006, as in previous years, a change in the distribution of antibacterial agents used, with an increasing consumption of combinations of penicillins with beta-lactamase inhibitors, cephalosporins, fluoroquinolones and carbapenems on behalf of beta-lactamase sensitive penicillins, broad spectrum penicillins, aminoglycosides and macrolides, was observed. In 2006, cephalosporins, fluoroquinolones and carbapenems represented 28.8% of hospital antibacterial consumption compared to 15.4% in 1997. Recent data suggest that this shift towards newer, broad-spectrum antimicrobial agents is already resulting in increased resistance in hospitals (see below).

Resistance in zoonotic bacteria

From 2005 to 2006, the occurrence of antimicrobial resistance in *Salmonella* Typhimurium isolates from food animals (pigs, cattle and poultry) remained unchanged except for a significant increase in resistance to tetracycline and sulfonamide among isolates from cattle. In addition, the first ESBL-producing *S.* Typhimurium was isolated from a healthy pig in Denmark in 2006. From 1999 to 2006 resistance to tetracycline, chloramphenicol, sulfonamide and ampicillin increased significantly in *S*. Typhimurium isolates from pigs, and the proportion of fully sensitive *S*. Typhimurium isolates has declined steadily in the same period. This increase in resistance coincided with an increased consumption of tetracycline, sulfonamides and broad-spectrum penicillin in pigs. Among *S*. Typhimurium from food, resistance to five of the tested antimicrobial agents was significantly higher in isolates from imported pork than in isolates from Danish pork. In isolates from imported turkey meat high levels of resistance to several antimicrobial agents were observed.

Resistance to ciprofloxacin and nalidixic acid in *S*. Enteritidis isolates from infections in humans was significantly higher among isolates from infections acquired abroad than among isolates from infections acquired in Denmark.

Resistance in *Campylobacter jejuni* isolates from broilers and *C. coli* isolates from pigs remained unchanged from 2005 to 2006. Results for *Campylobacter* isolates from food were not available for 2006. Among *C. jejuni* isolates from infections in humans, resistance to ciprofloxacin/nalidixic acid and tetracycline was significantly higher in isolates from infections acquired abroad, as compared to isolates from infections acquired domestically.

Resistance in indicator bacteria

The occurrence of resistance remained unchanged from 2005 to 2006 for both indicator *E. coli* and enterococci from pigs, cattle and broilers. The only exceptions were a significant decrease in sulfonamide resistance in *E. coli* from broilers and a significant increase in resistance to avilamycin in *E. faecium* from broilers. In indicator isolates from food, the occurrence of resistance was significantly higher in *E. faecium*, *E. faecalis* and *E. coli* isolates from imported broiler meat compared with isolates from Danish broiler meat.

In DANMAP 2006, the first isolation of vancomycin resistant *E. faecalis* from meat was reported. The vancomycin resistant *E. faecalis* isolates were detected in samples from turkey meat imported from Germany in 2005 and 2006. Vancomycin resistant *E. faecalis* isolates have not previously been reported from meat or food animals during the 11 years DANMAP has existed.

For *E. coli* and enterococcal isolates from healthy human volunteers, no significant changes in resistance were observed from 2005 to 2006. The resistance levels in *E. faecium, E. faecalis* and *E. coli* from healthy humans were in general similar to the levels observed in isolates from Danish broiler meat, whereas the resistances level was higher in isolates from imported turkey meat and imported broiler meat compared with isolates from healthy humans.

Resistance in bacteria from diagnostic submissions

The number of methicillin resistant *S. aureus* (MRSA) isolates decreased from 851 isolates in 2005 to 706 isolates in 2006 (these numbers include both colonisation and infection with MRSA, one isolate per patient). Most of the decrease was due to reduction in the number of cases in a large hospital outbreak in Vejle County caused by ST22, which now seems to be under control. In the remaining part of the country a small increase from 540 to 561 new cases was observed.

Overall, in 64% of the MRSA cases, the patient had an infection at the time of diagnosis. 82% percent of the MRSA infections acquired in Denmark had community onset. Skin and soft tissue infections were the most frequent types of infections (72%), however MRSA was also responsible for serious infections such as bacteraemia. In 2006, there were 19 cases of MRSA bacteraemia, which corresponded to 1.4% of S. aureus bacteraemia cases. This was comparable to 2005 where 23 MRSA represented 1.5% of S. aureus bacteraemia cases.

Resistance to penicillins and macrolides in *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Group A streptococci) remained low in 2006.

Among *E. coli* urine isolates from primary health care, resistance to ciprofloxacin increased significantly, reaching 5.0% in 2006. In *E. coli* urine isolates from hospitals, ciprofloxacin resistance also increased significantly to 6.3%. These increases in ciprofloxacin resistance were consistent with parallel increases in consumption of fluoroquinolones (mainly ciprofloxacin) observed in recent years, both in primary health care and hospitals. Among *E. coli* urine isolates from hospitals, resistance to sulfonamides and mecillinam increased significantly in 2006, reaching 33.8% and 5.0%, respectively. In *E. coli* urine isolates from primary health care, resistance to sulfonamides decreased significantly, in contrast to last years increase, reaching 36.4% in 2006, compared to 37.6% in 2005. Among *E. coli* blood isolates, gentamicin resistance increased significantly to 2.5% in 2006, compared to 1.7% in 2004. This increase in gentamicin resistance was seen despite no similar changes in gentamicin consumption. Although antimicrobial resistance generally remains low for most antimicrobial agents and most bacteria commonly isolated from clinical samples from infected patients in Denmark, the increases observed in recent years suggest that this is changing and underline the importance of close monitoring of antimicrobial resistance, both in primary health care and in hospitals.

Demographic data

Demographic data

Demographic information is presented in order to show the magnitude of animal and human populations in which antimicrobial agents were used during 2006.

Table 1 shows the production of food animals (including animals for live export), meat, and the population of dairy cattle. For turkeys, data on export of live animals is not included.

Table 2 shows distribution of import, export, production, provision and the import ratio of fresh and frozen meat in Denmark.

In Denmark, the consumption of imported meat has increased in recent years. Of all pork consumed, 47% was imported in 2006 compared to 22% in 2003. For beef and poultry the figures were 49% and 39% in 2006 compared with 33% and 17%, respectively in 2003. By 2004, 95% of all turkeys raised in Denmark were slaughtered abroad (Table 1). Therefore most of the turkey meat sold in Denmark has been imported since 2004 (Data from Statistics Denmark, www.dst.dk).

Table 3 provides information on the distribution of the human population in Denmark and on the Danish health care system by county. Figure 1 shows the counties of Denmark.

Table 1. Production of food animals (including export of live animals) and the production of meat and milk, Denmark

DANMAP 2006

Year	Broilers		Turkeys a)		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	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	1,038	12.6	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	965	11.5	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	510	7.4	625	161	596	4,540	24,434	1,898	34	8
2004	130,674	181	55	1.0	632	165	563	4,434	25,141	1,965	34	9
2005	120,498	180	158	0.5	549	145	558	4,449	25,758	1,988	31	8
2006	105,888	163	32	0.1	509	140	563	4,492	25,763	1,957	-	-

Data from Statistics Denmark (www.dst.dk) and The Danish Directorate for Fisheries

a) From 2002, the export of live turkeys for slaughter increased. By 2004, 95% of all turkeys raised in Denmark were slaughtered abroad. For turkeys, data on export of live animals is not included in the table

Table 2. Distribution of import, export, production, provision and the import ratio of fresh and frozen meat in Denmark

DANMAP 2006

	Broiler meat				Turkey meat				Pork				Beef			
	2003	2004	2005	2006 a)	2003	2004	2005	2006 a)	2003	2004	2005	2006 a)	2003	2004	2005	2006 a)
	tonnes															
Import	14,107	20,077	25,609	29,327	8,379	13,949	12,918	11,287	37,171	42,479	52,053	63,080	42,161	54,891	56,412	60,243
Export	116,616	122,960	115,783	102,842	3,229	1,101	2,123	2,306	1,082,895	1,168,096	1,164,178	1,207,453	55,220	58,344	52,665	53,446
Production	187,827	183,272	164,554	161,305	7,232	4,495	3,054	1,310	1,215,592	1,274,265	1,240,182	1,280,013	121,093	133,123	120,730	114,016
Provisions b)	85,318	80,389	74,380	87,791	12,383	17,344	13,849	10,290	169,868	148,649	128,057	135,640	108,035	129,670	129,670	120,814
import ratio c)	17%	25%	34%	33%	68%	80%	93%	110% d)	22%	29%	41%	47%	39%	42%	44%	50%

Source: Statistics Denmark

a) Figures from 2006 are provisionally

b) Provision = Production + import – export

c) Import ratio = Import/provision

d) Re-export is a possible explanation for an import ratio above 100% because figures from 2006 are still preliminary

Table 3. Distribution of the human population and health care structure by county, Denmark DANMAP 2006

County name	No. inhabitants (01/01/2006)	No. inh./km2 (2006)	No. inh./GP c) (2006)	No. bed-days d) (2005)	No. hospitals d) (2006)
Copenhagen Municipality a)	501,158	5,679	1,803	799,957	4
Frederiksberg Municipality a)	91,855	10,474	1,733	94,502	1
Copenhagen County b)	618,529	1,171	1,189	560,304	3
Frederiksborg	378,686	281	1,319	296,957	4
Roskilde	241,523	271	1,491	224,013	2
West Zealand	307,207	103	1,384	239,751	4
Storstrøm	262,781	77	1,334	234,255	4
Bornholm	43,245	74	1,169	36,952	1
Funen	478,347	137	1,432	461,406	6
South Jutland	252,433	64	1,629	192,737	4
Ribe	224,261	72	1,232	161,568	3
Vejle	360,921	120	1,399	309,726	6
Ringkøbing	275,065	57	1,846	208,667	5
Aarhus	661,37	145	1,366	601,218	8
Viborg	234,896	57	1,078	210,52	4
North Jutland	495,09	80	1,514	464,264	7
Denmark	5,427,367	126	1,405	5,096,797	66

a) Inner Copenhagen
b) Outer Copenhagen
c) GP=general practitioner
d) Excluding private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices.

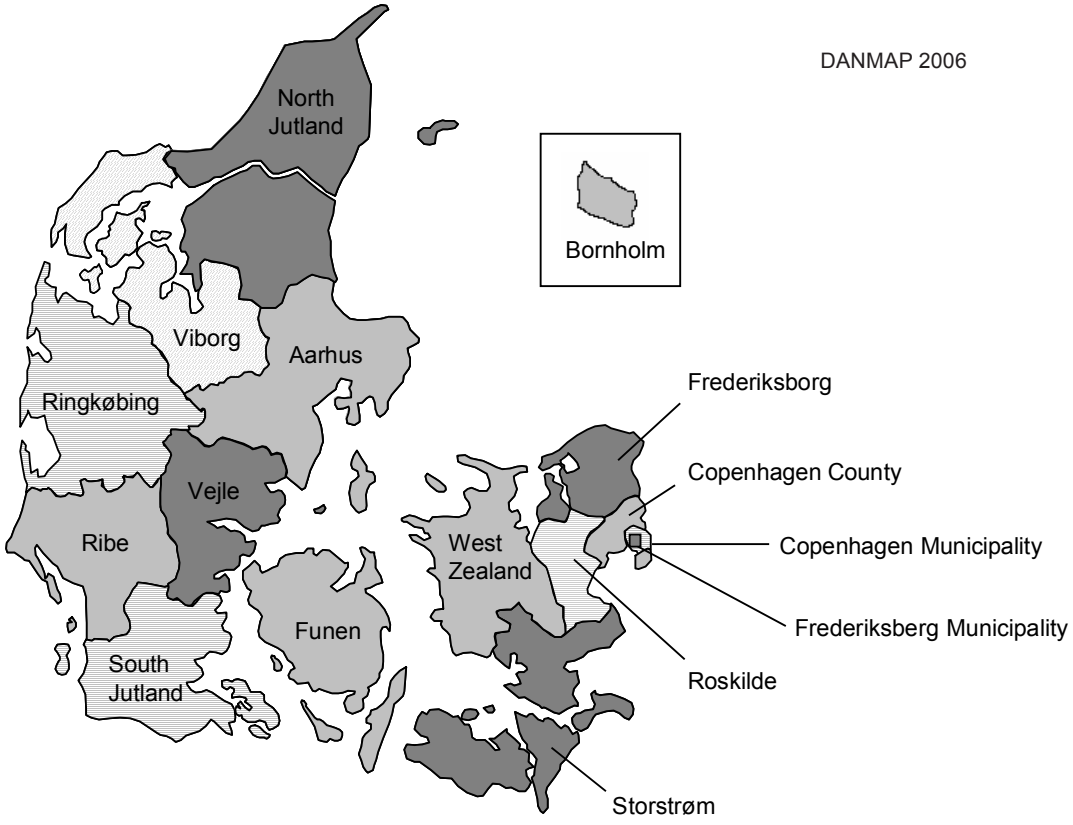


Figure 1. Counties in Denmark

Antimicrobial consumption

Antimicrobial consumption in animals

In Denmark, all antimicrobials are prescription only medicines, and must either be purchased through a pharmacy (95% of the consumption) or through a feed mill (5% of the consumption). Antimicrobial drugs purchased at pharmacies are either administered or sold to the end user by veterinary practitioners (13% of the purchased antimicrobials), or purchased by farmers/animal owners according to veterinary prescription (87% of the purchased antimicrobials). From 1st of April 2007, the pharmacy monopoly on sales of prescription antimicrobials was no longer maintained in Denmark.

From 2001 onwards, detailed data on usage of antimicrobials in each animal species were based on the Danish register of veterinary medicines, VetStat (see Appendix 1 for further details). Prior to 2001, data were based on overall sales data from the pharmaceutical industry (see Table 4).

Consumption of antimicrobials as kg active compound

Table 5 shows the total veterinary consumption of prescribed antimicrobial drugs in 2006 in kg active compound by animal species and age groups, including consumption in companion animals. The total veterinary consumption increased by 2% from 113.9 tonnes in 2005 to 116.5 tonnes in 2006 (Table 5). The antimicrobial consumption in pigs comprised 78% of the total veterinary consumption, while consumption in poultry and fish comprised 0.4% and 4%, respectively. An estimated 13% of the total antimicrobial consumption is used in cattle however, only 20% of the prescribed antimicrobials for cattle are purchased at pharmacies. This amount is registered in Table 5 as used in cattle. The remaining 80% are either administered or sold by veterinary practitioners. This consumption is registered as miscellaneous in Table 5. Therefore approximately 80% of the total antimicrobial consumption in cattle is registered as miscellaneous or intramammaria (Table 5).

During the 1990'ies considerable variation in the yearly veterinary consumption of prescribed antimicrobials was observed (Table 4). Depending on which year during the 1990'ies that the 2006 consumption figures are compared with, the increase in consumption varies considerably from 28% increase when comparing 1994 and 2006 to 140% increase when comparing 1996 and 2006 (Table 4). The increase in antimicrobial consumption was mainly due to increased consumption in pigs. During the same period the production of pigs increased approximately 25% (Table 1).

Figure 2 shows the trends in consumption of prescribed antimicrobials and growth promoters in production animals compared to antimicrobials prescribed for humans. The antimicrobial consumption in production animals is still low compared to the total consumption before the use of antimicrobial growth promoters (AGPs) was discontinued.
Following the official ban of virginiamycin in Denmark in 1998, the industry decided to voluntary discontinue all further use of AGPs. This became effective in broilers, finisher pigs and cattle in 1998. In weaning pigs the use of AGPs was phased out during 1999. Since 2000, there has been no reported use of AGPs for animals

Table 4. Trends in the estimated total consumption (kg active compound) of prescribed antimicrobials for production animals, Denmark

DANMAP 2006

ATC_{vet} group b)	Therapeutic group	1990	1992	1994	1996	1998	2000	2001	2002	2003	2004	2005	2006
QJ01AA	Tetracyclines	9,300 a)	22,000	36,500	12,900	12,100	24,000	28,500	24,500	27,300	29,500	30,050	32,650
QJ01CE	Penicillins, β–lactamase sensitive	5,000	6,700	9,400	7,200	14,300	15,100	16,400	17,400	19,000	20,900	22,250	22,600
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	7,300	8,800	9,900	11,100	12,900	12,300	11,550
QJ01EW	Sulfonamides + trimethoprim	3,800	7,900	9,500	4,800	7,700	7,000	9,200	10,600	10,600	11,500	12,200	13,800
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	950	900	850	850	750	750
QJ01F/QJ01XX	Macrolides, lincosamides, pleuromutilins	10,900	12,900	11,400	7,600	7,100	15,600	18,400	19,200	20,700	24,200	22,350	22,050
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	10,400	11,600	11,700	11,700	11,600	10,800	10,500
	Others c)	6,700	6,800	4,400	600	650	300	900	1,600	1,500	1,000	1,950	1,250
Total		53,400	73,200	89,900	48,000	57,300	80,700	94,700	95,900	102,500	112,500	112,650	115,150

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, used in production animals, is the only topical drug included

a) Kg active compound rounded to nearest 50 or 100

b) Only the major contributing ATC_{vet} groups are mentioned

c) Consumption in aquaculture was not included before 2001

Table 5. Antimicrobials sold (kg active compound) from pharmacies and feedmills by animal species and age group, Denmark

DANMAP 2006

Therapeutic group	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macro	Pleuro	Pen-β-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATC_{vet} groups a)	QJ01B	QJ01G	QJ01DA	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XX	QJ01CE	QJ01CA	QJ01E	QJ01AA	QJ01X	
Pigs														
- Sows and piglets	27	2,227	79	2	0	816	742	1,160	8,284	3,297	4,952	2,709	24	24,318
- Weaners	11	5,595	11	<1	0	801	5,547	2,554	1,417	2,661	1,839	14,060	210	34,706
- Finishers	25	587	6	0	0	1,272	3,539	3,544	5,852	2,067	170	12,690	4	29,756
- Age not given	2	196	2	<1	0	81	279	269	425	286	190	890	5	2,625
Cattle b)														
- Cows and bulls	3	14	7	<1	0	4	21	1	188	23	26	38	<1	326
- Calves<12 months	88	223	2	<1	0	5	37	0	278	153	279	305	3	1,373
- Heifers, Steers	2	<1	<1	0	0	<1	<1	0	6	<1	4	6	<1	21
- Age not given	6	85	3	<1	0	23	96	65	139	134	69	229	2	851
Poultry														
- Broilers	0	<1	0	6	0	<1	0	0	<1	32	3	0	0	40
- Rearing, broilers	0	0	0	<1	0	0	0	0	0	84	<1	0	0	85
- Layers, primatily rearing	0	0	0	0	0	0	<1	0	0	6	8	0	0	14
- Turkeys	0	0	0	10	0	0	0	0	0	174	3	8	0	194
- Geese and ducks	0	0	0	0	0	0	<1	0	0	16	0	0	0	16
- Gamebirds	0	1	0	<1	0	<1	3	0	0	16	15	4	0	39
-Production category not given	0	2	0	1	0	0	<1	0	0	17	5	7	0	33
Other species														
- Small ruminants	<1	20	<1	0	0	5	29	9	38	19	15	58	<1	195
- Fur animals	0	369	<1	<1	0	63	215	5	<1	643	308	89	<1	1,694
- Aquaculture	161	0	0	0	535	0	0	0	<1	8	3,475	1	0	4,180
- Other production animals	<1	<1	<1	<1	0	<1	<1	0	2	2	8	<1	<1	13
- Horses	<1	6	29	1	0	4	4	2	16	33	175	15	3	288
- Pet animals	<1	8	88	4	0	10	4	2	11	87	57	15	10	296
- Farm identified c)	<1	30	<1	<1	0	6	0	11	43	14	40	34	<1	180
For use in vet. practice d)														
- Small animal practice	<1	<1	266	9	0	36	<1	0	53	404	58	37	20	885
-Topical drugs	<1	4	0	0	0	0	0	0	0	0	56	47	10	117
- Intramammaries	0	33	91	0	0	4	0	0	73	153	13	0	<1	367
- Micellaneous b)	76	1,172	56	14	0	79	688	63	5,853	1,496	2,920	1,485	4	13,905
Total	402	10,573	638	49	535	3,211	11,207	7,685	22,681	11,825	14,687	32,727	296	116,516

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 is shown. 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. Therefore 80% of the antimicrobial consumption in cattle is registered as miscellaneous

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

d) These figures are not split on animal species but represent all medicine purchased by the veterinary practitioners for use in practice

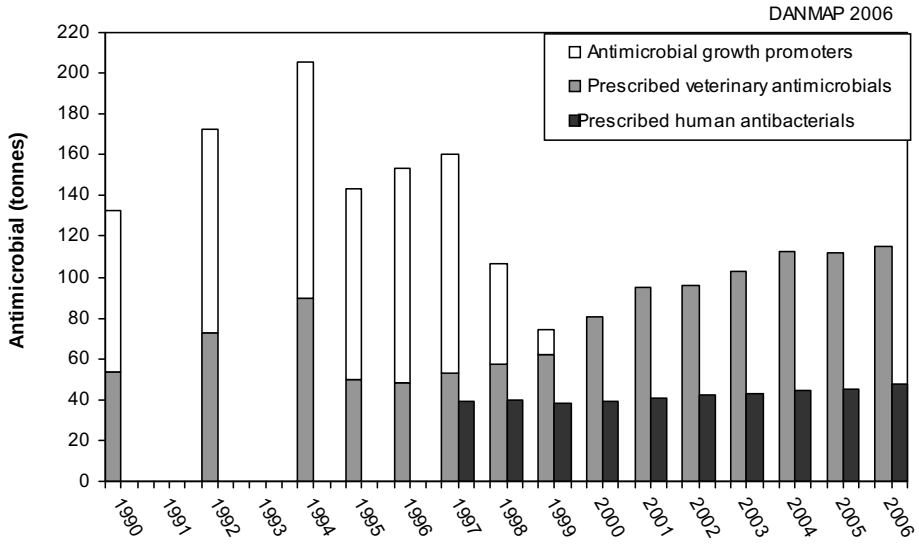


Figure 2. Consumption of prescribed antimicrobials and growth promoters in animal production and prescribed antibacterials in humans, Denmark

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). 2001-2006: Data from VetStat.

produced in Denmark. In the EU, the only antimicrobial agents approved for growth promotion after 1999, were flavomycin, avilamycin, salinomycin, and monensin. By 2006, these agents were no longer approved for use as growth promoters in the EU.

Antimicrobial consumption in pigs

The total consumption of antimicrobials in pigs decreased slightly from 2005 to 2006. Table 5 shows a decrease in consumption by 1.2% to 91.4 tonnes in 2006, while the number of pigs produced remained unchanged (Table 1). The prescription of 91.4 tonnes of antimicrobials in pigs amounts to 249 million Defined Animal Doses (ADD), mainly administrated to weaners (75%) followed by finishers (20%) (Table 6). Macrolides, tetracyclines and pleuromutilins remained the most commonly used antimicrobials in pigs, and the use of tetracyclines continued to rise in 2006, while macrolides decrease further compared to 2004 (Figure 3). From 2005 to 2006, the number of ADDs prescribed for sows/piglets increased by 2.2% while the number of ADDs prescribed to weaners and finishers decreased by 1.1% and 3.9%, respectively (Table 6).

Trends in the most commonly used antimicrobials for treatment of pigs from 2001 to 2006 are shown in Figure 3 as ADD_{kg}/pig produced. There are three general patterns of consumption visible; 1) a near-constant increase (tetracycline, beta-lactamase sensitive penicillin and cephalosporins), 2) a near-constant reduction (fluoroquinolones and aminoglycosides), and 3) an increase with a peak in 2004/05 followed by a reduction (macrolides, lincosamides, pleuromutilin, and other penicillins). We assume that the changes in trends occurring in 2004/05 can be attributed to the introduction of treatment guidelines by the veterinary authorities.

The consumption of fluoroquinolones in pigs further decreased to 2.6 kg in 2006, as compared to 94 kg in 2001. The decrease from 2001 and onwards was most likely due to restrictions on use of fluoroquinolones in food animals, implemented in 2002. Consumption of cephalosporins in pigs comprises 0.1% of the total antimicrobial consumption in pigs in 2006. However, the consumption of cephalosporins has increased by 300% from 24 kg in 2001 to 98 kg in 2006. The majority of all kg active compound cephalosporin prescribed for pigs were for sow/piglets (Figure 4). The main indication for prescribing cephalosporins in all age groups was diseases in extremities, central nervous system and skin. Cephalosporins are like fluoroquinolones regarded as criticaly important antimicrobials in human medicine and usage of 3rd and

Table 6. Consumption of antimicrobials in pigs given as Animal Daily Doses (ADDs) from 2003 to 2006, Denmark

DANMAP 2006

		Pharmacies and feed mills a)													
Age group		Sows/piglets				Weaners				Finishers				Age not given	
Animal standard weight		200 kg				15 kg				50 kg				50 kg	
		2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006	2005	2006
ATC_{vet} group	Therapeutic group	ADD (1,000s)													
QJ01A	Tetracyclines	915	927	877	1,013	32,367	38,207	43,419	52,339	11,138	12,212	13,096	14,843	824	1,154
QJ01B	Amphenicols	6	7	7	7	84	105	71	36	22	32	26	25	3	1
QJ01CE	Penicillins, β−lact. sen. b)	2,015	2,230	2,315	2,321	2,903	3,969	4,089	3,865	5,121	6,323	7,262	7,426	549	512
QJ01CA/CR	Penicillins, other	1,118	1,201	1,169	1,150	12,720	16,348	14,414	11,933	2,420	3,500	3,282	2,805	318	392
QJ01DA	Cephalosporins	99	113	132	148	254	263	267	290	56	60	62	49	10	12
QJ01E/QP51	Sulfonam./trimeth.	1,084	1,217	1,301	1,353	4,146	5,454	6,124	4,483	174	232	238	151	181	180
QJ01FA	Macrolides	746	773	774	803	41,173	52,157	50,484	47,924	12,255	12,274	12,676	10,875	881	885
QJ01FF	Lincosamides c)	580	588	574	541	19,821	22,411	19,192	16,492	4,412	4,622	4,382	3,607	330	319
QJ01G/A07AA	Aminoglycosides	237	220	171	154	22,231	21,469	19,782	19,410	194	124	237	213	75	148
QA07AA10	Colistin (local GI)	23	24	23	24	2,910	3,017	2,656	2,793	18	14	13	18	21	22
QJ01MA	Fluoroquinolones	21	3	4	3	11	7	3	<1	5	3	2	0	<1	2
QJ01R	Combinations	703	669	661	643	2,211	3,075	3,589	3,478	423	380	369	292	84	75
QJ01X	Pleuromutilins	946	987	811	856	19,759	24,811	23,494	22,539	8,478	10,177	10,157	9,479	735	699
QJ51	Intramammaries	4	2	1	<1	<1	<1	0	<1	<1	1	<1	<1	<1	<1
QG01AA	Gynecologic (local)	0	<1	<1	-	0	0	0	-	0	0	<1	<1	0	<1
Total		8,498	8,958	8,820	9,016	160,590	191,294	187,585	185,581	44,717	49,956	51,802	49,784	4,009	4,401

a) Consumption in veterinary practice comprises an estimated 1-2%. These data are not included
b) Beta-lactamase sensitive penicillins
c) Lincosamide/spectinomycin combinations

4th generation cephalosporins in animals might select for bacteria producing extended-spectrum beta-lactamases (ESBLs). ESBL mediated resistance is of increasing concern and concomitant with the observed increase in cephalosporin consumption in pigs, isolation of ESBL-producing pathogenic *E. coli* from diseased pigs is occurring more often than in previous years. In addition, the first ESBL-producing *Salmonella* Typhimurium was isolated from a healthy pig in Denmark in 2006 (Further details are presented in the textbox page 68).

Antimicrobial consumption in cattle

In 2006, the antimicrobial consumption in cattle was estimated at 15 tonnes, or 13% of the total consumption in production animals, which is approximately the same level of consumptions as in 2005. Only 20% of the prescribed antimicrobials for cattle are purchased at pharmacies. This amount is registered in Table 5 as used in cattle. The remaining approximately 80% was either administered or sold by veterinary practitioners to the farmer. This consumption is registered as miscellaneous in Table 5. In addition, 99% of all intramammaries are used in cattle.

In both 2005 and 2006, a total of 367 kg antimicrobials were used as intramammaries of which 25% or 91 kg was cephalosporins (Table 5). In total, 104 kg cephalosporins were used in cattle in 2006. Currently there is no national surveillance of antimicrobial resistance in mastitis pathogens in Denmark and therefore the occurrence of ESBL producing mastitis pathogens are unknown. In Denmark, It is common practice that after mastitis treatment and while there is still antimicrobial residues in the milk young calves are feed this milk. This practice might pose a risk that calves in a young age are exposed to cephalosporins.

Consumption of antimicrobials in poultry

In 2006, 421 kg active compound of the total veterinary antimicrobial consumption was prescribed for use in poultry production (Table 5). In addition, an estimated 81 kg were either administered or handed out by veterinary practitioners and therefore registered as miscellaneous, which made the total consumption amount to 502 kg, in 2006. This is a 20% increase compared to 2005. The most commonly used antimicrobial in the Danish poultry production was amoxicillin and it accounted for 84% of all prescribed antimicrobials in poultry in 2006. The second most

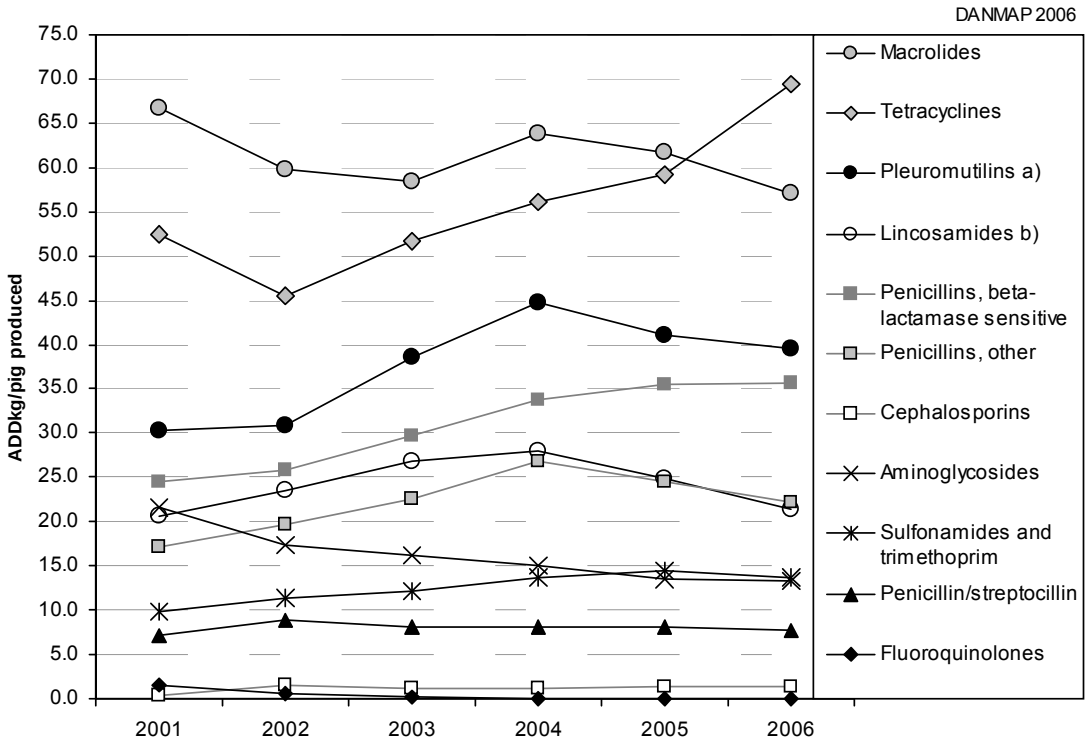


Figure 3. Trends in antimicrobial consumption (in ADDkg) in pigs, 2001-2006, Denmark

Amphenicols, colistin, intramammaries and gynecologicals are not included in the figure. Data from veterinary practice are not included (amounts to <2%)

a) Pleuromutilins comprise primarily tiamulin

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

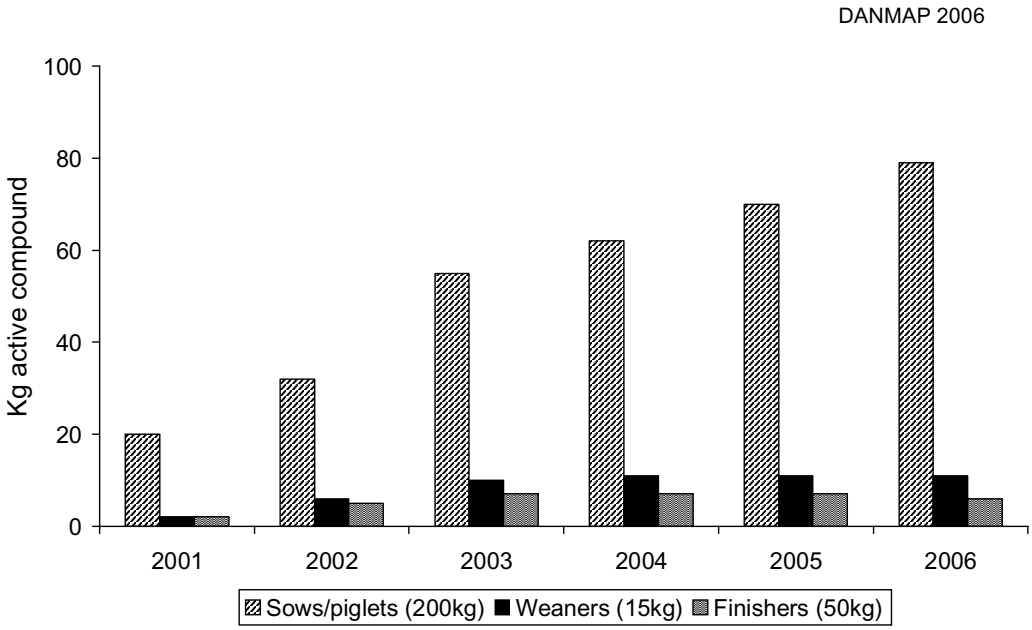


Figure 4 . Consumption of cephalosporins in pigs given as kg active compound from 2001 to 2006, Denmark

used antimicrobial in poultry was fluoroquinolones (21 kg), which made poultry the type of production animal with the highest fluoroquinolone consumption in 2006. Most of the fluoroquinolones prescribed for poultry are in Table 5 registered as used in poultry. The rest is registered as miscellaneous in Table 5.

The consumption of prescribed antimicrobials in turkeys increased from 93 kg active compound (10 million ADD_{kg}) in 2005 to 194 kg (15 million ADD_{kg}) in 2006 (Table 5 and Table 8). This increase accounted for most of the observed increase in antimicrobial consumption in poultry from 2005 to 2006.

The consumption of prescribed antimicrobials in broilers decreased by 20% from 2005 to 2006, however in rearing for broiler production, consumption increased by 45% and reached the same level as in 2004 (Table 7). In layers and rearing for layers, the consumption decreased further by 37% from 2005 to 2006 (Table 7).

In 2005, it was estimated that 1 mill pheasants, 0.5 mill ducks and 0.1 mill other birds was raised as game birds in Denmark. In 2006, an estimated 2.1 million ADDkg antimicrobials were prescribed for this type of birds, which is high compared to the size of the production. The same high consumption was observed in previous years (Table 8).

Antimicrobial consumption in fish, fur animals and companion animals

Based on sales from pharmacies antimicrobial consumption in fur animals increased by an estimated 19% from 2005 to 2006. This apparent increase should be interpreted with caution, as the figures do not include sales in veterinary practice. However, the registered sales of antimicrobials for fur animals from pharmacies have increased by 157% from 659 kg in 2001 to 1,694 kg in 2006. In the same period the number of fur breeding animals (mink, fox, chinchilla and castor rex) has increased by 17% from 2.3 million to 2.7 million breeding animals.

In 2006, the consumption of antimicrobials in aquaculture increased by 73% to 4,180 kg compared to 2,406 kg in 2005. The most likely reason for this increase was the relative high temperatures during most of 2006. The increased consumption was equally distributed between freshwater and marine

aquaculture. However, the antimicrobial consumption per kg fish produced was significantly higher in marine aquaculture (287 mg/kg fish) compared to freshwater aquaculture (64 mg/kg fish). The most commonly used antimicrobial in aquaculture was sulfonamide/ trimethoprim, which accounted for approximately 75% of the total consumption.

The use of antimicrobials in pet animals was an estimated 1.5 - 2 tonnes, including consumption in practice. The most commonly used antimicrobials in pet animals were broad-spectrum penicillins followed by cephalosporins. The consumption of cephalosporins increased further to 354 kg compared to 313 kg in 2005, corresponding to 55% of the total veterinary use of cephalosporins in both 2005 and 2006. Fluoroquinolone consumption in pet animals amounted to an estimated 24 kg or 49% of the total veterinary consumption of fluoroquinolones in 2006 (In Table 5 the 24 kg are composed of „Pet animals“, „Small animal practice“ and partly „miscellaneous“).

Table 7. Consumption of prescribed antimicrobials in domestic fowl given as Animal Daily Doses per kg (ADDkg), Denmark

DANMAP 2006

Production type		Broilers				Rearing for broiler production				Layers and layer rearing				Production type unknown a)			
		2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
ATC_{vet} code	Therapeutic group	ADD_{kg} (1,000s)															
QA07AA	Aminoglycosides	0	0	0	0	0	0	0	0	0	0	0	0	200	300	81	133
QJ01A	Tetracyclines	60	2	32	0	0	0	0	0	540	2	8	0	91	106	56	148
QJ01CA	Amoxicillin	2,988	4,469	3,708	2,570	1,358	5,760	3,896	6,100	350	1,066	675	437	2,342	3,657	2,223	3,538
QJ01E/QP51	Sulfonamides b)	8	56	48	40	0	0	0	15	328	210	228	125	348	439	165	178
QJ01FA	Macrolides	0	29	3	0	0	0	0	0	0	0	0	11	186	90	3	4
QJ01FF	Lincosamides c)	0	20	0	0	0	0	0	0	0	8	0	0	0	4	40	0
QJ01MA	Fluoroquinolones	270	603	171	550	80	420	400	104	0	100	0	0	411	131	40	162
QJ01X	Pleuromutilins	0	75	0	0	0	0	0	0	3	0	3	0	5	3	5	0
Total		3,325	5,254	3,962	3,160	1,438	6,180	4,296	6,219	1,220	1,386	913	573	3,582	4,729	2,613	4,162

Includes data from all sources (pharmacies, feedmills and veterinary practice).
a) May include other species than domestic fowl
b) Includes sulfaclozin, a coccidiostat/antibacterial on prescription
c) Lincomycin in combination with spectinomycin

Table 8. Consumption of antimicrobials in other than domestic fowl given as Animal Daily Doses per kg (ADDkg), Denmark

DANMAP 2006

Production type		Turkeys				Ducks, geese				Game birds			
		2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
ATC_{vet} code	Therapeutic group	ADD_{kg} (1,000s)											
QA07AA	Aminoglycosides	0	200	100	0	0	0	0	0	100	167	100	12
QJ01A	Tetracyclines	0	0	60	150	154	14	0	0	128	148	94	76
QJ01CA	Amoxicillin	10,867	4,871	8,363	14,083	250	400	375	1,025	904	966	1,852	1,750
QJ01E/QP51	Sulfonamides a)	58	36	68	45	0	0	0	0	316	459	398	235
QJ01FA	Macrolides	0	7	0	0	0	11	12	1	273	113	177	36
QJ01FF	Lincosamides b)	0	0	100	0	0	0	0	0	0	0	14	8
QA07AA10	Colistin (local GI)	0	0	0	0	0	0	0	0	0	0	0	15
QJ01MA	Fluoroquinolones	340	1,607	1,260	1,040	0	150	0	0	1	30	0	10
QJ01X	Pleuromutilins	0	0	0	0	0	3	0	0	10	18	13	0
Total		11,264	6,721	9,950	15,318	404	578	387	1,026	1,732	1,900	2,647	2,141

a) Includes sulfaclozin, a coccidiostat/antibacaterial on prescription
b) Lincomycin in combination with spectinomycin

Antimicrobial consumption in humans

Overall

In 2006, the overall consumption of antibacterials for systemic use (ATC group J01, 2006 definition) in humans in Denmark increased to 33.5 million DDDs or 16.9 DDD/1,000 inhabitant-days representing an increase of 3.1% compared to 2005. The percentage of DDDs prescribed in the primary health care sector remained stable at 90%. The distribution of the different classes of antibacterials used in the primary health care sector and in hospitals is shown in Figure 5. The two most used classes in both sectors were beta-lactamase sensitive penicillins and penicillins with extended spectrum. The third and fourth most used classes were macrolides-lincosamides-streptogramins and tetracyclines in primary health care, and cephalosporins and fluoroquinolones in hospitals. Tetracyclines, nitrofuran derivatives, methenamine, macrolides-lincosamides-streptogramins and beta-lactamase sensitive penicillins were mostly used in the primary health care sector, whereas imidazoles, glycopeptides, cephalosporins and aminoglycosides were mostly used in hospitals (Figure 6). For combinations of penicillins with beta-lactamase inhibitors and for fluoroquinolones, the ratio consumption in primary health care / consumption in hospitals was around 2 / 1.

To follow overall changes in the consumption of antibacterials and to allow comparison with consumption of antibacterials in animals, total human consumption is presented in kilograms (Table 9). In 2006, 47.6 tonnes of antibacterials for systemic use were used in humans in Denmark, representing an increase of 2.3% as compared to 2005 and of 19.3% compared to 1997.

Primary health care sector

The consumption of antibacterials for systemic use in primary health care is presented in Table 10 as a number of DDDs per 1,000 inhabitant-days. In 2006, the overall consumption of antibacterials for systemic use in the primary health care sector was 15.2 DDD/ 1,000 inhabitant-days. Beta-lactamase sensitive penicillins still represented 35.4% of the total consumption of antibacterials followed by penicillins with extended spectrum (19.4%) and macrolides (15.2%). This distribution was similar to previous years.

Total consumption expressed in DDD/1,000 inhabitant-days increased by 3.1% as compared to 2005. Since 2000, there has been a small but steady increase in antibacterial consumption in DDD/1,000 inhabitant-days ranging from 2 to 5% yearly. Overall, antibacterial consumption increased by 24.3% between 2000 and 2006.

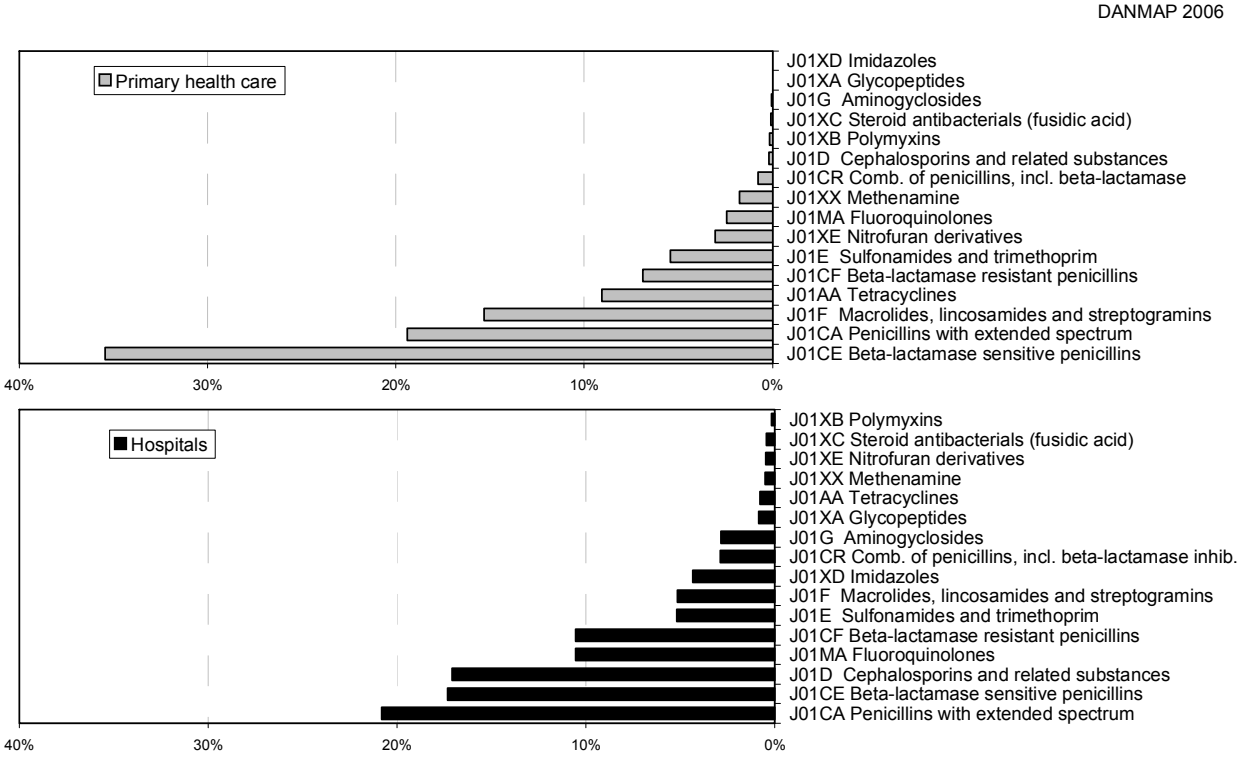


Figure 5. Distribution of the total number of DDDs of antibacterials in the primary health care sector and in hospitals, Denmark

DANMAP 2006

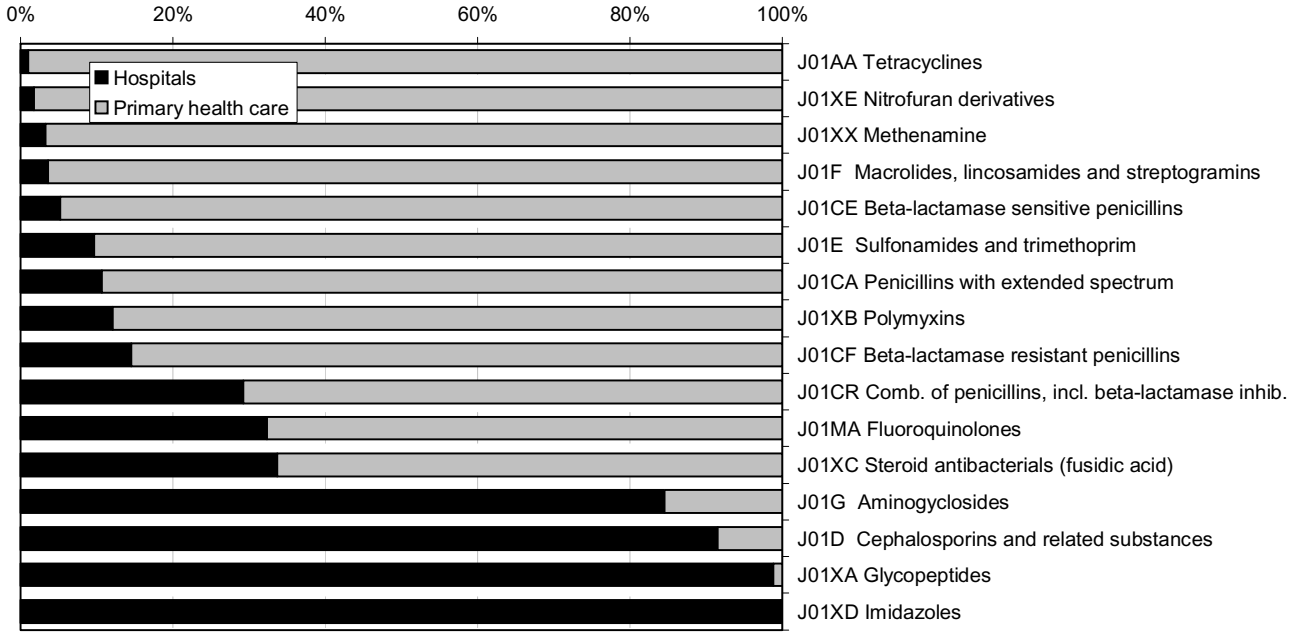


Figure 6. Distribution of the total number of DDDs of antibacterials between the primary health care sector and hospitals, Denmark

Table 9. Consumption of antibacterials for systemic use in humans (kg active compound), Denmark

These data include data from both primary health care and hospitals and have been re-calculated from original data expressed as a number of DDDs. For monitoring human primary health care and hospitals, the recommended way of expressing consumption is DDD per 1,000 inhabitant-days and DDDs per 1,000 occupied bed-days, respectively (see Tables 10 and 14)

DANMAP 2006

ATC group a)	Therapeutic group	Year									
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
J01AA	Tetracyclines	1,519	1,486	1,383	1,486	1,475	1,501	1,542	1,636	1,747	1,838
J01B	Amphenicols	1	1	0	0	1	0	0	0	0	0
J01CA	Penicillins with extended spectrum	5,525	5,477	5,202	5,141	5,385	5,356	5,295	5,346	5,561	5,740
J01CE	Beta-lactamase sensitive penicillins	18,840	19,969	18,825	19,749	20,730	21,263	21,630	22,230	22,520	22,853
J01CF	Beta-lactamase resistant penicillins	1,919	2,120	2,425	2,655	3,230	3,738	4,075	4,377	4,564	4,856
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	49	56	52	93	146	249	336	480	533	724
J01D	Cephalosporins and related substances	626	614	650	692	739	811	830	894	1,475	1,649 b)
J01EA	Trimethoprim and derivatives	245	256	258	262	280	293	307	334	351	383
J01EB	Short-acting sulfonamides	3,503	3,497	3,296	3,142	3,113	3,092	3,064	3,067	2,987	2,874
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	350	330	286	291	289	288	273	185	208	208
J01FA	Macrolides	4,227	4,536	4,147	4,040	4,089	4,150	3,876	3,743	3,772	3,524 c)
J01FF	Lincosamides	25	34	29	29	37	40	45	53	52	66 b)
J01G	Aminoglycosides	61	35	42	32	30	31	28	31	32	28
J01MA	Fluoroquinolones	384	405	383	344	398	451	611	722	866	982 b)
J01MB	Other quinolones	15	17	16	0	0	0	0	0	0	0
J01XA	Glycopeptides	25	27	33	37	36	42	43	46	51	55
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70	59	59	58	52	62	65
J01XD	Imidazoles	129	129	142	155	168	179	191	195	206	219
J01XE	Nitrofuran derivatives (nitrofurantoin)	141	144	145	151	155	163	166	171	177	185
J01XX05	Methenamine	2,234	2,132	1,956	1,788	1,637	1,662	1,590	1,473	1,383	1,346 d)
J01XX08	Linezolid	0	0	0	0	0	3	4	5	10	14
J01	Antibacterials for systemic use (total) e)	39,892	41,338	39,348	40,157	41,997	43,371	43,964	45,040	46,557	47,608

a) From the 2006 edition of the ATC classification system

b) Since 2005, the kg active compound 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

c) When two different DDDs of an antimicrobial existed e.g. for erythromycin, an average DDD was used. The lowest and the highest claculated limits are (2,701 - 4,348)

d) When two different DDDs of an antimicrobial existed e.g. for methenamine, an average DDD was used. The lowest and the highest claculated limits are (1,090 - 1,614)

e) Does not include polymyxins

Table 10. Consumption of antibacterials for systemic use in human primary health care (DDD/1,000 inhabitant-days), Denmark

DANMAP 2006

ATC group a)	Therapeutic group	Year									
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	0.99	1.04	1.07	1.17	1.27	1.38
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	2.47	2.51	2.52	2.63	2.78	2.95
J01CE	Beta-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	4.91	5.00	5.07	5.20	5.28	5.39
J01CF	Beta-lactamase resistant penicillins	0.34	0.40	0.48	0.52	0.65	0.77	0.85	0.92	0.97	1.05
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.02	0.03	0.02	0.02	0.03	0.04	0.05	0.06	0.07	0.12
J01D	Cephalosporins and related substances	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.03
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	0.35	0.36	0.38	0.41	0.44	0.47
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	0.36	0.36	0.36	0.36	0.35	0.35
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	0.08	0.04	0.03	0.03	0.04	0.03	0.03	0.00	0.00	0.00
J01FA	Macrolides	2.03	2.28	2.17	2.02	2.10	2.15	2.13	2.23	2.41	2.31
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
J01GB	Aminoglycosides	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.22	0.23	0.20	0.15	0.17	0.18	0.25	0.28	0.32	0.37
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	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	0.39	0.41	0.42	0.43	0.45	0.46
J01XX05	Methenamine	0.46	0.43	0.40	0.36	0.33	0.34	0.32	0.30	0.28	0.27
J01XX08	Linezolid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01	Antibacterials for systemic use (total)	12.24	12.76	12.14	12.24	12.86	13.26	13.53	14.06	14.75	15.21

a) From the 2006 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Almost 75% of the increase between 2005 and 2006 was due to an increased consumption of penicillins with extended spectrum (+0.17 DDD/1,000 inhabitant-days), tetracyclines (+0.10 DDD/1,000 inhabitant-days), beta-lactamase sensitive penicillins (+0.11 DDD/1,000 inhabitant-days) and beta-lactamase resistant penicillins (+0.08 DDD/1,000 inhabitant-days).The increase in consumption of penicillins with extended spectrum was mainly due to pivmecillinam, of which the use increased from 0.97 to 1.14 DDD/1,000 inhabitant-days between 2005 and 2006. The most important part of consumption of tetracyclines in Denmark was composed by tetracycline, which is mainly used for the treatment of acne, and doxycycline which is recommended for malaria prophylaxis. During the same period, the antimicrobial classes that increased the most were combinations of penicillins including inhibitors and fluoroquinolones with increases by 71.4% from 0.07 to 0.12 DDD/1,000 inhabitant-days and by 15.6% from 0.32 to 0.37 DDD/1,000 inhabitant-days, respectively. The overall increases of these classes between 1997 and 2006 were 500.0% and 68.2%, respectively. The use of beta-lactamase resistant penicillins also increased by 208.8%. Conversely, the use of methenamine decreased by 41.6% between 1997 and 2006.

Consumption in counties in Eastern Denmark was more homogenous than consumption in Western Denmark, which showed wider variations. Most of the Eastern counties had an overall consumption higher than the Danish average (Figure 7). All the Danish counties have shown an overall increase in antibacterial consumption and similar variations during the period 1997-2006 with the exception of Bornholm County. This suggests that the determinants behind these variations were shared by most counties, although these determinants are presently not known. Inversely, Bornholm County, which had the lowest consumption in 1997 showed the sharpest increase during 1997-2006 and now has one of the highest levels of consumption in Denmark. The reasons behind this rapid increase presently are unknown. Among other counties, the difference between the county with the lowest and the highest consumption remained about 1.2 times, i.e. the county with the highest consumption used about 20% more antibacterials in primary health care than the county with the lowest consumption. Finally, the ranking of counties according to their consumption was the same in 1997 and in 2006 (Spearman's rho = 0.63, p = 0.01). While some counties always had a high consumption during 1997-2006, e.g. Ribe County (second highest in 1997 and fifth in 2006), others always had a low consumption, e.g. Aarhus County (the lowest in 1997 and in 2006). This suggests the existence of county-specific determinants that influence the prescription of antibacterials in Danish counties.

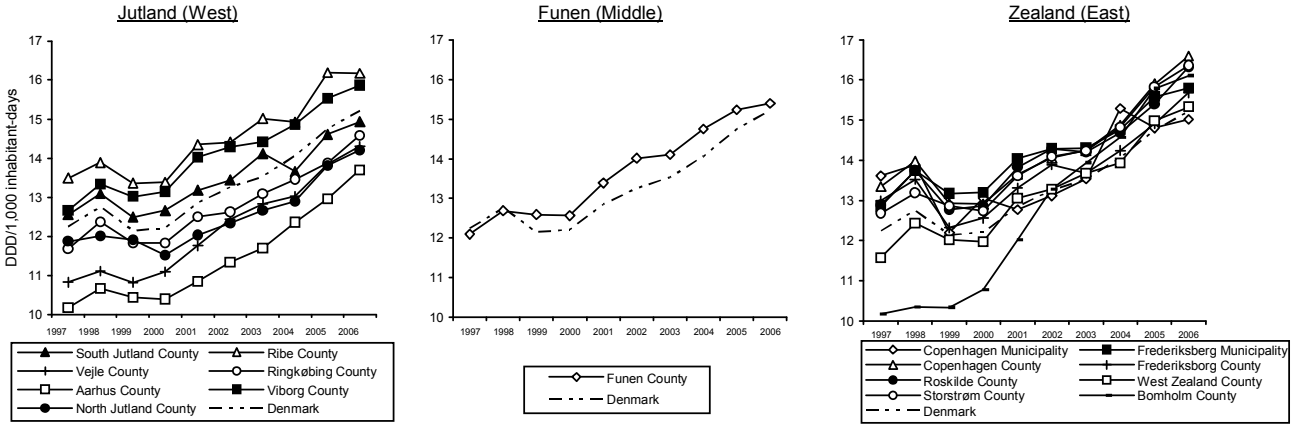


Figure 7. Trends in total use of antibacterials in primary health care in individual counties, Denmark

The increase in antibacterial consumption in primary health care was more important for some classes than others. Figure 8 shows the changes in consumption for selected classes of antibacterials for 1997-2006. The consumption of these selected classes was relatively stable between 1997 and 2000. However, consumption of fluoroquinolones and of combinations of penicillins with beta-lactamase inhibitors more than doubled since 2000.

Consumption of fluoroquinolones (primarily ciprofloxacin) continued to increase, from 0.32 DDD/ 1,000 inhabitant-days in 2005 to 0.35 in 2006. The most likely explanation for this is a markedly reduced price per DDD due to the opening of the market to generic ciprofloxacin (EPI-NEWS 2004, no. 41: http:// www.ssi.dk/sw18090.asp). Price is an important issue when prescribing antibiotics, however, the choice of antibiotic treatment should be based on recommendations rather than on price. Ciprofloxacin, as well as other fluoroquinolones, are potent antibiotics which should be reserved for treatment of serious infections, primarily in hospitals. It is thus essential that fluoroquinolones do not replace narrow spectrum antibiotics in the treatment of uncomplicated infections. National guidelines recommend prescribing ciprofloxacin for complicated and recurrent urinary tract infections, infections caused by bacteria resistant to other antibiotics, pyelonephritis, and certain gastrointestinal infections. In the case of serious infections caused by *Pseudomonas* or mycobacteria, fluoroquinolones should be used in combination with another antibiotic to prevent emergence of resistance. In general, the advice of a clinical microbiologist is recommended before using fluoroquinolones for the treatment of complicated infections.

Concomitantly, there has been a rapid and worrisome increase in fluoroquinolone resistance in *E. coli* obtained from urine isolates during the past years (see page 72). Prescribers should again be alerted on the ecological risks of a continued increase in fluoroquinolone consumption. Rational prescribing of ciprofloxacin and other fluoroquinolones is required to avoid unnecessary use and development of resistance, thus preserving the unique role of fluoroquinolones in the treatment of complicated infections.

In 2006, consumption of macrolides decreased by 4.2% compared to 2005. This decrease was due, firstly to the continued decrease in erythromycin consumption and secondly to a halt in the increase in roxithromycin consumption (Figure 9). In 2004 and 2005, the increase in roxithromycin consumption was likely due to an outbreak of *Mycoplasma pneumoniae*. In 2006, roxythromycin became the most used macrolide in Denmark. This coincided with a change in the guide-lines, which occurred in September 2006 and now recommend clarithromycin as first-choice macrolide. Antimicrobial consumption in primary health care is also presented in Table 11 as a number of packages per 1,000 inhabitants, and in Table 12 as a number of treated patients per 1,000 inhabitants. In 2006, the overall consumption of antibacterials for systemic use in the primary health care sector was 635 packages/ 1,000 inhabitants or 310 treated patients/1,000 inhabitants. Total consumption expressed as a number of packages/1,000 inhabitants showed a decrease by 2.2% as compared to 2005, but an 8-year increase by 9.2% as compared to 1999. Total consumption expressed as a number of treated patients/1,000 inhabitants increased by 0.7% as compared to 2005, and by 5.3% as compared to 1999.

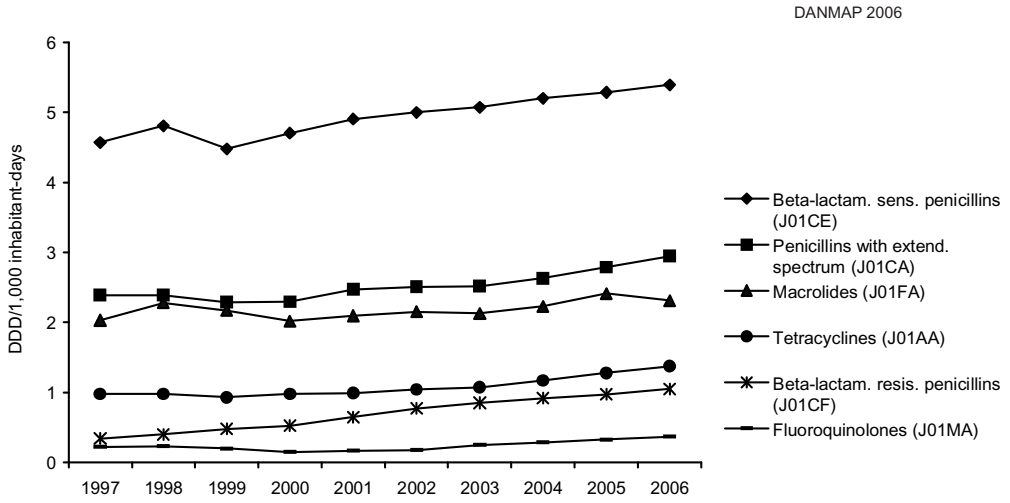


Figure 8. Consumption of selected antibacterials for systemic use in primary health care, Denmark

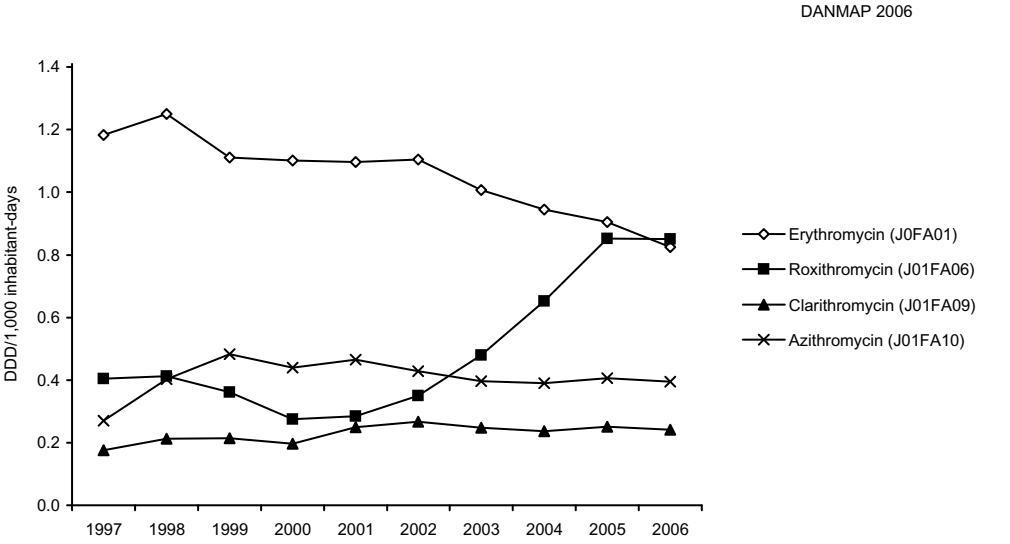


Figure 9. Consumption of macrolides in primary heath care, Denmark

Table 11. Consumption of antibacterials for systemic use in human primary health care (No. packages/1,000 inhabitants), Denmark

DANMAP 2006

ATC group a)	Therapeutic group	Year									
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
J01AA	Tetracyclines	24.3	24.0	22.2	22.8	22.4	21.7	21.6	22.5	23.8	23.9
J01CA	Penicillins with extended spectrum	111.0	111.2	102.9	103.7	110.9	111.8	111.5	115.3	119.9	120.1
J01CE	Beta-lactamase sensitive penicillins	246.4	256.0	232.5	243.7	251.0	254.4	254.5	253.7	251.1	244.4
J01CF	Beta-lactamase resistant penicillins	15.0	17.3	21.5	24.0	30.1	37.5	41.9	43.0	44.4	44.1
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	1.1	1.3	1.1	1.1	1.2	1.7	2.0	2.5	3.0	4.0
J01D	Cephalosporins and related substances	0.9	1.0	1.0	1.0	1.3	1.4	1.3	1.4	1.6	1.7
J01EA	Trimethoprim and derivatives	7.6	7.9	7.8	7.9	8.2	8.8	9.3	10.2	10.6	10.8
J01EB	Short-acting sulfonamides	51.0	51.4	48.9	47.8	47.8	47.6	47.9	48.3	47.5	45.9
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	2.6	1.6	1.3	1.4	1.4	1.3	1.0	0.0	0.0	0.0
J01FA	Macrolides	91.8	108.0	106.3	97.3	102.2	102.8	99.8	102.7	110.3	102.1
J01FF	Lincosamides	0.3	0.3	0.3	0.4	0.5	0.6	0.6	0.7	1.1	1.4
J01GB	Aminoglycosides	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
J01MA	Fluoroquinolones	13.9	14.6	12.7	9.7	10.6	11.0	13.8	16.2	18.3	19.5
J01XA	Glycopeptides	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2
J01XB	Polymyxins	2.8	2.8	2.9	2.8	2.1	2.0	2.0	2.1	2.0	1.5
J01XC	Steroid antibacterials (fusidic acid)	1.0	0.9	1.1	0.9	0.8	0.8	0.7	0.6	0.7	0.7
J01XE	Nitrofuran derivatives (nitrofurantoin)	9.7	10.0	9.8	10.4	10.4	11.1	11.3	11.7	12.3	12.5
J01XX05	Methenamine	4.5	4.2	3.8	3.5	3.2	3.2	2.6	2.4	2.3	2.0
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01	Antibacterials for systemic use (total)	584.6	612.9	576.6	578.5	604.4	618.0	622.3	633.6	649.3	635.0

a) From the 2006 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Between 1999 and 2006, the average total number of DDDs per treated patient increased from 15 to nearly 18, a relative increase of 18.9%, whereas the number of packages per treated patient remained stable at around 2 (Table 13).

Overall, each patient received an average 17.9 DDDs in 2 packages. When assuming that one package is prescribed for one prescription, the number of packages could be considered as a surrogate for the number of prescriptions when the latter are not available. For all classes, with the exception of aminoglycosides, polymyxins and methenamine, the number of DDDs per treated patient ranged between 3.9 and 41.1 and the number of packages per treated patient ranged between 1.4 and 3.5. For aminoglycosides, polymixins and methenamine, the number of DDDs per treated patient was 182.5, 182.5 and 234.6, respectively, and the number of packages per treated patient was 5.0, 37.5 and 4.8, respectively. Two hypotheses could explain the very high number of DDDs per treated patient for these three antimicrobial classes: the use of aminoglycosides and polymyxins by inhalation and the prophylactic use of methenamine for the treatment of chronic urinary tract infections. In general, the trends of the number of DDDs per treated patient and the number of packages per treated patient between 1999 and 2006 were identical to the trends in total use, with an increase in the number of DDDs per treated patient and no trend or a slight increase of the number of packages per treated patient. However, short-acting sulfonamides, lincosamides and steroid antibacterials showed a decrease or no trend in the number of DDDs per treated patient, and cephalosporins and lincosamides showed an increase in the number of packages per treated patient. Combinations of penicillins with beta-latamase inhibitors and tetracyclines showed the largest discrepancies between relative trends in the number of DDDs per treated patient and in the number of packages per treated patient (Figure 10). Indeed, the number of DDDs per treated patient increased by 46% and 67%, respectively, whereas the number of packages only increased by 6% and 1%, respectively. A change in the packaging, i.e. higher number of tablets per package or higher doses per tablet, could be explanations for these trends.

Hospitals

The consumption of antibacterials for systemic use in hospitals is presented in Table 14 as a number of DDD per 1,000 occupied bed-days and in Table 15 as a number of DDD per 1,000 discharged patients. Data on the number of hospital bed-days from the National Board of Health has been updated and corrected for 2004 and 2005. This update has led to only minor changes in the reported consumption.

Table 12. Consumption of antibacterials for systemic use in human primary health care (No. treated patients/1,000 inhabitants), Denmark.

DANMAP 2006

ATC group a)	Therapeutic group	Year							
		1999	2000	2001	2002	2003	2004	2005	2006
J01AA	Tetracyclines	12.1	12.0	11.8	11.5	11.4	11.6	12.0	12.3
J01CA	Penicillins with extended spectrum	66.7	65.6	69.4	69.2	68.8	70.6	73.0	75.8
J01CE	Beta-lactamase sensitive penicillins	163.9	168.9	173.3	173.4	172.6	171.2	170.2	171.3
J01CF	Beta-lactamase resistant penicillins	14.0	15.6	19.2	23.9	26.4	27.1	27.8	29.4
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.6	0.6	0.7	1.0	1.1	1.3	1.5	2.3
J01D	Cephalosporins and related substances	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5
J01EA	Trimethoprim and derivatives	4.1	4.1	4.2	4.5	4.6	5.0	5.4	5.6
J01EB	Short-acting sulfonamides	34.4	33.5	33.2	33.0	33.1	33.3	32.7	33.0
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.8	0.8	0.8	0.7	0.6	0.0	0.0	0.0
J01FA	Macrolides	73.5	65.7	67.7	66.9	64.1	65.9	70.7	67.0
J01FF	Lincosamides	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.5
J01GB	Aminoglycosides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01MA	Fluoroquinolones	9.2	7.0	7.5	7.7	8.9	10.8	12.2	13.1
J01XA	Glycopeptides	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
J01XC	Steroid antibacterials (fusidic acid)	0.6	0.5	0.5	0.4	0.3	0.3	0.3	0.4
J01XE	Nitrofuran derivatives (nitrofurantoin)	5.7	5.8	5.7	6.1	6.2	6.4	6.7	7.0
J01XX05	Methenamine	0.7	0.6	0.5	0.6	0.5	0.5	0.5	0.4
J01XX08	Linezolid	-	-	-	0.0	0.0	0.0	0.0	0.0
J01 b)	Antibacterials for systemic use (total)	294.6	292.0	300.6	301.5	301.4	302.6	308.0	310.3

a) From the 2006 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 13. Number of DDD per treated patient and of packages per treated patient in primary health care, Denmark

DANMAP 2006

ATC group a)	Therapeutic group	Indicator	Year							
			1999	2000	2001	2002	2003	2004	2005	2006
J01AA	Tetracyclines	DDDs / patient	28.1	29.8	30.6	33.0	34.4	36.9	38.8	41.1
		Packages / patient	1.8	1.9	1.9	1.9	1.9	1.9	2.0	1.9
J01CA	Penicillins with extended spectrum	DDDs / patient	12.5	12.8	13.0	13.2	13.4	13.6	13.9	14.2
		Packages / patient	1.5	1.6	1.6	1.6	1.6	1.6	1.6	1.6
J01CE	Beta-lactamase sensitive penicillins	DDDs / patient	10.0	10.2	10.3	10.5	10.7	11.1	11.3	11.5
		Packages / patient	1.4	1.4	1.4	1.5	1.5	1.5	1.5	1.4
J01CF	Beta-lactamase resistant penicillins	DDDs / patient	12.5	12.2	12.4	11.8	11.8	12.4	12.7	13.0
		Packages / patient	1.5	1.5	1.6	1.6	1.6	1.6	1.6	1.5
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDDs / patient	11.6	11.8	15.9	14.7	16.6	17.2	16.8	19.3
		Packages / patient	1.7	1.8	1.7	1.7	1.8	2.0	2.0	1.8
J01D	Cephalosporins and related substances	DDDs / patient	19.7	18.8	25.5	24.9	18.3	15.9	23.8	22.8
		Packages / patient	2.7	2.6	3.0	3.2	3.3	3.0	3.5	3.5
J01EA	Trimethoprim and derivatives	DDDs / patient	28.2	29.5	30.4	29.3	30.0	29.8	30.0	30.6
		Packages / patient	1.9	1.9	2.0	2.0	2.0	2.0	2.0	1.9
J01EB	Short-acting sulfonamides	DDDs / patient	4.0	4.0	4.0	4.0	4.0	4.0	3.9	3.9
		Packages / patient	1.4	1.4	1.4	1.4	1.4	1.5	1.5	1.4
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	DDDs / patient	14.2	14.3	19.5	15.6	18.3	0.0	0.0	0.0
		Packages / patient	1.7	1.8	1.9	1.9	1.7	0.0	0.0	0.0
J01FA	Macrolides	DDDs / patient	10.8	11.3	11.3	11.7	12.1	12.4	12.4	12.6
		Packages / patient	1.4	1.5	1.5	1.5	1.6	1.6	1.6	1.5
J01FF	Lincosamides	DDDs / patient	20.3	17.4	15.2	11.1	11.1	9.6	9.1	14.9
		Packages / patient	1.7	1.9	2.1	1.8	1.8	1.8	2.8	2.9
J01GB	Aminoglycosides	DDDs / patient	0.0	0.0	0.0	121.7	121.7	122.0	121.7	182.5
		Packages / patient	0.0	0.0	0.0	6.7	3.3	3.3	3.3	5.0
J01MA	Fluoroquinolones	DDDs / patient	8.0	7.8	8.3	8.6	10.3	9.5	9.6	10.3
		Packages / patient	1.4	1.4	1.4	1.4	1.6	1.5	1.5	1.5
J01XB	Polymyxins	DDDs / patient	273.8	274.5	182.5	243.3	243.3	183.0	182.5	182.5
		Packages / patient	72.5	70.0	52.5	66.7	66.7	52.5	50.0	37.5
J01XC	Steroid antibacterials (fusidic acid)	DDDs / patient	11.8	14.9	7.6	8.7	11.1	12.2	11.1	10.4
		Packages / patient	1.8	1.8	1.7	1.9	2.1	2.0	2.1	2.0
J01XE	Nitrofuran derivatives (nitrofurantoin)	DDDs / patient	23.1	23.9	24.8	24.5	24.8	24.6	24.5	24.0
		Packages / patient	1.7	1.8	1.8	1.8	1.8	1.8	1.8	1.8
J01XX05	Methenamine	DDDs / patient	202.8	212.5	227.3	225.6	220.4	224.1	222.2	234.6
		Packages / patient	5.3	5.6	6.0	5.8	4.9	4.9	5.0	4.8
J01	Antibacterials for systemic use (total)	DDDs / patient	15.0	15.3	15.6	16.0	16.4	17.0	17.5	17.9
		Packages / patient	2.0	2.0	2.0	2.0	2.1	2.1	2.1	2.0

a) From the 2006 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Total consumption in hospitals increased by 52% from 421 to 639 DDD/1,000 occupied bed-days between 1997 and 2005 and increased by 54% from 421 to an estimated 649 DDD/1,000 occupied bed-days between 1997 and 2006. The increase from 1997 to 2005 in consumption was due to a 24.7% increase in the number of DDDs of antibacterials registered by hospital pharmacies (from 2.6 million DDDs in 1997 to 3.2 million DDDs in 2005), while there was a concurrent 15.5% decrease in the total number of hospital bed-days registered in Denmark in the same period. When expressed as a number of DDD per 1,000 discharged patients the total consumption in hospitals increased by 12.6% from 2,413 to 2,717 DDD/1,000 discharged patients in 2005 and by 13.6% from 2,413 to an estimated 2,738 DDD/1,000 discharged patients in 2006.

Between 2005 and 2006, antibacterial use in hospitals continued to increase whether it was expressed as a number of DDDs, in DDD/1,000 occupied bed-days or in DDD/1,000 discharged patients with increases of 1.7%, 4.5% and 0.7%, respectively. This increase, however, should be interpreted with caution since data for 2006 were calculated with an estimate of the numbers of occupied bed-days and of the numbers of discharged patients based on 2004 and 2005 data.

The increase in the number of DDDs of antibacterials used in Danish hospitals could be explained by an increase in the number of antibacterial treatments because of the admission of patients who more frequently required an antibiotic, e.g. for peri-operative antibiotic prophylaxis, by an increase in the daily dosage or by an increase in the frequency of combination therapies prescribed in hospitals. However, more detailed data on the quality of

antibacterial prescriptions including information on the indication for treatment, the dosage and the duration of treatment, are necessary to verify these hypotheses and interpret these changes in consumption.

In 2006, penicillins still represented more than 51% of hospital antimicrobial use in DDDs followed by cephalosporins and related substances (15.1%) and fluoroquinolones (10.8%). Since 1997, there has been a progressive switch towards newer antibacterial classes. The percentage of „broad-spectrum" antibacterials, i.e. cephalosporins, fluoroquinolones, combination of penicillins including beta-lactamase inhibitors and carbapenems, which represented 15.4% of the hospital antibacterial consumption in 1997, increased to 22.4% in 2003 and then to 28.8% in 2006. Glycopeptides (mainly vancomycin) still represented less than 1% of total hospital use in 2006 but their use increased from 3.3 to 5.8 DDD/1,000 occupied bed-days, and from 17.2 to 24.4 DDD/1,000 discharged patients between 2000 and 2006. This could be related to an increased frequency of patients infected with methicillin resistant staphylococci in Danish hospitals (see pages 74 and 75). Figure 11 illustrates this steady shift towards increasing consumption of newer, broad-spectrum antibacterials in Danish hospitals. In 1997, consumption of penicillins with extended spectrum still represented 26.6% of total hospital antibacterial consumption in Denmark, but this percentage decreased to 20.8% in 2005 and remained stable in 2006. The decrease mainly concerned amoxicillin whereas consumption of pivmecillinam increased. Consumption of macrolides and aminoglycosides continued to decrease and these classes represented less than 4.5% and 2.9% of the total hospital consumption in 2006, respectively. The increase of hospital use of combination of penicillins including beta-lactamase inhibitors by 61.2% between 2005 and 2006 is parallel to the increase by 71.4% in primary care.
The consequences of these changes in the pattern of antibacterial use could be a better coverage by empirical treatment of bacteria responsible for infection. However, this potential gain seems to be rapidly counterbalanced by the emergence of resistance towards newer classes of antibacterials.

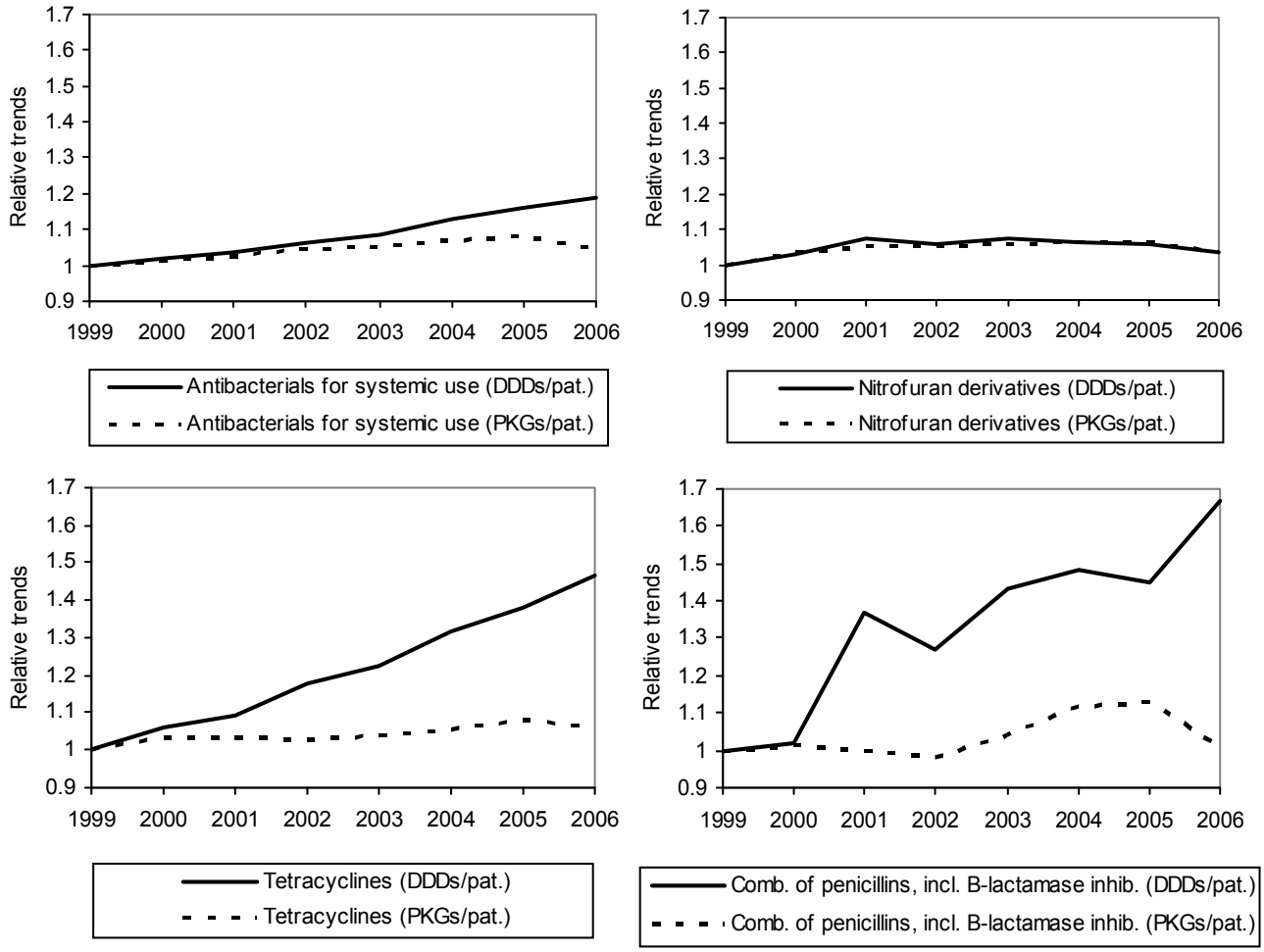


Figure 10. Examples of relative trends of the number of DDDs per treated patient (DDDs/pat.) and of the number of packages per treated patient (PKGs/pat.), Denmark

Table 14. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 occupied bed-days), Denmark

Data represented 97.5% of the total DDDs used in Danish hospitals in 2006. Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were excluded.

DANMAP 2006

ATC group a)	Therapeutic group	Year									
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006 b)
J01AA	Tetracyclines	3.4	3.3	2.8	2.9	2.8	3.2	3.1	3.5	3.4	4.1
J01CA	Penicillins with extended spectrum	112.1	113.4	112.7	115.7	116.1	115.2	119.2	117.9	133.1	135.4
J01CE	Beta-lactamase sensitive penicillins	80.2	89.2	95.3	100.3	106.5	114.3	121.2	123.1	126.0	111.8
J01CF	Beta-lactamase resistant penicillins	44.4	45.8	48.3	53.5	60.2	62.8	66.8	69.9	69.3	67.2
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.3	0.4	0.4	0.9	1.7	3.1	5.0	8.5	12.0	19.3
J01DB	First-generation cephalosporins	1.3	1.0	1.2	1.0	1.2	1.4	1.4	1.7	1.6	1.5
J01DC	Second-generation cephalosporins	39.9	41.9	44.0	47.4	52.1	58.5	63.9	70.6	81.0	88.7
J01DD	Third-generation cephalosporins	5.0	5.4	6.4	6.7	6.5	6.5	6.7	6.8	7.7	7.7
J01DF	Monobactams	0.6	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.0	0.0
J01DH	Carbapenems	3.6	2.4	3.2	3.9	4.2	6.0	6.9	8.6	11.5	13.5
J01EA	Trimethoprim and derivatives	4.2	4.4	3.8	3.7	4.3	4.2	4.4	4.2	4.2	4.3
J01EB	Short-acting sulfonamides	12.9	13.3	12.9	12.3	12.5	12.4	11.8	10.8	10.1	7.8
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	4.4	13.9	13.7	14.0	13.4	14.6	15.4	18.3	21.7	21.8
J01FA	Macrolides	34.5	35.3	33.5	32.8	32.6	32.3	30.9	29.4	29.7	29.5
J01FF	Lincosamides	1.3	1.8	1.5	1.6	1.7	1.9	1.9	2.3	2.5	3.2
J01GB	Aminoglycosides	33.8	23.6	27.6	21.3	18.5	17.7	17.4	20.3	20.1	18.8
J01MA	Fluoroquinolones	14.6	15.5	18.8	23.1	28.4	35.2	39.6	59.8	63.3	69.8
J01XA	Glycopeptides	2.1	2.3	2.8	3.3	3.2	3.7	4.2	4.7	5.4	5.8
J01XB	Polymyxins	0.4	0.2	0.3	0.4	0.3	0.3	0.3	0.6	1.3	1.3
J01XC	Steroid antibacterials (fusidic acid)	2.5	2.5	2.6	2.3	2.0	1.9	2.2	2.2	2.6	2.9
J01XD	Imidazole derivatives	14.2	14.4	16.2	17.9	19.6	21.1	23.7	24.7	27.0	28.7
J01XE	Nitrofuran derivatives (nitrofurantoin)	3.7	3.5	3.0	2.9	2.9	2.8	2.8	2.8	3.0	3.0
J01XX05	Methenamine	1.8	1.8	1.6	1.4	1.3	1.2	0.8	1.0	0.8	1.1
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.7	1.5	2.1
J01	Antibacterials for systemic use (total)	421.3	435.3	452.9	469.5	492.1	521.0	550.0	582.4	638.6	649.3

a) From the 2006 edition of the Anatomical Therapeutic Chemical (ATC) classification system

b) Estimate calculated with the actual number of DDDs and the estimated number of occupied bed-days based on the variation observed previously between 2004 and 2005

Table 15. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 discharged patients), Denmark

Data represented 97.5% of the total DDDs used in Danish hospitals in 2006. Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were excluded.

DANMAP 2006

ATC group a)	Therapeutic group	Year									
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006 b)
J01AA	Tetracyclines	19.3	18.2	15.4	15.4	14.8	16.3	14.8	15.8	14.5	17.1
J01CA	Penicillins with extended spectrum	641.8	634.4	608.7	610.5	604.9	578.0	566.2	535.2	566.1	570.6
J01CE	Beta-lactamase sensitive penicillins	459.3	498.9	514.6	529.1	555.0	573.5	575.9	558.5	535.8	471.4
J01CF	Beta-lactamase resistant penicillins	254.4	256.3	260.9	282.5	313.7	314.9	317.3	317.0	294.9	283.3
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	1.8	2.1	2.4	4.9	8.9	15.6	23.6	38.5	50.9	81.5
J01DB	First-generation cephalosporins	7.4	5.4	6.7	5.2	6.1	7.2	6.8	7.7	6.7	6.5
J01DC	Second-generation cephalosporins	228.7	234.2	237.7	250.2	271.5	293.6	303.6	320.5	344.7	274.0
J01DD	Third-generation cephalosporins	28.6	29.9	34.8	35.6	34.0	32.5	32.0	30.9	32.7	32.4
J01DF	Monobactams	3.3	0.7	0.8	0.9	0.5	0.2	0.2	0.2	0.2	0.0
J01DH	Carbapenems	20.6	13.5	17.2	20.6	21.9	29.9	32.7	38.8	48.8	57.0
J01EA	Trimethoprim and derivatives	24.0	24.6	20.7	19.5	22.6	21.0	21.0	19.0	17.8	18.3
J01EB	Short-acting sulfonamides	73.9	74.4	69.7	64.9	64.9	62.2	55.8	49.2	43.2	33.1
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	25.1	77.5	74.1	73.6	70.0	73.2	73.1	83.2	92.1	91.9
J01FA	Macrolides	197.6	197.3	180.8	173.1	170.1	161.9	146.7	133.3	126.5	124.4
J01FF	Lincosamides	7.6	10.0	8.1	8.5	9.0	9.5	9.0	10.4	10.5	13.4
J01GB	Aminoglycosides	193.6	131.9	149.0	112.5	96.4	88.6	82.8	91.9	85.5	79.3
J01MA	Fluoroquinolones	83.4	86.8	101.4	121.8	148.1	176.7	188.0	226.0	269.1	294.3
J01XA	Glycopeptides	12.3	13.1	15.3	17.2	16.6	18.8	19.9	21.2	22.8	24.4
J01XB	Polymyxins	2.5	1.4	1.8	2.1	1.5	1.7	1.5	2.7	5.4	5.4
J01XC	Steroid antibacterials (fusidic acid)	14.4	14.1	14.2	12.1	10.2	9.7	10.5	10.2	11.1	12.2
J01XD	Imidazole derivatives	81.5	80.6	87.6	94.5	102.0	106.0	112.5	112.1	114.7	120.8
J01XE	Nitrofuran derivatives (nitrofurantoin)	21.3	19.5	16.3	15.5	15.0	14.2	13.1	12.8	12.8	12.8
J01XX05	Methenamine	10.2	10.3	8.6	7.5	6.7	6.1	3.9	4.6	3.6	4.7
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	2.2	2.1	3.3	6.4	8.9
J01	Antibacterials for systemic use (total)	2,412.6	2,435.2	2,446.2	2,477.6	2,564.6	2,613.3	2,613.0	2,642.8	2,717.6	2,737.6

a) From the 2006 edition of the Anatomical Therapeutic Chemical (ATC) classification system

b) Estimate calculated with the actual number of DDDs and the estimated number of discharged patients based on the variation observed previously between 2004 and 2005

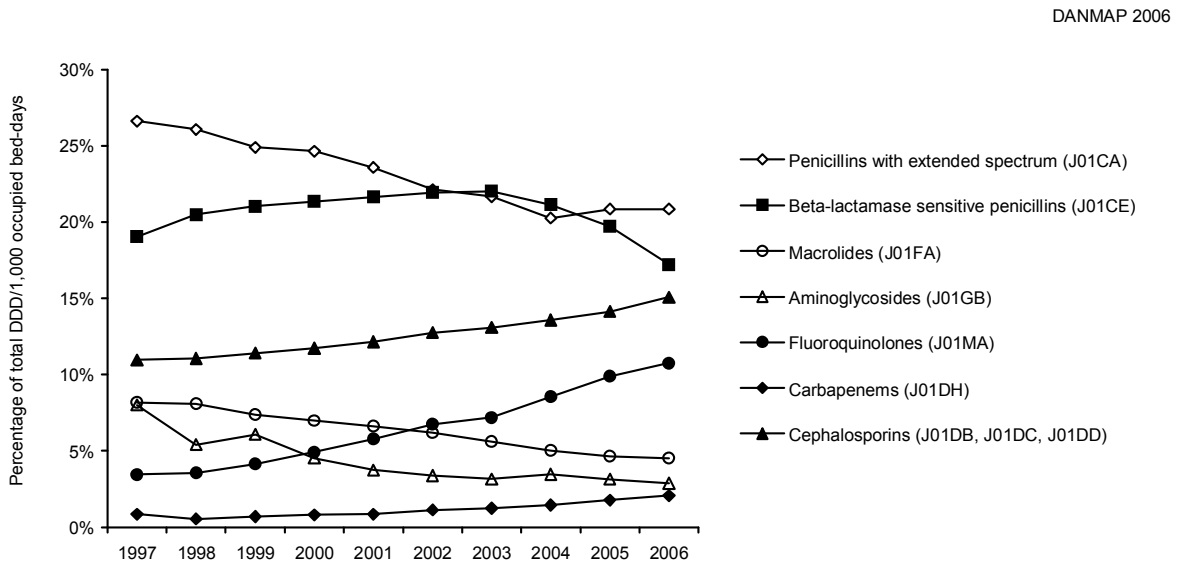


Figure 11. Percentages of total hospital consumption represented by selected classes of antibacterials for systemic use, Denmark

Resistance in zoonotic bacteria

Salmonella

Salmonella Typhimurium (24.8%) and *Salmonella* Enteritidis (33.9%) are the most common serovars among the *Salmonella* serovars isolated from human infections. Susceptibility data on these two serovares are presented in this, and in the previous DANMAP reports. In this report, we furthermore present susceptibility data on other less frequent *Salmonella* serovars in humans (see textbox page 42).

The phage type distributions of *Salmonella* Typhimurium and *Salmonella* Enteritidis are presented in Tables 16 and 17.

Salmonella from food animals

Salmonella isolates from pigs and poultry (broilers and layers) were mainly from sub-clinical infections, while the majority of isolates from cattle were from clinical cases of salmonellosis. Only one isolate per farm of each serotype was included in this report.

Table 18 shows the MIC distributions and the occurrence of antimicrobial resistance in *S.* Typhimurium from poultry, cattle and pigs in 2006.

From 1999 to 2006 a significant increase in resistance to tetracycline ($P<0.0001$), chloramphenicol ($P<0.0001$), ampicillin ($P<0.0001$), and sulfonamide ($P<0.0001$) was observed among *S.* Typhimurium

Table 16. Distribution (%) of Salmonella *Typhimurium phage types from food animals, food of Danish and imported origin and human cases categorized as acquired domestically or reported as associated with travel abroad among the isolates selected for susceptibility testing, Denmark*

DANMAP 2006

Phage types	Poultry	Cattle	Pigs	Turkey meat	Pork		Humans a)	
	Danish	Danish	Danish	Imported	Danish	Imported	Domestically acquired b) %	Travel abroad reported
	%	%	%	%	%	%		%
12	0	0	17	0	8	0	8	0
15a	6	4	2	0	0	0	1	4
17	6	8	5	0	2	0	<1	0
41	33	0	0	0	0	0	1	0
104/104b/104c	6	15	9	58	11	13	25	20
120	0	23	18	0	31	5	23	36
170	6	0	12	0	11	0	3	0
193	6	38	6	0	3	16	9	8
U302	0	0	3	0	6	3	3	4
Others including non-typeable	39	12	28	42	28	62	27	28
Number of isolates	18	26	509	19	64	37	357	25

a) Not all isolates selected for susceptibility testing were phage typed
b) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

Table 17. Distribution (%) of Salmonella *Enteritidis phage types from imported broiler meat and human cases categorized as acquired domestically or reported as associated with travel abroad among the isolates selected for susceptibility testing, Denmark*

DANMAP 2006

Phage type	Broiler meat	Humans a)	
	Imported	Domestically acquired b)	Travel abroad reported
	%	%	%
1	0	11	24
2	0	2	
4	40	18	13
4b	0	3	
6	0	5	2
6a	0	2	9
8	0	29	17
13a	0	2	
14b	0	4	2
21/21b	60	7	17
Others including non-typeable	0	17	16
Number of isolates	25	287	46

a) Not all isolates selected for susceptibility testing were phage typed
b) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

isolates from pigs (Figure 12) and from 2003 to 2006, streptomycin resistance (*P*=0.003) also increased significantly. During the same period, the consumption in pigs of tetracyclines, and sulfonamide/trimethoprim increased, whereas the consumption of macrolides penicillins with extended spectrum and aminoglycosides was slightly reduced (Figure 3). Although the overall consumption of antimicrobials in pigs has decreased marginally from 2004 to 2006 the proportion of fully susceptible isolates continued to decrease in 2005 and 2006, while the resistance patterns STR-SUL-TET and AMP-STR-SUL-TET continued to increase (Figure 13). Due to co-resistance, the increase in consumption of some antimicrobial agents (e.g. tetracycline and sulfonamides) may select for these resistance patterns and thus be the reason for the observed increase. The consumption of critically important antimicrobial agents (e.g. cephalosporins and fluoroquinolones) in pigs in Denmark is low compared to the total consumption of antimicrobials, however, in 2006, the first ESBL-producing *S*. Typhimurium isolate was obtained from a healthy Danish pig.

Only a low number of *S*. Typhimurium isolates were available from poultry and cattle, and any changes in the occurrence of resistance should be interpreted with

Table 18. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from poultry (n=18), cattle (n=26) and pigs (n=509), Denmark*

DANMAP 2006

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution of MICs (%)																
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Poultry	5.6	[0.1-27.3]							94.4					5.6					
	Cattle	69.2	[48.2-85.7]							30.8				19.2	50.0					
	Pigs	43.0	[38.7-47.5]							55.0	2.0		0.2	6.3	36.5					
Chloramphenicol	Poultry	0	[0-18.5]							5.6	72.2	22.2								
	Cattle	19.2	[6.6-39.4]								69.2	7.7	3.9			19.2				
	Pigs	12.6	[9.8-15.8]							0.4	61.5	24.4	1.2	0.4	1.8	10.4				
Florfenicol	Poultry	0	[0-18.5]							44.4	55.6									
	Cattle	19.2	[6.6-39.4]								65.4	15.4		15.4		3.9				
	Pigs	6.9	[4.8-9.4]							1.6	77.8	12.0	1.8	6.1		0.8				
Ampicillin	Poultry	11.1	[1.4-34.7]						83.3	5.6					11.1					
	Cattle	69.2	[48.2-85.7]						26.9	3.9					69.2					
	Pigs	32.2	[28.2-36.5]						52.7	13.4	1.6	0.2		0.2	32.0					
Amoxicillin/clavulanic acid a)	Poultry	0	[0-18.5]							88.9		5.6	5.6							
	Cattle	0	[0-13.2]							30.8	11.5	42.3	15.4							
	Pigs	0	[0-0.7]							68.0	7.5	16.9	7.7							
Cephalothin	Poultry	0	[0-18.5]								94.4	5.6								
	Cattle	3.8	[0.1-19.6]								53.9	42.3		3.9						
	Pigs	1.0	[0.3-2.3]								74.1	20.0	4.9	0.6	0.4					
Ceftiofur	Poultry	0	[0-18.5]					77.8	22.2											
	Cattle	0	[0-13.2]					65.4	34.6											
	Pigs	0.2	[0.005-1.1]					54.6	40.3	4.9			0.2							
Cepodoxime	Poultry	0	[0-18.5]			27.8	72.2													
	Cattle	0	[0-13.2]				76.9	19.2	3.9											
	Pigs	0.4	[0.05-1.4]			0.6	69.7	25.3	3.9	0.2		0.2								
Sulfonamide	Poultry	16.7	[3.6-41.4]												83.3					16.7
	Cattle	73.1	[52.2-88.4]												26.9					73.1
	Pigs	43.4	[39.1-47.9]												56.2	0.4			1.0	42.4
Trimethoprim	Poultry	5.6	[0.1-27.3]								94.4				5.6					
	Cattle	0	[0-13.2]								100									
	Pigs	9.2	[6.9-12.1]								90.8				9.2					
Apramycin	Poultry	0	[0-18.5]								100									
	Cattle	0	[0-13.2]								96.2	3.9								
	Pigs	1.6	[0.7-3.1]								97.1	1.2	0.2		1.6					
Gentamicin	Poultry	0	[0-18.5]						100											
	Cattle	0	[0-13.2]						100											
	Pigs	1.8	[0.8-3.3]						97.3	1.0			0.6	0.8	0.4					
Neomycin	Poultry	0	[0-18.5]							100										
	Cattle	0	[0-13.2]							96.2	3.9									
	Pigs	8.8	[6.5-11.7]							89.4	1.4	0.4		0.8	8.1					
Spectinomycin	Poultry	5.6	[0.1-27.3]											27.8	66.7		5.6			
	Cattle	23.1	[9.0-43.6]											61.5	15.4			23.1		
	Pigs	20.0	[16.6-23.8]										0.2	51.3	28.5	2.4	2.2	15.5		
Streptomycin	Poultry	11.1	[1.4-34.7]									33.3	55.6			11.1				
	Cattle	69.2	[48.2-85.7]									30.8			7.7	61.5				
	Pigs	42.2	[37.9-46.7]								1.4	43.2	13.2	0.8	5.7	35.8				
Ciprofloxacin	Poultry	5.6	[0.1-27.3]	94.4			5.6													
	Cattle	0	[0-13.2]	96.2	3.9															
	Pigs	1.4	[0.6-2.8]	89.2	9.4		1.0	0.4												
Nalidixic acid	Poultry	5.6	[0.1-27.3]								94.4					5.6				
	Cattle	0	[0-13.2]								88.5	7.7	3.9							
	Pigs	1.4	[0.6-2.8]								87.0	10.8	0.8			1.4				
Colistin	Poultry	0	[0-18.5]								100									
	Cattle	0	[0-13.2]								100									
	Pigs	0	[0-0.7]								100									

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilusions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. Mics equal to or lower than the lowest contration tested are given as the lowest contration

a) Concentration of amoxicillin given, tested with clavulanic acid in concentration 2:1

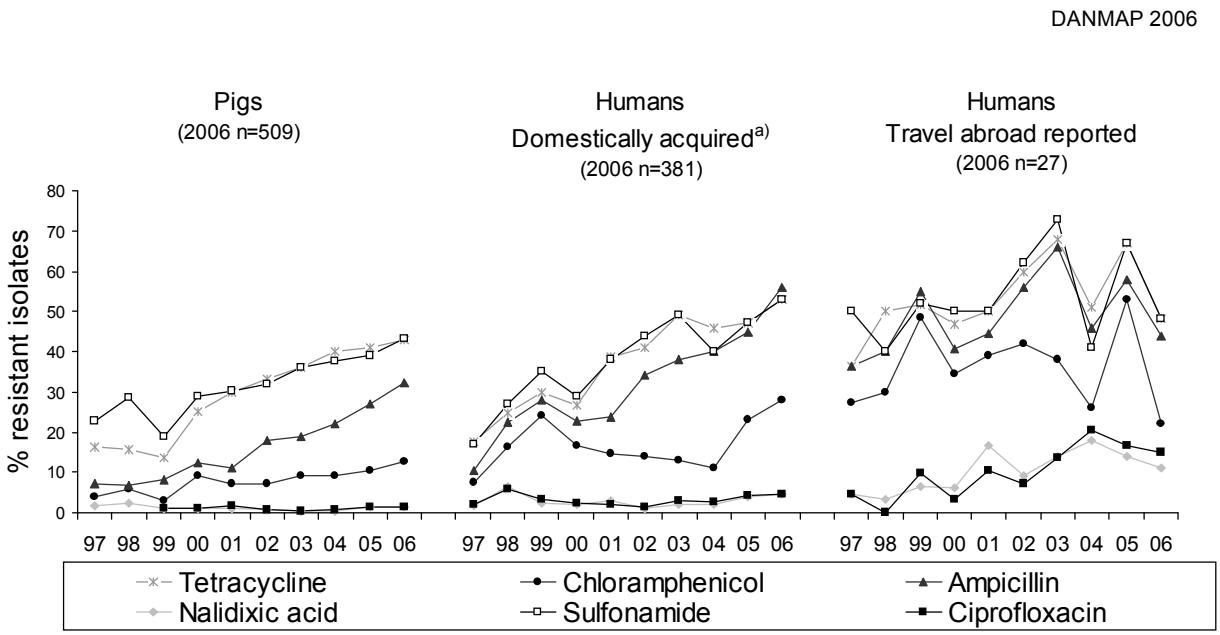


Figure 12. Trends in resistance to selected antimicrobials among Salmonella *Typhimurium isolated from pigs and from human cases, Denmark*

a) Includes cases where origin of infection is not documented and may therefore include some isolates acquired abroad but not documented as such

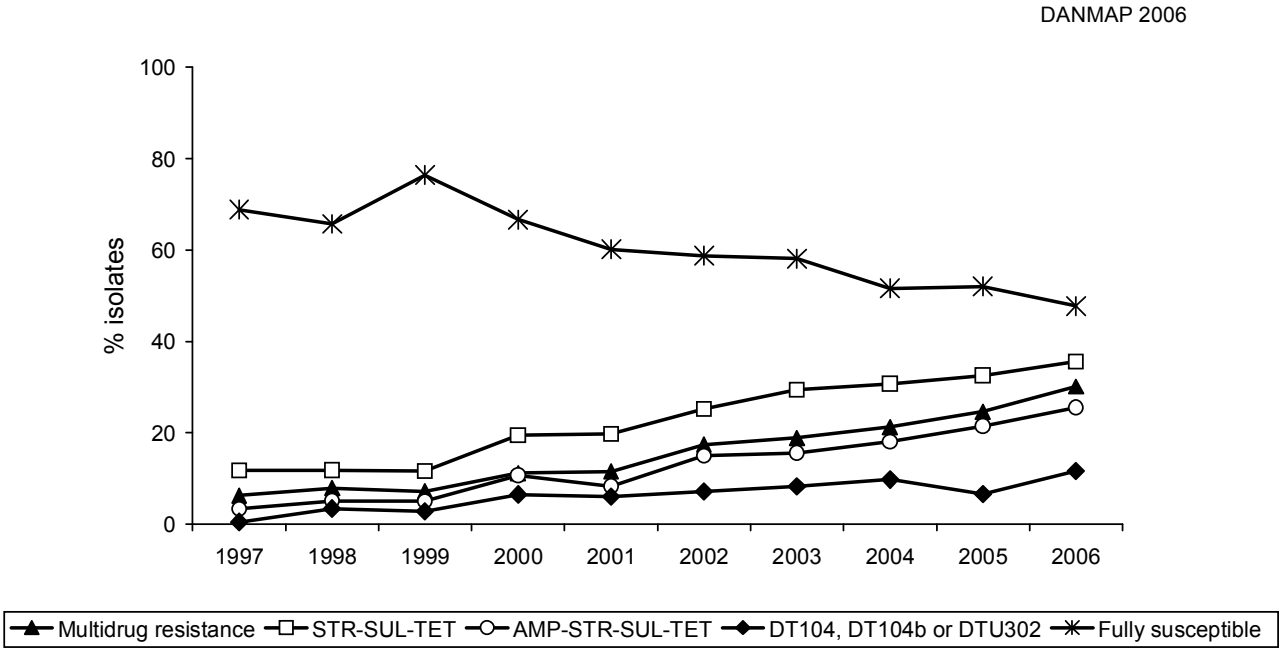


Figure 13. Trends in multidrug resistance and selected resistance patterns among Salmonella *Typhimurium isolated from pigs, Denmark*

Multidrug resistance; is defined as isolates resistant to ≥ 4 of 8 antimicrobial agents (ampicillin, chloramphenicol, gentamicin, naldixic acid, sulfonamide, streptomycin, tetracycline or trimethoprim). STR-SUL-TET; at least resistant to streptomycin, sulfonamide and tetracycline. AMP-STR-SUL-TET; at least resistant to ampicillin, streptomycin, sulfonamide and tetracycline. DT104, DT104b or DTU302; occurrence of these three phage types. Fully susceptible; isolates which were susceptible to all 8 antimicrobial agents

caution. From 2005 to 2006, a significant increase in resistance to tetracycline (*P*=0.003) and sulfonamide (*P*=0.032) was observed among *S*. Typhimurium isolates from cattle. Like previous years, a low level of resistance was observed among *S*. Typhimurium isolates from poultry.

Salmonella from food

In 2006, *Salmonella* isolates from food were obtained from Danish pork at slaughterhouses and from imported pork. Furthermore, *Salmonella* isolates were obtained from imported broiler meat and imported turkey meat sold at wholesale and retail outlets. The results of the susceptibility testing of *S.* Typhimurium and *S*. Enteritidis are shown in Tables 19 and 20. For further discussion of the occurrence of resistance in food isolates please see „Comparison of resistance in *Salmonella* isolates from animals, food and human infections".

Salmonella in humans

In 2006, 1,658 cases of human salmonellosis occurring in Denmark were reported to Statens Serum Institut. This represents a slight decrease in incidence from 33 cases per 100,000 inhabitants in 2005 to 31 cases per 100,000 inhabitants in 2006. The incidence of salmonellosis in humans has been relatively stable during the last four years [EPI-NEWS 2007, no. 12: http://www.ssi.dk/sw38271.asp]. For 12% of the Salmonella infections, travel abroad was reported and therefore these were most likely acquired in a foreign country. For the majority of the remaining infections no data on travel history were available. In the following, infections that were reported not to be travel-related

Table 19. Distribution of MICs and occurence of resistance in Salmonella *Typhimurium from turkey meat (imported n=19) and pork (Danish n=64; imported n=37), Denmark*

DANMAP 2006

Compound	Food type	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs															
					0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Turkey meat	Imported	63.2	[38.4-83.7]							36.8				31.6	31.6					
	Pork	Danish	45.3	[32.8-58.2]							53.1		1.6		10.9	34.4					
		Imported	83.8	[68.0-93.8]							16.2			5.4	21.6	56.8					
Chloramphenicol	Turkey meat	Imported	52.6	[28.9-75.6]								42.1	5.3				52.6				
	Pork	Danish	14.1	[6.6-25.0]								67.2	18.8				14.1				
		Imported	37.8	[22.5-55.2]								40.5	16.2	5.4	8.1	5.4	24.3				
Florfenicol	Turkey meat	Imported	52.6	[28.9-75.6]								47.4			47.4	5.3					
	Pork	Danish	10.9	[4.5-21.3]								79.7	7.8	1.6	4.7	1.6	4.7				
		Imported	24.3	[11.8-41.2]								51.4	18.9	5.4	24.3						
Ampicillin	Turkey meat	Imported	63.2	[38.4-83.7]						36.8						63.2					
	Pork	Danish	53.1	[40.2-65.7]						37.5	9.4					53.1					
		Imported	73.0	[55.9-86.2]						27.0						73.0					
Amoxicillin/ clavulanic acid a)	Turkey meat	Imported	0	[0-17.7]							36.8		15.8	47.4							
	Pork	Danish	0	[0-5.6]							46.9	23.4	20.3	9.4							
		Imported	0	[0-9.5]							27.0	5.4	46.0	21.6							
Cephalothin	Turkey meat	Imported	5.3	[0.1-26.0]								79.0	10.5	5.3	5.3						
	Pork	Danish	0	[0-5.6]								71.9	23.4	4.7							
		Imported	0	[0-9.5]								67.6	29.7	2.7							
Ceftiofur	Turkey meat	Imported	0	[0-17.7]					68.4	26.3		5.3									
	Pork	Danish	0	[0-5.6]					51.6	43.8	4.7										
		Imported	0	[0-9.5]					75.7	18.9	5.4										
Cefpodoxime	Turkey meat	Imported	5.3	[0.1-26.0]			10.5	73.7	10.5				5.3								
	Pork	Danish	0	[0-5.6]				75.0	20.3	4.7											
		Imported	0	[0-9.5]				67.6	32.4												
Sulfonamide	Turkey meat	Imported	63.2	[38.4-83.7]												31.6	5.3				63.2
	Pork	Danish	51.6	[38.7-64.3]												48.4					51.6
		Imported	81.1	[64.8-92.0]												18.9					81.1
Trimethoprim	Turkey meat	Imported	10.5	[1.3-33.1]								89.5				10.5					
	Pork	Danish	10.9	[4.5-21.3]								89.1				10.9					
		Imported	32.4	[18.0-49.8]								67.6				32.4					
Apramycin	Turkey meat	Imported	0	[0-17.7]								89.5	10.5								
	Pork	Danish	1.6	[0.04-8.4]								93.8	4.7			1.6					
		Imported	8.1	[1.7-21.9]								86.5	5.4			8.1					
Gentamicin	Turkey meat	Imported	0	[0-17.7]						100											
	Pork	Danish	1.6	[0.04-8.4]						98.4				1.6							
		Imported	8.1	[1.7-21.9]						86.5	5.4				8.1						
Neomycin	Turkey meat	Imported	26.3	[9.2-51.2]							73.7				5.3	21.1					
	Pork	Danish	7.8	[2.6-17.3]							92.2			1.6	1.6	4.7					
		Imported	5.4	[0.7-18.2]							86.5	8.1				5.4					
Spectinomycin	Turkey meat	Imported	57.9	[33.5-79.8]										5.3	36.8			5.3	52.6		
	Pork	Danish	25.0	[15.0-37.4]											45.3	29.7	1.6	1.6	21.9		
		Imported	51.4	[34.4-68.1]											32.4	16.2	2.7	5.4	43.2		
Streptomycin	Turkey meat	Imported	63.2	[38.4-83.7]									15.8	21.1		31.6	31.6				
	Pork	Danish	50.0	[37.3-67.8]									35.9	14.1	1.6	9.4	39.1				
		Imported	70.3	[53.0-84.1]									16.2	13.5	2.7	18.9	48.7				
Ciprofloxacin	Turkey meat	Imported	31.6	[12.6-56.6]	68.4			31.6													
	Pork	Danish	1.6	[0.04-8.4]	93.8	4.7		1.6													
		Imported	5.4	[0.7-18.2]	89.2	5.4		5.4													
Nalidixic acid	Turkey meat	Imported	-																		
	Pork	Danish	1.6	[0.04-8.4]								90.6	7.8				1.6				
		Imported	5.4	[0.7-18.2]								78.4	16.2				5.4				
Colistin	Turkey meat	Imported	0	[0-17.7]								100									
	Pork	Danish	0	[0-5.6]								100									
		Imported	0	[0-9.5]								100									

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

Table 20. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from imported broiler meat (n=25), Denmark*

DANMAP 2006

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs															
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	4.0	[0.1-20.4]							96.0					4.0					
Chloramphenicol	0	[0-13.7]							8.0	76.0	16.0								
Florfenicol	0	[0-13.7]							12.0	88.0									
Ampicillin	16.0	[4.5-36.1]						56.0	24.0			4.0		16.0					
Amoxicillin/clavulanic acid a)	0	[0-13.7]							80.0	4.0	4.0	12.0							
Cephalothin	4.0	[0.1-20.4]								80.0		16.0	4.0						
Ceftiofur	0	[0-13.7]					72.0	24.0	4.0										
Cefpodoxime	8.0	[1.0-26.0]			4.0	72.0	12.0	4.0		8.0									
Sulfonamide	8.0	[1.0-26.0]												88.0		4.0			8.0
Trimethoprim	4.0	[0.1-20.4]								96.0				4.0					
Apramycin	0	[0-13.7]								100									
Gentamicin	4.0	[0.1-20.4]						96.0				4.0							
Neomycin	4.0	[0.1-20.4]							96.0				4.0						
Spectinomycin	4.0	[0.1-20.4]										92.0	4.0				4.0		
Streptomycin	12.0	[2.6-31.2]								88.0			4.0	4.0	4.0				
Ciprofloxacin	24.0	[9.4-45.1]	76.0		4.0	16.0	4.0												
Colistin	0	[0-13.7]								100									

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

and infections with unknown travel history are categorized as domestically acquired, though it is known that many patients with an unknown travel history were in fact infected abroad. Therefore comparisons of data between those infections for which travel abroad was reported and those categorized as acquired domestically should be interpreted with caution.

Susceptibility testing was performed for 408 (>99%) of the *S.* Typhimurium isolates and for 356 (64%) of *S.* Enteritidis isolates submitted to Statens Serum Institut. Table 21 presents the MIC distributions and occurrence of antimicrobial resistance among *S.* Typhimurium isolates from humans by origin of infection in 2006.

In *S.* Typhimurium isolates acquired domestically, a significant increase in resistance to florfenicol (P<0.0001), ampicillin (P=0.001), trimethoprim (P=0.002) and spectinomycin (P=0.01) occurred from 2005 to 2006.

Among *S.* Typhimurium isolates from domestically acquired infections, a highly significant increase in the proportion of DT104 and related phage types (DT104b, DTU302) from 14% to 27% was observed from 2004 to 2006 (P<0.0001). This could in part explain the significant increase in resistance to chloramphenicol from 10.6% in 2004 to 28.1% in 2006 (P<0.0001), within this group (Figure 12).

Two domestically aquired *S.* Typhimurium isolates were resistant to both cefpodoxime and ceftiofur (Table 21). When tested phenotypically for extended-spectrum beta-lactamase (ESBL) activity using the Etest, one of the two isolates was confirmed as ESBL-

Table 21. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from human cases categorized as acquired domestically (n=381) or reported as associated with travel abroad (n=27), Denmark*

DANMAP 2006

Compound	Origin a)	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Domestically acquired	53.3	[48.1-58.4]							45.1	1.3	0.3	0.3	19.4	33.6					
	Travel abroad reported	48.1	[28.7-68.1]							51.9				14.8	33.3					
Chloramphenicol	Domestically acquired	28.1	[23.6-32.9]							1.8	56.7	12.9	0.5	1.0	4.7	22.3				
	Travel abroad reported	22.2	[8.6-42.3]							7.4	66.7	3.7				22.2				
Florfenicol	Domestically acquired	24.3	[20.2-29.1]							10.5	59.8	4.2	1.0	22.8	1.0	0.5				
	Travel abroad reported	22.2	[8.6-42.3]							7.4	70.4			18.5		3.7				
Ampicillin	Domestically acquired	56.2	[51.0-61.2]						34.9	8.1	0.8				56.2					
	Travel abroad reported	44.4	[25.5-64.7]						51.9	3.7					44.4					
Amoxicillin/ clavulanic acid b)	Domestically acquired	1.3	[0.4-3.0]							43.3	9.2	19.7	26.5	0.8	0.5					
	Travel abroad reported	0	[0-12.8]							55.6		22.2	22.2							
Cephalothin	Domestically acquired	1.3	[0.4-3.0]								80.3	17.6	0.8	0.5	0.8					
	Travel abroad reported	0	[0-12.8]								81.5	18.5								
Cefpodoxime	Domestically acquired	0.8	[0.2-2.3]			2.6	88.5	7.9	0.3			0.8								
	Travel abroad reported	0	[0-12.8]				100													
Ceftiofur	Domestically acquired	0.5	[0.1-1.9]					85.3	13.9	0.3			0.5							
	Travel abroad reported	0	[0-12.8]					96.3	3.7											
Sulfonamide	Domestically acquired	53.3	[48.1-58.4]												45.7	0.8	0.3			53.3
	Travel abroad reported	48.1	[28.7-68.1]												51.9					48.1
Trimethoprim	Domestically acquired	9.2	[6.5-12.5]								90.8		0.3		8.9					
	Travel abroad reported	7.4	[0.9-24.3]								92.6				7.4					
Apramycin	Domestically acquired	0.5	[0.1-1.9]								98.2	1.0	0.3		0.5					
	Travel abroad reported	3.7	[0.1-19.0]								96.3				3.7					
Gentamicin	Domestically acquired	1.8	[0.7-3.8]						97.1	1.0		0.5	0.5	0.8						
	Travel abroad reported	7.4	[0.9-24.3]						92.6			3.7			3.7					
Neomycin	Domestically acquired	1.6	[0.6-3.4]							97.9	0.5			0.3	1.3					
	Travel abroad reported	11.1	[2.4-29.2]							85.2	3.7			3.7	7.4					
Spectinomycin	Domestically acquired	32.0	[27.4-37.0]										0.8	63.0	4.2		0.5	31.5		
	Travel abroad reported	25.9	[11.1-46.3]											74.1			3.7	22.2		
Streptomycin	Domestically acquired	50.1	[45.0-55.3]								7.3	38.8	3.7	5.2	21.3	23.6				
	Travel abroad reported	44.4	[25.5-64.7]								11.1	40.7	3.7		14.8	29.6				
Ciprofloxacin	Domestically acquired	4.8	[2.8-7.4]	93.2	2.1	1.3	2.9		0.3			0.3								
	Travel abroad reported	14.8	[4.2-33.7]	81.5	3.7		11.1	3.7												
Nalidixic acid	Domestically acquired	4.5	[2.6-7.1]								92.1	3.4			0.3	4.2				
	Travel abroad reported	11.1	[2.4-29.2]								85.2		3.7			11.1				
Colistin	Domestically acquired	0.3	[0.01-1.5]								99.7			0.3						
	Travel abroad reported	0	[0-12.8]								96.3	3.7								

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Domestically acquired includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

b) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

positive (according to Etest technical guide 3B, Gram-negative aerobic specific AES 004, from AB BIODISK, Solna, Sweden). Resistance to fosfomycin and mecillinam was 0% in all tested *S.* Typhimurium isolates.

Table 22 presents the MIC distributions and occurrence of antimicrobial resistance among *S.* Enteritidis isolates from humans by origin of infection in 2006. One domestically acquired *S.* Enteritidis isolate was resistant to both cefpodoxime and ceftiofur (Table 22). When tested phenotypically for extended-spectrum beta-lactamase (ESBL) activity using the Etest, this isolate was confirmed ESBL-positive. Resistance to fosfomycin and mecillinam was 0% in all tested *S.* Enteritidis isolates.

Comparison of resistance in *Salmonella* isolates from animals, food and human infections

Imported meat is more often contaminated with *Salmonella* than meat of Danish origin. A survey conducted as part of an intensified control programme targeting *Salmonella* in fresh meat of imported and Danish origin in 2006/2007, showed that 25% of 32 batches of imported pork and 13% of 52 batches of imported poultry meat contained *S*. Typhimurium or *S*. Enteritidis. For comparison *Salmonella* was not found in 18 batches of Danish pork and 17 batches of Danish broiler meat, sampled in the same survey. *Salmonella* was not found in 14 batches of Danish beef and 5 batches of imported beef [Annual report on Zoonoses in Denmark 2006: http://www.dfvf.dk/Default.aspx?ID=9202#74145].

Table 22. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from human cases categorized as acquired domestically (n=304) or reported as associated with travel abroad (n=52), Denmark*

DANMAP 2006

Compound	Origin a)	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
				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	2.0	[0.7-4.3]							98.0					2.0					
	Travel abroad reported	3.8	[0.5-13.2]							96.2					3.8					
Chloramphenicol	Domestically acquired	0	[0-1.6]							1.0	86.2	12.5	0.3							
	Travel abroad reported	0	[0-6.8]								96.2	3.8								
Florfenicol	Domestically acquired	0	[0-1.6]							5.3	92.8	2.0								
	Travel abroad reported	0	[0-6.8]							9.6	90.4									
Ampicillin	Domestically acquired	3.0	[1.4-5.6]						56.3	38.8	1.6	0.3			3.0					
	Travel abroad reported	5.8	[1.2-15.9]						59.6	34.6					5.8					
Amoxicillin/ clavulanic acid b)	Domestically acquired	0	[0-1.6]							96.7	0.7	2.0	0.7							
	Travel abroad reported	0	[0-6.8]							94.2		5.8								
Cephalothin	Domestically acquired	1.3	[0.4-3.3]								95.4	2.3	1.0	1.0	0.3					
	Travel abroad reported	0	[0-6.8]								98.1	1.9								
Cefpodoxime	Domestically acquired	1.0	[0.2-2.9]			2.0	89.5	7.2	0.3	0.7		0.3								
	Travel abroad reported	0	[0-6.8]				98.1	1.9												
Ceftiofur c)	Domestically acquired	0.3	[0.01-1.8]					84.1	13.9	1.7			0.3							
	Travel abroad reported	0	[0-6.8]					88.5	11.5											
Sulfonamide	Domestically acquired	0.3	[0.01-1.8]												99.7					0.3
	Travel abroad reported	0	[0-6.8]												100					
Trimethoprim	Domestically acquired	0.3	[0.01-1.8]								99.7				0.3					
	Travel abroad reported	0	[0-6.8]								100									
Apramycin	Domestically acquired	0	[0-1.6]								100									
	Travel abroad reported	0	[0-6.8]								100									
Gentamicin	Domestically acquired	0	[0-1.6]						99.7	0.3										
	Travel abroad reported	0	[0-6.8]						100											
Neomycin	Domestically acquired	0.3	[0.01-1.8]							99.0	0.7				0.3					
	Travel abroad reported	0	[0-6.8]							100										
Spectinomycin	Domestically acquired	0.3	[0.01-1.8]										55.0	43.1	1.6			0.3		
	Travel abroad reported	0	[0-6.8]										44.2	55.8						
Streptomycin	Domestically acquired	0	[0-1.6]								95.7	3.6	0.7							
	Travel abroad reported	0	[0-6.8]								98.1	1.9								
Ciprofloxacin	Domestically acquired	15.2	[11.3-19.7]	84.2	0.7	5.3	8.9	0.7	0.3											
	Travel abroad reported	28.8	[17.1-43.1]	71.2		13.5	15.4													
Nalidixic acid c)	Domestically acquired	14.8	[11.0-19.3]								83.5	1.7			0.3	14.5				
	Travel abroad reported	28.8	[17.1-43.1]								69.2	1.9				28.8				
Colistin	Domestically acquired	0	[0-1.6]								100									
	Travel abroad reported	0	[0-6.8]								100									

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Domestically acquired includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

b) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

c) For nalidixic acid and ceftiofur the number of tested isolates was n = 52 for cases associated with reported travel abroad and n = 303 for human cases where travel was not declared

The occurrence of resistance in *S*. Typhimurium isolates from Danish food animals, imported and Danish pork, imported turkey meat and human cases acquired domestically or abroad are compared in Table 23. The occurrence of resistance in *S.* Typhimurium isolates from Danish pigs was similar to the occurrence in Danish pork. The occurrence of resistance in *S*. Typhimurium from imported pork was significantly higher than the occurrence in isolates from Danish pork for the following five antimicrobial agents: Tetracycline (*P*=0.0002), chloramphenicol (*P*=0.012), sulfonamide (*P*=0.003), trimethoprim (*P*=0.008) and spectinomycin (*P*=0.05). The occurrence of resistance in domestically acquired human isolates was similar to the occurrence in Danish pork, but lower than the occurrence in imported pork. The consumption of imported pork has increased in Denmark from 22% in 2003 to 47% in 2006. This increase may contribute to a further increase in resistance in *S.* Typimurium isolates from domestically acquired human infections. In *S.* Typhimurium isolates, only resistance to neomycin (*P*=0.01) was significantly higher in travel associated isolates, compared to isolates acquired domestically (Table 23).

In humans, *S.* Typhimurium, DT104 and related phage types (DT104b, DTU302) were the most prevalent phage types in Denmark, accounting for 28% of the cases in 2006. The chromosome of DT104 isolates includes a multidrug resistance (MDR) region, which normally consists of five antimicrobial resistance genes encoding resistance towards ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. However, variations of the antibiogram can occur [Mulvey *et al.* 2006. Microbes Infect. 8:1915-22]. The proportion of multi-resistant *S*. Typhimurium isolates can have a strong influence on the total frequency of resistance. Therefore, resistance among *S.* Typhimurium other than phage types DT104/104b and DTU302 are presented in Table 24.

The occurrence of resistance among *S.* Enteritidis isolates from imported broiler meat and human cases acquired domestically or abroad are compared in Table 25. As in 2005, *S*. Enteritidis was rare in layers and broilers in Denmark in 2006 therefore no susceptibility results are shown. Comparison of resistance in *S*. Enteritidis from imported broiler meat and human cases acquired domestically or abroad is presented in Table 25. Except for resistance to ciprofloxacin, low levels of resistance were observed in isolates from imported broiler meat and from humans. As in 2005, resistance to ciprofloxacin (*P*=0.02) and nalidixic acid (*P*=0.01) was significantly higher in travel associated *S.* Enteritidis isolates compared to domestically acquired isolates.

The occurrence of resistance was significantly higher in isolates from imported broiler meat compared to isolates from domestically acquired human cases for ampicillin (*P*=0.01), cefpodoxime (*P*=0.05), sulfonamide (*P*=0.02), and streptomycin (*P*=0.0004). However, the Danish Salmonella source account points out eggs as the primary source of *S.* Enteritidis infections in humans.

Table 23. Comparison of resistance (%) among Salmonella *Typhimurium from food animals, food of Danish and imported origin and human cases categorized as acquired domestically or reported as associated with travel abroad, Denmark*

DANMAP 2006

Compound	Poultry	Cattle	Pigs	Turkey meat	Pork		Humans	
	Danish	Danish	Danish	Imported	Danish	Imported	Domestically acquired a)	Travel abroad reported
	%	%	%	%	%	%	%	%
Tetracycline	6	69	43	63	45	84	53	48
Chloramphenicol	0	19	13	53	14	38	28	22
Florfenicol	0	19	7	53	11	24	24	22
Ampicillin	11	69	32	63	53	73	56	44
Amoxicillin/clavulanic acid	0	0	0	0	0	0	1	0
Cephalothin	0	4	<1	5	0	0	1	0
Cefpodoxime	0	0	<1	0	0	0	1	0
Ceftiofur	0	0	<1	5	0	0	<1	0
Sulfonamide	17	73	43	63	52	81	53	48
Trimethoprim	6	0	9	11	11	32	9	7
Apramycin	0	0	2	0	2	8	<1	4
Gentamicin	0	0	2	0	2	8	2	7
Neomycin	0	0	9	26	8	5	2	11
Spectinomycin	6	23	20	58	25	51	32	26
Streptomycin	11	69	42	63	50	70	50	44
Ciprofloxacin	6	0	1	32	2	5	5	15
Nalidixic acid	6	0	1	-	2	5	4	11
Colistin	0	0	0	0	0	0	<1	0
Number of isolates	18	26	509	19	64	37	381	27

a) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

Table 24. Comparison of resistance (%) among Salmonella *Typhimurium other than DT104, DT104b and DTU302 from food animals, food of Danish and imported origin and human cases categorized as acquired domestically or reported as associated with travel abroad, Denmark*

DANMAP 2006

Compound	Poultry	Cattle	Pigs	Turkey meat	Pork		Humans	
	Danish	Danish	Danish	Imported	Danish	Imported	Domestically acquired a)	Travel abroad reported
	%	%	%	%	%	%	%	%
Tetracycline	6	64	40	63	38	81	42	32
Chloramphenicol	0	5	5	50	4	29	8	5
Florfenicol	0	5	<1	50	0	13	4	5
Ampicillin	6	64	28	63	43	71	46	32
Amoxicillin/clavulanic acid	0	0	0	0	0	0	2	0
Cephalothin	0	5	<1	0	0	0	2	0
Cefpodoxime	0	0	<1	0	0	0	1	0
Ceftiofur	0	0	0	0	0	0	1	0
Sulfonamide	12	68	40	63	43	77	41	37
Trimethoprim	6	0	10	0	13	35	9	11
Apramycin	0	0	2	0	2	10	<1	5
Gentamicin	0	0	2	0	2	10	2	11
Neomycin	0	0	8	13	6	6	2	16
Spectinomycin	6	9	14	50	17	45	10	11
Streptomycin	6	64	39	63	42	68	37	32
Ciprofloxacin	6	0	2	50	0	0	4	11
Nalidixic acid	6	0	2	-	0	0	3	5
Colistin	0	0	0	0	0	0	<1	0
Number of isolates	17	22	450	8	53	31	256	19

a) Includes cases where origin of infection is not documented and may therefore include some isolates acquired abroad but not documented as such

Table 25. Comparison of resistance (%) among Salmonella *Enteritidis from imported broiler meat and human cases categorized as acquired domestically or reported as associated with travel abroad, Denmark*

DANMAP 2006

Compound	Broiler meat	Humans	
	Imported	Domestically acquired a) %	Travel abroad reported
	%		%
Tetracycline	4	2	4
Chloramphenicol	0	0	0
Florfenicol	0	0	0
Ampicillin	4	3	6
Amoxicillin/clavulanic acid	0	0	0
Cephalothin	4	1	0
Cefpodoxime	8	1	0
Ceftiofur b)	0	<1	0
Sulfonamide	8	<1	0
Trimethoprim	4	<1	0
Apramycin	0	0	0
Gentamicin	4	0	0
Neomycin	4	<1	0
Spectinomycin	4	<1	0
Streptomycin	12	0	0
Ciprofloxacin	24	15	29
Nalidixic acid b)	-	15	29
Colistin	0	0	0
Number of isolates	25	304	52

a) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such
b) For nalidixic acid and ceftiofur the number of tested isolates was n = 52 for cases associated with reported travel abroad and n = 303 for human cases where travel was not declared

Less frequent *Salmonella* serovars as a reservoir of antimicrobial resistance

Salmonella enterica serovars Enteritidis and Typhimurium are the most frequent *Salmonella* serovars isolated from humans. However, a significant proportion of human *Salmonella* isolates belong to other serovars, for which antimicrobial susceptibility patterns are rarely reported. In Denmark, the frequency of isolation of these locally uncommon *Salmonella* serovars from human clinical samples has increased in recent years. They represented approximately 30% of all human *Salmonella* isolates, which corresponded to an incidence of 10.5 per 100,000 inhabitants in 2005. In the present study, 530 isolates belonging to these *Salmonella* serovars were tested for their antimicrobial susceptibility by broth microdilution with the same panel as used for other *Salmonella* isolates in DANMAP. These included 106 *S.* Virchow, 95 *S.* Newport, 91 *S.* Stanley, 80 *S.* Dublin, 53 *S.* Hadar, 38 *S.* Saintpaul, 32 *S.* Derby, 20 *S.* Uganda, and 15 *S.* Anatum obtained from sporadic clinical infections isolated at 16 Clinical Microbiology Laboratories in the period from 2003 to 2005 [Bagger-Skjøt *et al*. J. Antimicrob. Chemother. 2007].
Isolates belonging to some serovars, e.g. *S.* Dublin, *S.* Saintpaul, *S.* Derby, were susceptible to most antimicrobials tested, while other serovars showed much higher frequencies of resistance, e.g. *S*. Virchow, *S*. Newport, *S*. Stanley (Table 1). A high prevalence of multidrug resistance (resistance to ≥ four antimicrobial agents) was observed in the following serovars: *S.* Hadar (30%), *S.* Virchow (25%), *S.* Stanley (22%) and *S.* Newport (19%). Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline was present in *S.* Newport (15%), *S.* Stanley (8%) and *S.* Virchow (1%). Resistance to these five antimicrobials could indicate the presence of the multidrug-resistant region *Salmonella* genomic island 1 (SGI1). This region is well described and common in *S.* Typhimurium phage type DT104.

Table 1. Number of tested isolates and percentages of resistance for each Salmonella *serovar*

DANMAP 2006

Serovar	No. isolates	% Resistant, by antimicrobial agent a)									
		AMP	CEF	CHL	CIP b)	GEN	NAL	SMX	STR	TET	TMP
S. Anatum	15	7	0	0	0 (0)	0	0	7	0	7	0
S. Derby	32	0	0	3	0 (0)	0	0	3	3	6	3
S. Dublin	80	4	0	0	0 (4)	0	4	0	10	4	0
S. Hadar	53	30	23	0	0 (89)	0	87	4	77	87	4
S. Newport	95	18	1	16	0 (20)	9	20	20	19	19	18
S. Saintpaul	38	8	0	0	0 (3)	3	3	5	5	8	8
S. Stanley	91	13	0	12	0 (9)	1	7	49	46	53	14
S. Uganda	20	0	0	0	0 (0)	0	0	0	0	0	0
S. Virchow	106	4	0	3	0 (80)	3	80	26	12	22	25

a) AMP, ampicillin; CEF, cefalotin; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; SMX, sulfamethoxazole; STR, streptomycin; TET, tetracycline; TMP, trimethoprim
b) The % of isolates resistant to ciprofloxacin is shown for both the CLSI breakpoint (4 µg/ml) and in parentheses for a breakpoint of 0.125 µg/ml.

Among the tested *S.* Hadar and *S.* Virchow isolates, 87% and 80%, respectively, were resistant to nalidixic acid. However, these isolates were not resistant to ciprofloxacin when the CLSI breakpoint (4 µg/ml) was used. When using the EUCAST breakpoint for ciprofloxacin (0.125 µg/ml) good consistency was observed between nalidixic acid and ciprofloxacin resistance levels. A Danish study has shown failures of treatment with ciprofloxacin for infection with *S*. Typhimurium DT104 with a MIC value below the CLSI breakpoint [Kristiansen *et al*. J. Clin. Microbiol. 2003]. Similar failures of treatment might be observed for *S.* Hadar and *S.* Virchow if the CLSI breakpoint for ciprofloxacin is used. This is of great concern, since ciprofloxacin is often the drug of choice when treating invasive *Salmonella* infections.
Prevalence of antimicrobial resistance varies greatly among less frequent *Salmonella* serovars isolated in Denmark and some serovars show a higher level of resistance than others.

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Campylobacter

Campylobacter from food animals

Table 26 presents the MIC distributions and occurrence of antimicrobial resistance among *C. jejuni* from broilers and cattle in 2006 and Table 27 presents data for *C. coli* from pigs in 2006. Trends in resistance to selected antimicrobial agents among *C. jejuni* and *C. coli* from 1996 to 2006 are presented in Figures 14 and 15, respectively. In 2006, the occurrence of nalidixic acid resistance in *C. jejuni* isolates from cattle was reduced to 19%, compared to 29% in 2005, however considerable variation in resistance was observed throughout 1996 to 2006 among *C. jejuni* isolates from cattle (Figure 14). From 1996 to 2006, resistance in *C. jejuni* isolates from broilers has remained at a low level.

Among *C. coli* isolates from pigs the occurrence of resistance remained unchanged from 2005 to 2006, except for streptomycin resistance, which increased from 48% to 61% (*P*=0.02) (Table 27). After withdrawal of the growth promoter tylosin from the Danish pig production in 1998-1999 erythromycin resistance decreased significantly in *C. coli* from pigs from 71% in 1997 to 39% in 2000. In the following years a further decrease in resistance was observed and in 2006 13% of *C. coli* isolates from pigs were resistant to erythromycin (Figure 15). The consumption of

Table 26. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *isolates from broilers (n=75) and cattle (n=74), Denmark*

DANMAP 2006

Compound	Animal species	% Resistant	[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	Broilers	6.7	[2.2-14.9]				52.0	41.3						6.7		
	Cattle	2.7	[0.3-9.4]				55.4	37.8	4.1					2.7		
Chloramphenicol	Broilers	0	[0-4.8]							20.0	76.0	2.7	1.3			
	Cattle	0	[0-4.9]							73.0	25.7	1.4				
Erythromycin	Broilers	0	[0-4.8]					4.0	28.0	45.3	22.7					
	Cattle	0	[0-4.9]					47.3	37.8	9.5	5.4					
Gentamicin	Broilers	0	[0-4.8]			10.7	66.7	22.7								
	Cattle	0	[0-4.9]			32.4	62.2	4.1	1.4							
Streptomycin	Broilers	2.7	[0.3-9.3]							97.3				2.7		
	Cattle	1.4	[0.03-7.3]							98.7				1.4		
Ciprofloxacin	Broilers	6.7	[2.2-14.9]		13.3	53.3	25.3	1.3			1.3	5.3				
	Cattle	18.9	[10.7-29.7]	1.4	28.4	43.2	6.8		1.4			18.9				
Nalidixic acid	Broilers	6.7	[2.2-14.9]							6.7	77.3	6.7	1.3	1.3		6.7
	Cattle	20.3	[11.8-31.2]							4.1	48.7	27.0			2.7	17.6

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.

Table 27. Distribution of MICs and occurrence of resistance among Campylobacter coli *isolates from cattle (n=10) and pigs (n=103), Denmark*

DANMAP 2006

Compound	Animal species	% Resistant	[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	Cattle	0	[0-30.8]				30.0	40.0			30.0					
	Pigs	3.9	[1.1-9.6]				45.6	34.0	6.8	4.9	3.9	1.0	1.0	2.9		
Chloramphenicol	Cattle	0	[0-30.8]							60.0	30.0	10.0				
	Pigs	0	[0-3.5]							24.3	50.5	24.3	1.0			
Erythromycin	Cattle	30.0	[6.7-65.2]					30.0	30.0		10.0				30.0	
	Pigs	12.6	[6.9-20.6]					23.3	22.3	32.0	9.7			1.0	11.7	
Gentamicin	Cattle	0	[0-30.8]			50.0	50.0									
	Pigs	0	[0-3.5]			15.5	52.4	32.0								
Streptomycin	Cattle	20.0	[2.5-55.6]							80.0				20.0		
	Pigs	61.2	[51.1-70.6]							36.9	1.9		6.8	54.4		
Ciprofloxacin	Cattle	10.0	[0.3-44.5]			60.0	10.0	10.0	10.0			10.0				
	Pigs	11.7	[6.2-19.5]	9.7	39.8	24.3	12.6	1.0		1.0	4.9	6.8				
Nalidixic acid	Cattle	20.0	[2.5-55.6]								10.0	60.0		10.0		20.0
	Pigs	10.7	[5.5- 18.3]							6.8	36.9	29.1	13.6	2.9	1.0	9.7

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

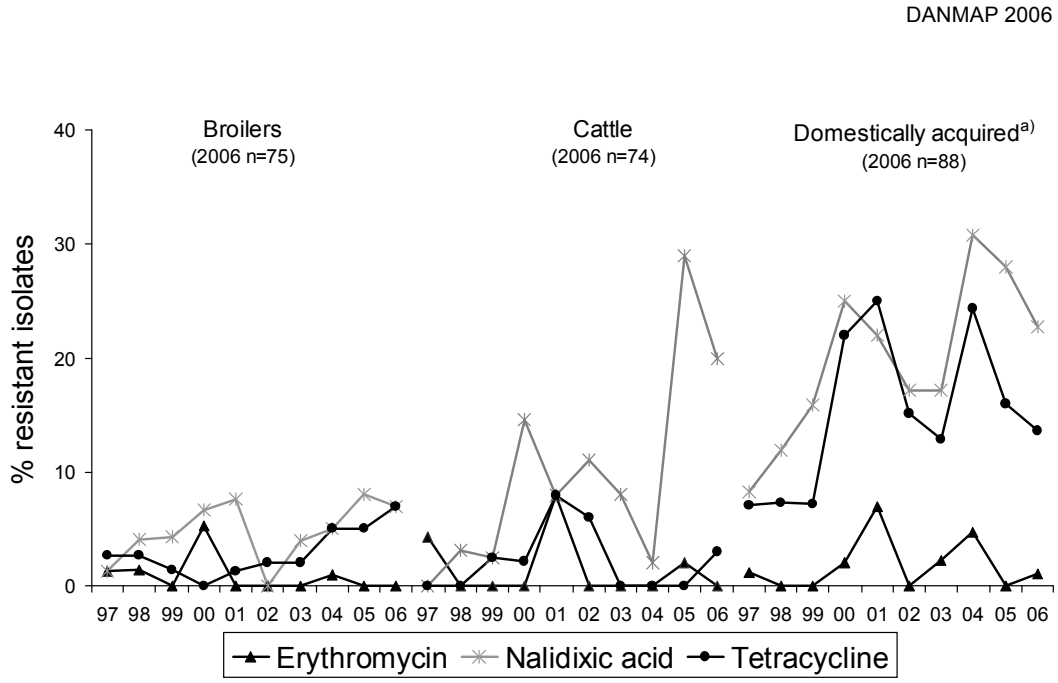

Figure 14. Trends in resistance to selected antimicrobials among Campylobacter jejuni *isolates from broilers, cattle and human cases categorized as acquired domestically, Denmark*

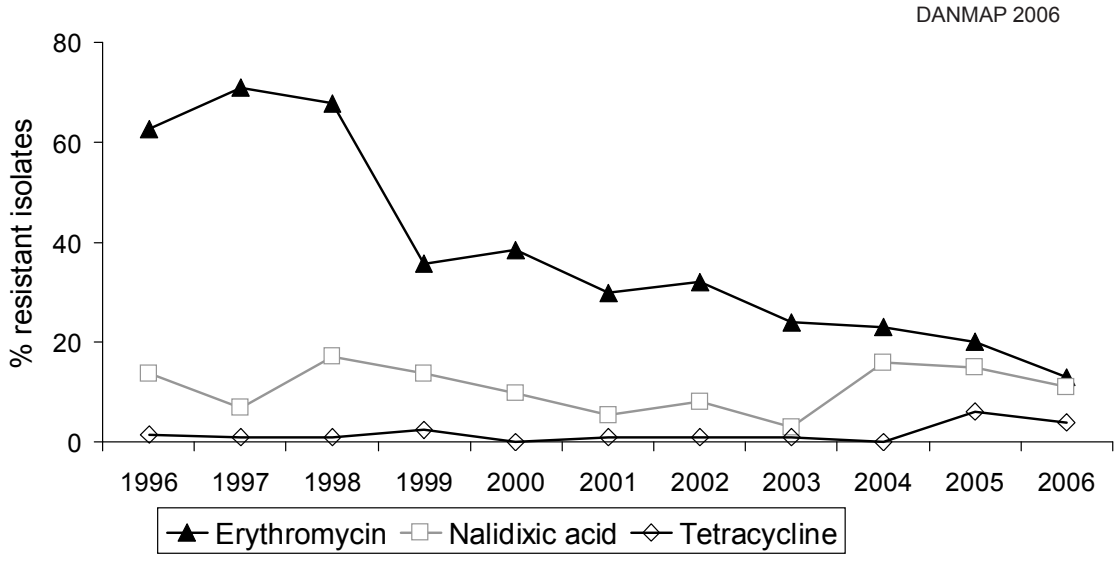

Figure 15. Trends in resistance to selected antimicrobials among Campylobacter coli *isolates from pigs, Denmark*

tetracycline in pigs has increased from 1996 to 2006 (Table 4). However, tetracycline resistance in *C. coli* remained at a low level (Figure 15).

Campylobacter from food

Results from susceptibility testing of *Campylobacter* isolates from food were not available from the Danish Veterinary and Food Administration before the current version of this report was published.

Campylobacter in humans

In 2006, there were 3,242 laboratory confirmed cases of human campylobacteriosis occurring in Denmark making it the most common bacterial cause of diarrhoeal illness. This corresponds to an incidence of 60 per 100,000 inhabitants and reflects a substantial decrease in the number of cases by 12% from 2005 to 2006. The number of infections has decreased steadily during the past six years [EPI-NEWS 2007, no. 12: http://www.ssi.dk/sw48510.asp]. For 12% of the campylobacteriosis cases, travel abroad was reported and therefore these were most likely acquired in a foreign country. No data on travel history was available for the majority of the remaining infections. In the following, infections that were reported not to be travel-related and infections with unknown travel history are categorized as domestically acquired, although it is known that many patients with an unknown travel history were in fact infected abroad. Therefore comparisons of data between those infections for which travel abroad was reported and those categorized as acquired domestically should be interpreted with caution.

Species determination was available for 165 (5%) of all *Campylobacter* isolates reported to the Unit of Gastrointestinal Infections at the Statens Serum Institut. Among these isolates, 147 (89%) were *C. jejuni* and 18 (11%) were *C. coli*.

Tables 28 and 29 show the occurrence of resistance among *C. jejuni* and *C. coli* isolates from humans by origin of infection. Trends in resistance to selected antimicrobial agents among *C. jejuni* cases categorized as acquired domestically are shown in Figure 14.

Table 28. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *from human cases categorized as acquired domestically (n=88) or reported as associated with travel abroad (n=59), Denmark*

DANMAP 2006

Compound	Origin a)	% Resistant	[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	13.6	[7.2-22.6]				67.0	16.0	2.3	1.1				13.6		
	Travel abroad reported	35.6	[23.6-49.1]				45.8	15.3				3.4		35.6		
Chloramphenicol	Domestically acquired	0	[0-4.1]							55.7	30.7	12.5	1.1			
	Travel abroad reported	0	[0-6.1]							64.4	28.8	6.8				
Erythromycin	Domestically acquired	1.1	[0.03-6.2]					22.7	51.1	17.1	6.8	1.1			1.1	
	Travel abroad reported	1.7	[0.04-9.1]					35.6	44.1	13.6	5.1				1.7	
Gentamicin	Domestically acquired	3.4	[0.7-9.6]			35.2	48.9	9.1	2.3	1.1		2.3	1.1			
	Travel abroad reported	3.4	[0.4-11.7]			40.7	50.8	5.1				3.4				
Streptomycin	Domestically acquired	9.1	[4.0-17.1]							91.0			3.4	5.7		
	Travel abroad reported	3.4	[0.4-11.7]							94.9		1.7		3.4		
Ciprofloxacin	Domestically acquired	22.7	[14.5-32.9]	2.3	30.7	29.5	14.8					22.7				
	Travel abroad reported	54.2	[40.8-67.3]		25.4	15.3	5.1				10.2	44.1				
Nalidixic acid	Domestically acquired	22.7	[14.5-32.9]							13.6	47.7	11.4	2.3	2.3		22.7
	Travel abroad reported	55.9	[42.4-68.8]							16.9	23.7	1.7	1.7			55.9

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

Table 29. Distribution of MICs and occurrence of resistance among Campylobacter coli *from human cases reported as associated with travel abroad (n=14), Denmark*

DANMAP 2006

Compound	% Resistant	[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	7.1	[0.2-33.9]				42.9	28.6	14.3			7.1		7.1		
Chloramphenicol	7.1	[0.2-33.9]							28.6	28.6	35.7		7.1		
Erythromycin	0	[0-23.2]					64.3	28.6	7.1						
Gentamicin	0	[0-23.2]			14.3	64.3	21.4								
Streptomycin	7.1	[0.2-33.9]							85.7	7.1			7.1		
Ciprofloxacin	28.6	[8.4-58.1]	7.1	28.6	35.7					14.3	14.3				
Nalidixic acid	28.6	[8.4-58.1]							7.1	42.9	21.4			7.1	21.4

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

Table 30. Comparison of resistance (%) among Campylobacter jejuni *from Danish food animals and human cases categorized as acquired domestically or reported as associated with travel abroad, Denmark*

DANMAP 2006

Compound	Cattle	Broilers	Humans	
	Danish	Danish	Domestically acquired a)	Travel abroad reported
	%	%	%	%
Tetracycline	3	7	14	36
Chloramphenicol	0	0	0	0
Erythromycin	0	0	1	2
Gentamicin	0	0	3	3
Streptomycin	1	3	9	3
Ciprofloxacin	19	7	23	54
Nalidixic acid	20	7	23	56
Number of isolates	74	75	88	59

a) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such

Table 31. Comparison of resistance (%) among Campylobacter coli *from Danish food animals and human cases reported as associated with travel abroad, Denmark*

DANMAP 2006

Compound	Cattle	Pigs	Humans
	Danish	Danish	Travel abroad reported
	%	%	%
Tetracycline	0	4	7
Chloramphenicol	0	0	7
Erythromycin	30	13	0
Gentamicin	0	0	0
Streptomycin	20	61	7
Ciprofloxacin	10	12	29
Nalidixic acid	20	11	29
Number of isolates	10	103	14

Resistance to ciprofloxacin (P<0.0001), nalidixic acid (P<0.0001) and tetracycline (P=0.002) was significantly higher in travel associated *C. jejuni* isolates compared to isolates acquired domestically (Tables 28 and 30). Most *Campylobacter* infections do not require antimicrobial treatment; however, these results should be taken into account prior to prescribing any necessary antimicrobial treatment to patients with *Campylobacter* infections. Doctors should inquire into the patient's travel history before considering treatment with fluoroquinolones because of the high probability of resistance to these antimicrobial agents when *Campylobacter* infections are acquired outside Denmark. Among domestically acquired *C. jejuni* isolates resistance to tetracycline, erythromycin, nalidixic acid, and ciprofloxacin was at the same level in 2006 as in 2005 (Figure 14).

Comparison of resistance in *Campylobacter* isolates from animals and from human infections

Comparisons of the occurrence of resistance among *C. jejuni* and *C. coli* isolates from Danish food animals and human cases categorized as acquired domestically and reported as associated with travel abroad are presented in Tables 30 and 31, respectively.

As in previous years a low level of resistance was observed in *C. jejuni* isolates from Danish broilers (Table 30). The occurrence of resistance to ciprofloxacin (P=0.009) and nalidixic acid (P=0.009) was significantly higher in *C. jejuni* isolates from human cases acquired domestically than in isolates from Danish broilers. Poultry meat is regarded an important source of *Campylobacter* infections in humans. As stated in previous DANMAP reports, the consumption of imported broiler meat is increasing in Denmark (from 17% in 2003 to 33% in 2006) and imported broiler meat is more often contaminated with *Campylobacter* than Danish broiler meat. In 2006, 52% of imported broiler meat was found positive for *Campylobacter,* whereas 12% of Danish broiler meat was contaminated with *Campylobacter* [Annual report on Zoonoses in Denmark 2006: http://www.dfvf.dk/Default.aspx?ID=9202#74145]. In previous DANMAP reports, higher levels of ciprofloxacin and nalidixic acid resistance were reported in *Campylobacter* isolates from imported meat, compared to Danish meat. Imported broiler meat may therefore contribute to the high levels of resistance to certain antimicrobial agents in isolates from human infections categorized as domestically acquired.

Resistance in indicator bacteria

Enterococci

Enterococci from food animals

Enterococci from food animals were isolated from faecal samples from pigs and cloacal swabs from broilers. All samples were collected at slaughter. In 2006, enterococci were not collected from cattle.

The MIC distribution and the occurrence of resistance among enterococci from food animals are shown in Tables 32 and 33.

Trends in resistance among *E. faecium* isolates from broilers and pigs to antimicrobial growth promoters and tetracycline are presented in Figures 16-22. Figures 16 and 19 presenting streptogramin resistance in *E. faecium* were updated with a new breakpoint (> 4 µg/ml) for quinopristin/dalfopristin, which is further described in textbox page 49-50. Among *E. faecium* isolates from pigs no significant changes in resistance were observed between 2005 and 2006. The only significant change in resistance in *E. faecium* from broilers was an increase in avilamycin resistance from 2% in 2005 to 13% in 2006 (*P*=0.008) (Figure 18). In *E. faecalis* isolates from broilers and pigs, the occurrence of resistance remained unchanged from 2005 to 2006.

Enterococci from food

Tables 34-37 present the MIC distributions and occurrence of antimicrobial resistance in enterococci isolated from broiler meat and turkey meat sold at wholesale and retail outlets. Pork and beef were not sampled by the Danish Veterinary and Food Administration in 2006 and therefore no enterococcal isolates were available for the 2006 DANMAP report.

In general, the occurrence of resistance was significantly higher for several antimicrobial agents in *E. faecium* and *E. faecalis* isolates from imported broiler meat compared with isolates from Danish broiler meat (Table 34 and Table 35) and for the first time vancomycin resistance was detected in *E. faecalis* from imported turkey meat. For further details please see below.

Table 32. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broilers (n=72) and pigs (n=145), Denmark*

DANMAP 2006

Compound	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	6.9	[2.3-15.5]							88.9	2.8		1.4			6.9						
	Pigs	60.7	[52.2-68.7]							39.3					5.5	55.2						
Chloramphenicol	Broilers	0	[0-5.0]								9.7	54.2	36.1									
	Pigs	0.7	[0.02-3.8]								6.9	60.7	31.0	0.7	0.7							
Florfenicol	Broilers	0	[0-5.0]									100										
	Pigs	0	[0-2.5]									100										
Ampicillin	Broilers	0	[0-5.0]								70.8	20.8	8.3									
	Pigs	0	[0-2.5]								60.7	39.3										
Erythromycin	Broilers	29.2	[19.0-41.1]						34.7	16.7	11.1	8.3	6.9	11.1	2.8	8.3						
	Pigs	34.5	[26.8-42.8]						18.6	4.1	11.7	31.0	7.6	0.7		26.2						
Gentamicin	Broilers	0	[0-5.0]														100					
	Pigs	0	[0-2.5]														100					
Kanamycin	Broilers	0	[0-5.0]														38.9	51.4	8.3	1.4		
	Pigs	24.8	[18.0-32.7]														32.4	31.0	10.3	1.4		24.8
Streptomycin	Broilers	13.9	[6.9-24.1]														86.1					13.9
	Pigs	30.3	[23.0-38.5]														61.4	2.1	2.1	4.1	15.9	14.5
Vancomycin	Broilers	0	[0-5.0]								100											
	Pigs	3.4	[1.1-7.9]								91.0		5.5			3.5						
Quinupristin/dalfopristin	Broilers	1.4	[1.5-13.6]						29.2	20.8	44.4	4.2	1.4									
	Pigs	1.4	[13.8-27.4]						13.8	5.5	60.7	18.6	1.4									
Avilamycin	Broilers	12.5	[5.9-22.4]								56.9	27.8	2.8	9.7	2.8							
	Pigs	0	[0-2.5]								100											
Salinomycin	Broilers	0	[0-5.0]								9.7	40.3	50.0									
	Pigs	0	[0-2.5]								100											
Linezolid	Broilers	0	[0-5.0]							15.3	84.7											
	Pigs	0	[0-2.5]							15.9	83.5	0.7										
Daptomycin	Broilers	0	[0-5.0]							11.1	55.6	33.3										
	Pigs	0	[0-2.5]					1.4		40.7	44.1	13.8										

Vetical lines indicate breakpoints for resistance
Flavomycin is not listed, since Enterococcus faecium is naturally resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Enterococci from healthy human volunteers

In 2006, stool samples from 57 healthy human volunteers were collected. In total 24 *E. faecium* isolates and 31 *E. faecalis* isolates were obtained. The MIC distributions and occurrence of antimicrobial resistance among enterococci from humans are shown in Tables 38 and 39. From 2005 to 2006, no significant changes in resistance were observed in *E. faecium* and *E. faecalis* from humans.

Comparison of resistance in *E. faecium* from animals, food and healthy human volunteers

A comparison of resistance among *E. faecium* from Danish food animals, food and humans is presented in Table 40 and in Figures 16-22. Resistance to tetracycline, erythromycin and streptomycin was significantly higher in *E. faecium* isolates from pigs than in isolates from healthy humans (Table 40).

The occurrence of resistance in *E. faecium* isolates from Danish broilers and Danish broiler meat was similar except for resistance to streptomycin and avilamycin, which was significantly higher in isolates from Danish broiler meat.

In *E. faecium* isolates from imported broiler meat, resistance to tetracycline, ampicillin, erythromycin, kanamycin, streptomycin, quinupristin/dalfopristin and avilamycin was significantly higher than in isolates from Danish broiler meat.

The resistance levels for *E. faecium* isolates were similar for Danish broiler meat and healthy humans; whereas *E. faecium* isolates from imported broiler meat were significantly more resistant to tetracycline and erythromycin compared to isolates from healthy humans. The level of resistance in *E. faecium* isolates was significantly higher for tetracycline, chloramphenicol, erythromycin and avilamycin when comparing imported turkey meat with isolates from healthy humans.

Comparison of resistance in *E. faecalis* from animals, food and healthy human volunteers

A comparison of resistance among *E. faecalis* from Danish food animals, food and humans is presented in Table 41.

Table 33. Distribution of MICs and occurrence of resistance among Enterococcus faecalis from broilers (n=45) and pigs (n=154), Denmark

DANMAP 2006

Compound	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	26.7	[14.6-41.9]							73.3				2.2	15.6	8.9						
	Pigs	85.1	[78.4-90.3]							13.6	1.3			3.9	11.0	70.1						
Chloramphenicol	Broilers	2.2	[0.06-11.8]								2.2	57.8	37.8			2.2						
	Pigs	11.0	[6.6-17.1]								0.7	39.6	46.8	2.0		4.6	6.5					
Florfenicol	Broilers	0	[0-7.9]									100										
	Pigs	0	[0-2.4]									100										
Ampicillin	Broilers	0	[0-7.9]								100											
	Pigs	0	[0-2.4]								100											
Erythromycin	Broilers	20.0	[9.6-34.6]						31.1	8.9	35.6	4.4		4.4	2.2	13.3						
	Pigs	38.3	[30.6-46.5]						20.1	33.1	8.4			0.7		37.7						
Gentamicin	Broilers	0	[0-7.9]														100					
	Pigs	3.9	[1.4-8.3]														94.2	0.7	1.3	0.7	1.3	2.0
Kanamycin	Broilers	0	[0-7.9]														95.6	4.4				
	Pigs	22.7	[16.4-30.2]														74.7	1.3	0.7	0.7	0.7	22.1
Streptomycin	Broilers	0	[0-7.9]														97.8	2.2				
	Pigs	31.8	[24.6-39.8]														57.1	9.7		1.3	1.3	30.5
Vancomycin	Broilers	0	[0-7.9]								95.6	4.4										
	Pigs	0	[0-2.4]								97.4	2.6										
Avilamycin	Broilers	0	[0-7.9]								93.3	6.7										
	Pigs	0	[0-2.4]								99.4	0.7										
Flavomycin	Broilers	4.4	[0.5-15.1]									95.6				4.4						
	Pigs	0	[0-2.4]									100										
Salinomycin	Broilers	0	[0-7.9]								77.8	22.2										
	Pigs	0	[0-2.4]								100											
Linezolid	Broilers	0	[0-7.9]							37.8	62.2											
	Pigs	0	[0-2.4]							24.0	76.0											
Daptomycin	Broilers	0	[0-7.9]				4.4			73.3	20.0	2.2										
	Pigs	0	[0-2.4]				0.7			88.2	6.5	4.6										

Vertical lines indicate breakpoints for resistance

Virginiamycin and Quinupristin/dalfopristin are not listed, since *Enterococcus faecalis* is natural resistant

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Evaluation of the quinupristin/dalforpristin breakpoint for *Enterococcus faecium*

The streptogramin family consists of virginiamycin, pristinamycin and quinupristin/dalfopristin. Virginiamycin has been banned as a growth promoter in Europe; but is still used in other countries. Pristinamycin and quinupristin/dalfopristin are used for treatment of human infections caused by either *Enterococcus faecium* or *Staphylococcus aureus*.

All streptogramins-antibiotics consist of two unrelated compounds: streptogramin A and streptogramin B. Individually, A and B components are bacteriostatic, whereas in combination they are bactericidal. Therefore, streptogramin A and streptogramin B are used in combination due to their synergistic effect. [Cocito, Microbiol. Rev. 1979]. Quinupristin/dalfopristin is a mixture (30:70) of two streptogramins: Quinupristin (streptogramin B) and dalfopristin (streptogramin A).

Mechanisms conferring resistance against both components (A and B) are essential for resistance against the combination antibiotic in *E. faecium*. Two acetyltransferases encoded by *vat*(D) and *vat*(E) inactivating streptogramin A have been identified in *E. faecium*. In most cases resistance to the streptogramin B component in *E. faecium* is due to methylation of the 23rRNA encoded by *erm*(B), which also encodes resistance to macrolides and licosamides. Two less frequently observed genes, encoding streptogramin B, have been detected in *E. faecium* (*vgb*(B) and *mrs*(A)) [Werner *et al.* Int J Med Microbiol 2002]. Presumable unspecific mutations can create reduced susceptibility. Millichap *et al.* were able to induce reduced susceptibility towards quinupristin/dalfopristin by spreading isolates on plates with quinupristin/dalfopristin. Stable resistance was obtained for isolates with an MIC> 8 µg/ml [Millichap *et al*.Diagn Microbiol Infect Dis 1996].

Clinical and Laboratory Standards Institute (CLSI) has suggested a breakpoint of ≥ 4 µg/ml for quinupristin/dalfopristin [CLSI document M100-S15 (ISBN 1-56238-556-9) CLSI, Wayne, PA, USA, 2005]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has suggested >4 µg/ml as „Preliminary EUCAST breakpoint“ for quinupristin/dalfopristin; whereas isolates with MIC ≤ 4 µg/ml should be reported as sensitive [Personal communication, G Kahlmeter].

In the DANMAP reports, MICs for quinupristin/dalfopristin have been reported from 1998-2006 (Table 1). The CLSI breakpoint of ≥ 4 µg/ml for quinupristin/dalfopristin has been used.

In 2006, 9 of 27 (33%) of the *E. faecium* isolates from healthy humans, had a MIC = 4 µg/ml for quinupristin/dalfopristin; which categorized them as resistant to quinupristin/dalfopristin using the CLSI breakpoint. None of *E. faecium* isolates obtained from healthy humans had a MIC value >4 µg/ml for quinupristin/dalfopristin (Table 1). In 2006, 45 of the 250 (18%) susceptibility tested *E. faecium* isolates obtained from pigs had MIC = 4 µg/ml for quinupristin/dalfopristin and three isolates had MIC = 8 µg/ml. Among *E. faecium* isolates from broilers 17 out of 203 (8%) had MIC = 4 µg/ml for quinupristin/dalfopristin and four isolates had MIC = 8 µg/ml (Table 1).

The *E. faecium* isolates with MIC ≥ 4 µg/ml for quinupristin/dalfopristin obtained from healthy humans, pigs and broilers were screened for the presence of *vat*(D) and *vat*(E) by PCR. None of the *E. faecium* isolates from humans or pigs were positive for *vat*(D) or *vat*(E). Of the 17 isolates from broilers with MIC equal to 4 µg/ml *vat*(D) was detected in one isolate and *vat*(E) in three isolates; whereas the other were PCR-negative. Of the four isolates from broilers with MIC >4 µg/ml, one isolate had *vat*(D) and three isolates had *vat*(E). Streptogramins A resistance genes (together with streptogramin B resistance genes) are essential for resistance towards the combination antibiotic quinupristin/dalfopristin. The results presented here indicate that the isolates where none of the two genes were found, are either not truly resistant towards quinupristin/dalfopristin, or an unknown resistance mechanism is present in these isolates.

The reproducibility of MIC values for quinupristin/dalfopristin was studied by testing of the ATCC strain *E. faecalis* 29212 repeatedly. *E. faecalis* 29212 were susceptibility tested towards quinupristin/dalfopristin weekly for more than a year in one laboratory (65 times). The range for MICs obtained was between 0.5-8 µg/ml for quinupristin/dalfopristin with most frequently values of 2, 4 or 8 µg/ml (11%, 65% and 20%, respectively). The obtained MICs did not seem to be influenced by the age of the MIC-panels used.

In conclusion, variations in MICs due to the experimental setup, can lead to misinterpretation of isolates as resistance due to overlapping population structure of the sensitive and resistant populations. Based on the data reported above, we suggest a MIC value >4 µg/ml as the resistance breakpoint for quinupristin/dalfopristin for surveillance programs, whereas MIC = 4 µg/ml should be reported as intermediate resistant. Intermediate resistant isolates should be tested for the presence of resistance genes.

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Table 1. Distribution of MICs and occurrence of Quinupristin/dalfopristin resistance among Enterococcus faecium *from poultry, pigs and healthy humans from DANMAP reports (1998-2006), Denmark*

DANMAP 2006

Animal species	Year	Compound	% Resistant with use of two different breakpoints		Distribution (%) of MICs								
			4	8	0.25	0.5	1	2	4	8	16	32	64
Poultry	1998	Quinupristin/dalfopristin	74.0	59.7	0.7	3.3	5.2	16.9	14.3	21.4	32.5	5.8	
	1999	Quinupristin/dalfopristin	40.7	22.7		14.8	12.7	31.8	18.0	14.8	7.4	0.5	
	2000	Quinupristin/dalfopristin	37.1	27.6		13.2	6.9	42.9	9.5	26.5	1.1		
	2001	Quinupristin/dalfopristin	30.6	7.7		19.8	22.9	26.7	22.9	6.9	0.8		
	2002	Quinupristin/dalfopristin	28.5	21.6		20.6	10.8	40.2	6.9	18.6	2.0		1.0
	2003	Quinupristin/dalfopristin	25.2	6.5		22.0	15.4	37.4	18.7	6.5			
	2004	Quinupristin/dalfopristin	23.7	14.8		13.3	23.0	40.0	8.9	13.3	1.5		
	2005	Quinupristin/dalfopristin	13.0	2.3		46.6	11.5	29.0	10.7	2.3			
	2006	Quinupristin/dalfopristin	5.6	1.4		29.2	20.8	44.4	4.2	1.4			
Pigs	1998	Quinupristin/dalfopristin	46.4	11.1	3.9	9.2	7.8	32.7	35.3	6.5	3.9	0.7	
	1999	Quinupristin/dalfopristin	19.4	4.0		24.8	6.4	49.5	15.4	3.0	1.0		
	2000	Quinupristin/dalfopristin	23.6	15.4		15.9	15.9	44.5	8.2	1.1	7.7	6.6	
	2001	Quinupristin/dalfopristin	8.6	2.3		21.7	9.7	60.0	6.3	2.3			
	2002	Quinupristin/dalfopristin	12.9	0.5		23.7	7.7	55.7	12.4	0.5			
	2003	Quinupristin/dalfopristin	8.6	0.6		19.4	8.6	63.4	8.0		0.6		
	2004	Quinupristin/dalfopristin	12.9	0.7		19.6	6.8	60.8	12.2	0.7			
	2005	Quinupristin/dalfopristin	16.2	1.0		17.1	8.6	58.1	15.2	1.0			
	2006	Quinupristin/dalfopristin	20.0	1.4		13.8	5.5	60.7	18.6	1.4			
Healthy humans	2002	Quinupristin/dalfopristin	2.5	0		30.0	10.0	57.5	2.5				
	2003	Quinupristin/dalfopristin	7.3	0		23.6	14.6	54.5	7.3				
	2004	Quinupristin/dalfopristin	35.2	0		18.5	16.7	29.6	35.2				
	2005	Quinupristin/dalfopristin	54.0	0		26.0	8.0	12.0	54.0				
	2006	Quinupristin/dalfopristin	37.5	0		25.0	16.7	20.8	37.5				

The vertical lines indicate the two different breakpoints for resistance (see text for futher information)

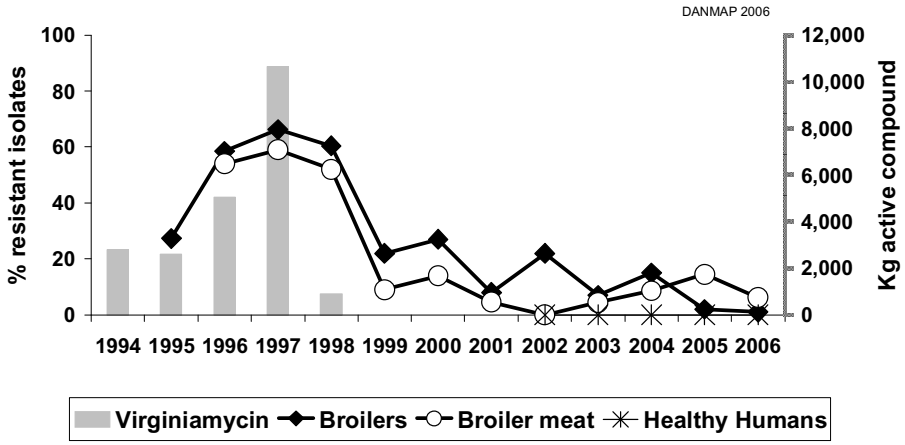

Figure 16. Trends in streptogramin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

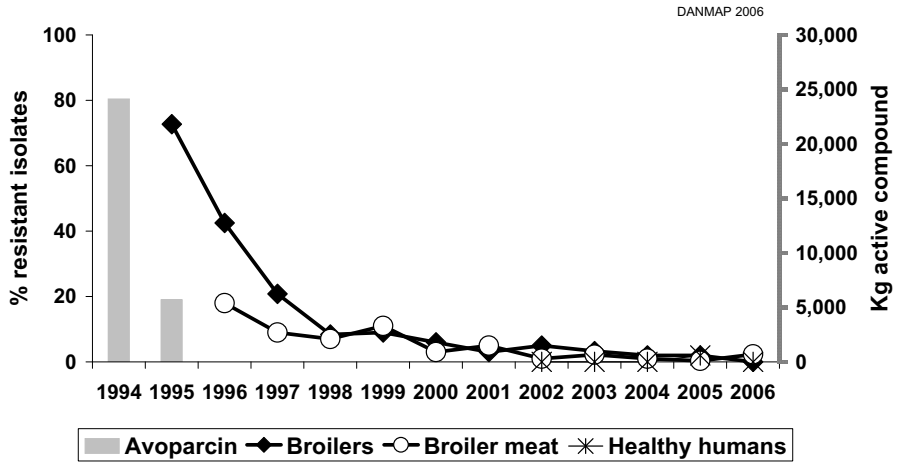

Figure 17. Trends in glycopeptide resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and consumption of the growth promoter avoparcin in animals, Denmark*

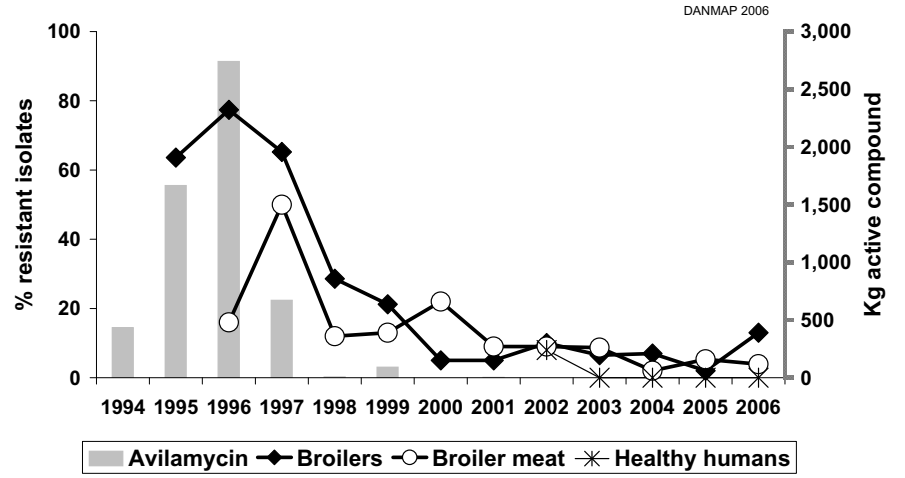

Figure 18. Trends in avilamycin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and consumption of the growth promoter avilamycin in animals, Denmark*

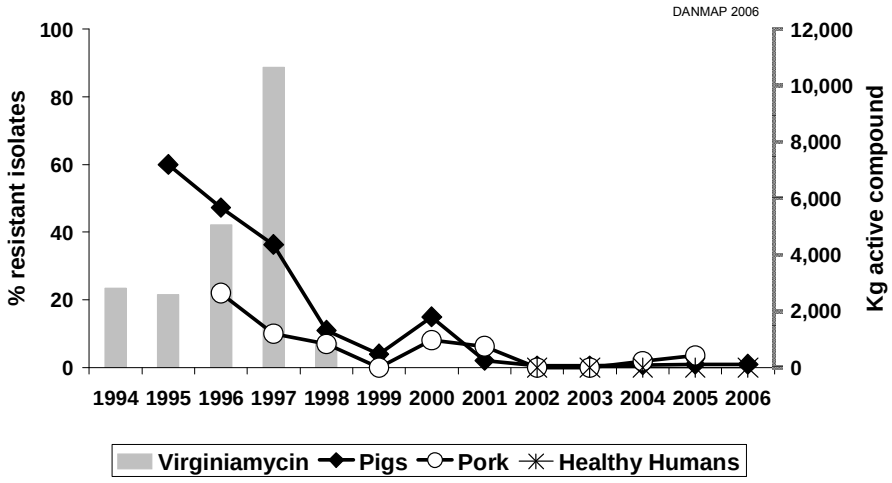

Figure 19. Trends in streptogramin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

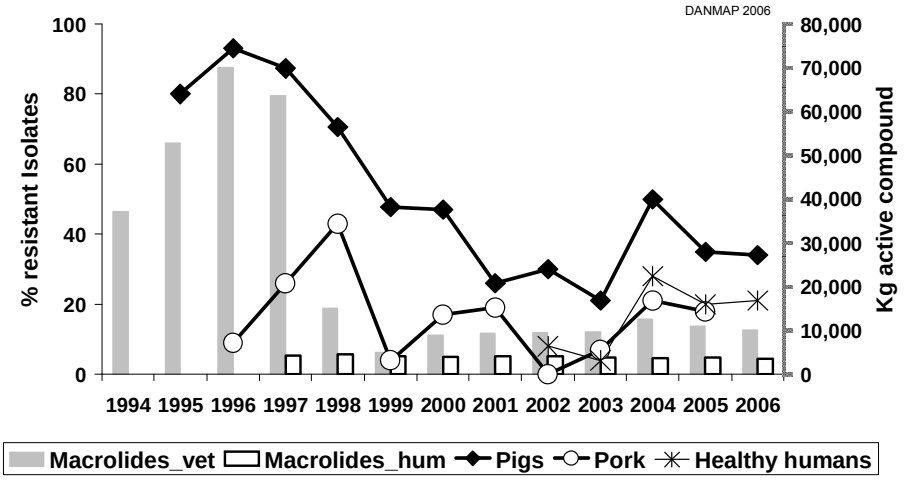

Figure 20. Trends in erythromycin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the total consumption of macrolides, both as growth promoters in animals and therapeutics in animals and humans, Denmark*

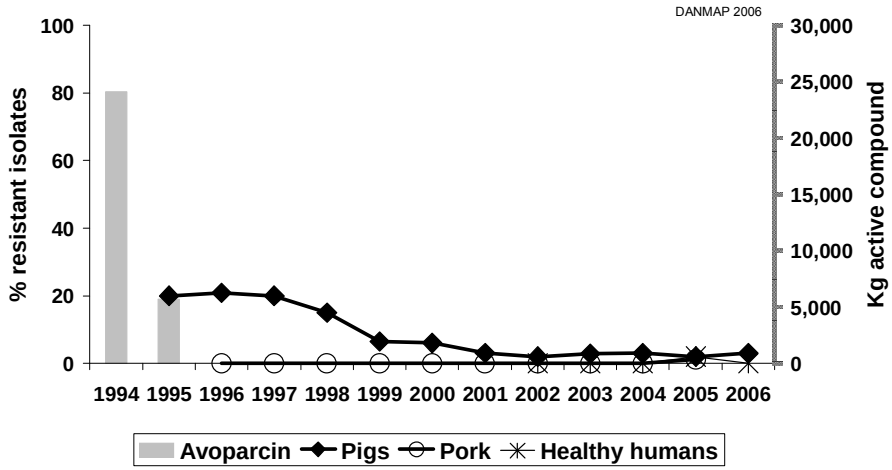

Figure 21. Trends in glycopeptide resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and consumption of the growth promoter avoparcin in animals, Denmark*

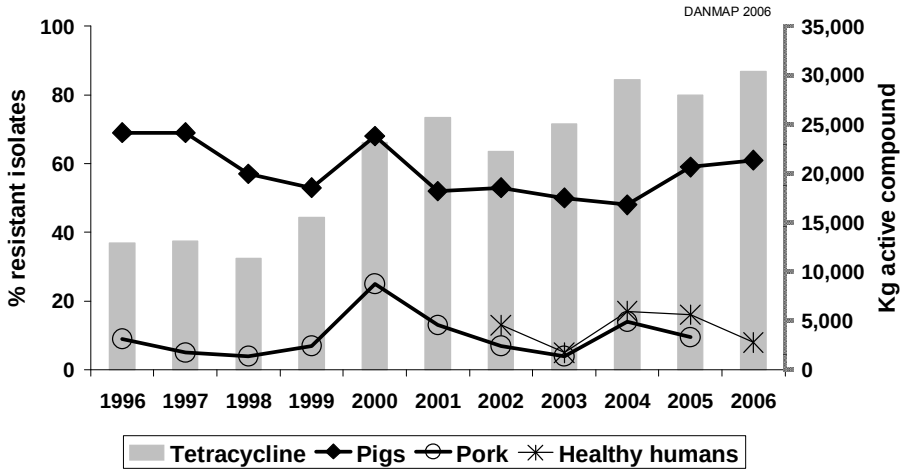

Figure 22. Trends in tetracycline resistance among Enterococcus faecium *from pigs, pork and healthy humans and the consumption of tetracycline in pig production, Denmark*

Turkey meat sold in Denmark was sampled for enterococci in both 2005 and 2006, however the results of the susceptibility tests were not available from the Danish Veterinary and Food Administration until the beginning of 2007. Vancomycin resistant *E. faecalis* were detected in samples from turkey meat imported from Germany in 2005 and 2006. This is the first isolation of vancomycin resistant *E. faecalis* from meat during 11 years of integrated antimicrobial resistance surveillance in the DANMAP programme. In 2005, the first two vancomycin resistant *E. faecalis* isolates were obtained from humans in Denmark [DANMAP 2005, www.danmap.org].

In *E. faecalis* isolates from broilers and Danish broiler meat, the resistance levels were similar except for streptomycin resistance, which was significantly higher in isolates from Danish broiler meat.

Among *E. faecalis* isolates from imported broiler meat the occurrence of resistance to tetracycline, chloramphenicol, erythromycin, gentamicin, kanamycin and streptomycin was significantly higher than in Danish broiler meat.

The resistance levels for *E. faecalis* isolates were similar for Danish broiler meat and healthy humans; whereas *E. faecalis* isolates from imported broiler meat were more resistant to tetracycline and erythromycin compared to isolates from healthy humans. Differences between levels of resistance were observed among *E. faecalis* isolates from imported turkey meat and healthy humans, where resistance to tetracycline, chloramphenicol and erythromycin was significantly higher in isolates from imported turkey meat.

Table 34. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broiler meat (Danish n=306; imported n=351), Denmark*

DANMAP 2006

Compound	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	Danish	14.4	[10.7-18.8]							82.4	2.6		0.7	0.3	2.0	12.1						
	Imported	51.3	[45.9-56.6]							47.6	0.6	0.3	0.3	1.1	2.9	47.3						
Chloramphenicol	Danish	4.6	[2.5-7.6]								2.9	39.9	49.0	3.6	4.3	0.3						
	Imported	8.0	[5.4-11.3]								4.6	23.9	55.0	8.6	7.1	0.6	0.3					
Florfenicol	Danish	0	[0-1.2]									98.4	1.6									
	Imported	0	[0-1.1]									98.9	1.1									
Ampicillin	Danish	0.7	[0.1-2.3]								86.6	9.2	3.6			0.3	0.3					
	Imported	9.7	[6.8-13.3]								74.6	11.7	4.0	1.4	2.6	1.7	4.0					
Erythromycin	Danish	20.6	[16.2-25.6]						23.5	6.2	22.9	26.8	7.2	5.6	1.0	6.9						
	Imported	39.9	[34.7-45.2]						15.7	11.1	21.4	12.0	6.6	1.1	1.1	31.1						
Gentamicin	Danish	0	[0-1.2]														99.4	0.7				
	Imported	0.6	[0-2.0]														98.6	0.9		0.3	0.3	
Kanamycin	Danish	2.9	[1.4-5.5]														19.9	49.0	19.9	8.2	1.6	1.3
	Imported	18.2	[14.3-22.7]														16.8	33.9	22.8	8.3	2.6	15.7
Streptomycin	Danish	3.3	[1.6-5.9]														95.1	0.7	0.7	0.3	0.7	2.6
	Imported	20.2	[16.2-24.8]														72.9	1.7	2.6	2.6	4.8	15.4
Vancomycin	Danish	2.3	[0.9-4.7]								90.9	6.9				2.3						
	Imported	0.9	[0.2-2.5]								93.5	5.4	0.3			0.9						
Quinupristin/dalfopristin	Danish	6.2	[3.8-9.5]						18.0	33.3	19.0	23.5	2.9	2.0	1.3							
	Imported	13.4	[10.0-17.4]						13.7	18.8	18.8	35.3	7.7	2.6	3.1							
Avilamycin	Danish	3.9	[2.0-6.8]								35.3	53.6	7.2	1.6	2.3							
	Imported	9.7	[6.8-13.3]								32.2	50.1	8.0	1.1	8.6							
Salinomycin	Danish	0.7	[0.1-2.3]								17.8	11.5	70.1	0.7								
	Imported	0.3	[0-1.6]								42.2	18.2	39.3	0.3								

Vertical lines indicate breakpoints for resistance
Flavomycin is not listed, since *Enterococcus faecium* is naturally resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Table 35. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broiler meat (Danish n=180; imported n=317), Denmark*

DANMAP 2006

Compound	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	Danish	29.4	[22.9-36.7]							70.0	0.6			1.1	5.0	23.3						
	Imported	64.4	[58.8-69.6]							33.8	1.3	0.3	0.3	1.0	7.3	56.2						
Chloramphenicol	Danish	0	[0-2.0]								1.7	16.1	81.1	1.1								
	Imported	5.4	[3.2-8.5]								1.3	10.1	78.9	4.4	1.0	2.2	2.2					
Florfenicol	Danish	0	[0-2.0]									100										
	Imported	0	[0-1.2]									99.7	0.3									
Ampicillin	Danish	0	[0-2.0]								98.9	0.6	0.6									
	Imported	0	[0-1.2]								98.7	1.3										
Erythromycin	Danish	11.1	[6.9-16.6]						29.4	16.7	36.1	6.7	0.6	1.1	0.6	8.9						
	Imported	35.7	[30.4-41.2]						34.7	12.9	14.5	2.2	1.0	2.5		32.2						
Gentamicin	Danish	0	[0-2.0]														98.9	1.1				
	Imported	3.2	[1.5-5.7]														95.9	0.6	0.3	1.0	0.6	1.6
Kanamycin	Danish	0.6	[0-3.1]														92.8	4.4	1.7	0.6		0.6
	Imported	16.1	[12.2-20.6]														79.8	2.2	1.3	0.6		16.1
Streptomycin	Danish	12.8	[8.3-18.6]														81.1	3.9	0.6	1.7	7.2	5.6
	Imported	21.8	[17.4-26.7]														75.1	1.3	1.0	1.0	4.1	17.7
Vancomycin	Danish	1.1	[0-2.0]								93.3	5.6			0.6	0.6						
	Imported	0	[1.7-6.1]								92.4	7.6										
Avilamycin	Danish	0.6	[0.1-4.0]								77.2	21.7	0.6		0.6							
	Imported	1.3	[0-1.2]								73.8	23.7	1.3		1.3							
Flavomycin	Danish	0	[0-2.0]									100										
	Imported	0.3	[0-1.7]									98.1	1.6			0.3						
Salinomycin	Danish	0	[0-2.0]								82.8	11.1	6.1									
	Imported	0	[0-1.2]								86.8	11.7	1.6									

Vertical lines indicate breakpoints for resistance
Virginiamycin and Quinupristin/dalfopristin are not listed, since *Enterococcus faecalis* is naturally resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Table 36. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from turkey meat (Danish n=6; imported n=244), Denmark*

DANMAP 2006

Compound	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	Danish	66.7	[22.3-95.7]							33.3						66.7						
	Imported	79.5	[73.9-84.4]							20.1		0.4		0.4	0.8	78.3						
Chloramphenicol	Danish	0	[0-45.9]										16.7	83.3								
	Imported	16.8	[12.3-22.1]								3.3	16.0	45.5	18.4	15.2	1.6						
Florfenicol	Danish	0	[0-45.9]									100										
	Imported	0	[0-1.5]									99.2	0.8									
Ampicillin	Danish	0	[0-45.9]								50.0	50.0										
	Imported	5.3	[2.9-8.9]								71.3	19.7	3.7	0.4	2.1	2.1	0.8					
Erythromycin	Danish	16.7	[0.4-64.1]						66.7		16.7		16.7									
	Imported	42.2	[35.9-48.7]						13.9	8.2	25.0	10.7	8.2	0.8	0.4	32.8						
Gentamicin	Danish	0	[0-45.9]														100					
	Imported	2.1	[0.7-4.7]														95.9	1.6	0.4		0.8	1.2
Kanamycin	Danish	0	[0-45.9]														33.3	66.7				
	Imported	13.5	[9.5-18.5]														18.0	28.7	29.5	10.3	4.5	9.0
Streptomycin	Danish	0	[0-45.9]														100					
	Imported	9.8	[6.4-14.3]														81.2	1.6	2.1	5.3	2.5	7.4
Vancomycin	Danish	0	[0-45.9]								100											
	Imported	0	[0-1.5]								96.3	3.3	0.4									
Quinupristin/dalfopristin	Danish	0	[0-45.9]								50.0	50.0										
	Imported	14.8	[10.6-19.8]						10.7	23.0	25.0	26.6	6.6	5.7	2.5							
Avilamycin	Danish	33.3	[4.3-77.7]									50.0	16.7		33.3							
	Imported	16.8	[12.3-22.1]								31.6	46.7	4.9	1.2	15.6							
Salinomycin	Danish	0	[0-45.9]								33.3	50.0	16.7									
	Imported	0	[0-1.5]								80.3	11.1	8.6									

Vertical lines indicate breakpoints for resistance
Flavomycin is not listed, since *Enterococcus faecium* is naturally resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Table 37. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from turkey meat (Imported n=235), Denmark*

DANMAP 2006

Compound	% 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	86.0	[80.9-90.1]							13.6	0.4			0.4	2.1	83.4						
Chloramphenicol	12.8	[8.8-17.7]								2.1	8.5	74.0	2.6	3.0	8.1	1.7					
Florfenicol	0	[0-1.6]									100										
Ampicillin	0	[0-1.6]								98.7	1.3										
Erythromycin	52.3	[45.8-58.9]						25.1	4.3	15.3	3.0		0.4	0.4	51.5						
Gentamicin	1.7	[0.5-4.3]														98	0.4			0.4	1.3
Kanamycin	7.6	[4.6-11.8]														85.5	4.7	0.9	1.3	0.9	6.8
Streptomycin	16.6	[12.1-22.0]														78.3	3.4	0.4	1.3	1.3	15.3
Vancomycin	0.4	[0.3-3.7]								93.2	6.4				0.4						
Avilamycin	1.7	[0.5-4.3]								70.6	26.8	0.9	0.9	0.9							
Flavomycin	0	[0-1.6]									97.0	3.0									
Salinomycin	0	[0-1.6]								92.3	6.4	1.3									

Vertical lines indicate breakpoints for resistance
Quinupristin/dalfopristin are not listed, since *Enterococcus faecalis* is natural resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest

Table 38. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from healthy humans (n=24), Denmark*

DANMAP 2006

Compound	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																	
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	8.3	[1.0-27.0]							87.5	4.2					8.3						
Tigecycline	0	[0-14.3]		47.7	4.2	54.2															
Chloramphenicol	0	[0-14.3]								8.3	45.8	45.8									
Florfenicol	0	[0-14.3]									100										
Ampicillin	4.2	[0.1-21.1]								91.7		4.2		4.2							
Erythromycin	20.8	[7.1-42.2]						12.5	12.5	29.2	25.0	12.5			8.3						
Gentamicin	0	[0-14.3]														100					
Kanamycin	12.5	[2.7-32.4]														41.7	25.0	20.8		4.2	8.3
Streptomycin	4.2	[0.1-21.1]														91.7	4.2				4.2
Vancomycin	0	[0-14.3]								100											
Quinupristin/dalfopristin	0	[0-14.3]						25.0	16.7	20.8	37.5										
Avilamycin	0	[0-14.3]								54.2	45.8										
Salinomycin	0	[0-14.3]								100											
Linezolid	0	[0-14.3]							16.7	83.3											
Daptomycin	0	[0-14.3]							29.2	62.5	8.3										

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

Table 39. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from healthy humans (n=31), Denmark*

DANMAP 2006

Compound	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																	
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	38.7	[21.9-57.8]							61.3						38.7						
Tigecycline	0	[0-11.2]	3.2	16.1	32.3	32.3	19.4														
Chloramphenico	0	[0-11.2]								9.7	9.7	80.6									
Florfenicol	0	[0-11.2]									100										
Ampicillin	0	[0-11.2]								100											
Erythromycin	6.5	[0.8-21.4]						16.1	41.5	32.3	3.2				6.5						
Gentamicin	0	[0-11.2]														100					
Kanamycin	9.7	[2.0-25.8]														90.3					9.7
Streptomycin	9.7	[2.0-25.8]														87.1	3.2				9.7
Vancomycin	0	[0-11.2]								93.5	6.5										
Avilamycin	0	[0-11.2]								100											
Flavomycin	0	[0-11.2]									100										
Salinomycin	0	[0-11.2]								100											
Linezolid	0	[0-11.2]							16.1	83.9											
Daptomycin	0	[0-11.2]				3.2		19.4	74.2	3.2											

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

Table 40. Comparison of resistance (%) among Enterococcus faecium *from food animals, foods of Danish and imported origin and healthy humans, Denmark*

DANMAP 2006

Compound	Pigs	Broilers	Broiler meat		Turkey meat		Healthy humans
	Danish	Danish	Danish	Imported	Danish	Imported	%
	%	%	%	%	%	%	
Tetracycline	61	7	14	51	67	80	8
Chloramphenicol	<1	0	5	8	0	17	0
Florfenicol	0	0	0	0	0	0	0
Ampicillin	0	0	<1	10	0	5	4
Erythromycin	34	29	21	40	17	42	8
Gentamicin	0	0	0	<1	0	2	0
Kanamycin	25	0	3	18	0	14	13
Streptomycin	30	14	3	20	0	10	4
Vancomycin	3	0	2	<1	0	0	0
Quinupristin/dalfopristin	1	1	6	13	0	15	0
Avilamycin	0	13	4	10	33	17	0
Salinomycin	0	0	<1	<1	0	0	0
Linezolid	0	0	<1	0	0	0	0
Daptomycin	0	0	2	<1	0	2	0
Number of isolates	145	72	306	351	6	244	24

Table 41. Comparison of resistance (%) among Enterococcus faecalis *from food animals, foods of Danish and imported origin and healthy humans, Denmark*

DANMAP 2006

Compound	Pigs	Broilers	Broiler meat		Turkey meat	Healthy humans
	Danish	Danish	Danish	Imported	Imported	%
	%	%	%	%	%	
Tetracycline	85	27	29	64	86	39
Chloramphenicol	11	2	0	5	13	0
Florfenicol	0	0	0	0	0	0
Ampicillin	0	0	0	0	0	0
Erythromycin	38	20	11	36	52	7
Gentamicin	4	0	0	3	2	0
Kanamycin	23	0	<1	16	8	10
Streptomycin	32	0	13	22	17	10
Vancomycin	0	0	1	0	<1	0
Avilamycin	0	0	<1	1	2	0
Flavomycin	0	4	0	<1	0	0
Salinomycin	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0
Daptomycin	0	0	0	0	<1	0
Number of isolates	153	45	180	317	235	31

Escherichia coli

Escherichia coli from food animals

Table 42 presents the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates from animals at slaughter. Figure 23 presents the trends in resistance to selected antimicrobial agents from 1996 to 2006.

From 2005 to 2006, the occurrence of resistance in *E. coli* from pigs remained unchanged. However, compared to 2004, a significant decrease in resistance to tetracycline, chloramphenicol, ampicillin, sulfonamide, trimethoprim and neomycin was observed. Concomitant with this decrease in resistance, the proportion of fully susceptible isolates increased from 35% in 2004 to 52% in 2006, and the occurrence of the resistance patterns STR-SUL-TET and AMP-STR-SUL-TET decreased (Figure 24).

Table 42. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy broilers (n=123), cattle (n=93) and pigs (n=148), Denmark*

DANMAP 2006

Compound	Animal species	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Broilers	6.5	[2.8-12.4]							93.5					6.5					
	Cattle	9.7	[4.5-17.6]							90.3				2.2	7.5					
	Pigs	28.4	[21.3-36.4]							69.6	2.0		0.7	0.7	27.0					
Chloramphenicol	Broilers	0	[0-3.0]							8.9	61.0	30.1								
	Cattle	0	[0-3.9]							1.1	36.6	62.4								
	Pigs	1.4	[0.2-4.8]							2.0	40.5	53.4	2.7			1.4				
Florfenicol	Broilers	0	[0-3.0]							14.6	70.7	14.6								
	Cattle	0	[0-3.9]							1.1	35.5	62.4	1.1							
	Pigs	0.7	[0.02-3.7]							0.7	35.8	59.5	3.4			0.7				
Ampicillin	Broilers	17.1	[10.9-24.9]						26.0	49.6	7.3			0.8	16.3					
	Cattle	2.2	[0.3-7.6]						2.2	36.6	58.1	1.1			2.2					
	Pigs	20.3	[14.1-27.7]						3.4	41.2	34.5	0.7			20.3					
Amoxicillin/ clavulanic acid a)	Broilers	0	[0-3.0]							60.2	30.1	9.8								
	Cattle	0	[0-3.9]							25.8	69.9	3.2	1.1							
	Pigs	0	[0-2.5]							36.5	43.9	19.6								
Cephalothin	Broilers	3.3	[0.9-8.1]								30.1	51.2	15.5	3.3						
	Cattle	2.2	[0.3-7.6]								11.8	73.1	12.9	2.2						
	Pigs	2.7	[0.7-6.8]								23.0	48.7	25.7	2.7						
Cefpodoxime	Broilers	0	[0-3.0]			21.1	59.4	15.5	4.1											
	Cattle	0	[0-3.9]			2.2	57.0	36.6	4.3											
	Pigs	0.7	[0.02-3.7]			4.1	46.6	41.9	6.8	0.7										
Ceftiofur	Broilers	0	[0-3.0]					100												
	Cattle	0	[0-3.9]					98.9	1.1											
	Pigs	0	[0-2.5]					100												
Sulfonamide	Broilers	8.9	[4.5-15.4]												90.2	0.8				8.9
	Cattle	11.8	[6.1-20.2]												88.2				1.1	10.8
	Pigs	25.7	[18.9-33.5]												73.7	0.7				25.7
Trimethoprim	Broilers	1.6	[0.2-5.8]								97.6	0.8			1.6					
	Cattle	3.2	[0.7-9.1]								96.8			1.1	2.2					
	Pigs	14.2	[9.0-20.9]								85.8				14.2					
Apramycin	Broilers	0	[0-3.0]								87.8	12.2								
	Cattle	0	[0-3.9]								95.7	4.3								
	Pigs	0.7	[0.02-3.7]								93.9	5.4			0.7					
Gentamicin	Broilers	0	[0-3.0]						99.2	0.8										
	Cattle	0	[0-3.9]						98.9	1.1										
	Pigs	0.7	[0.02-3.7]						98.0	1.4		0.7								
Neomycin	Broilers	2.4	[0.5-7.0]							96.8	0.8			0.8	1.6					
	Cattle	0	[0-3.9]							98.9	1.1									
	Pigs	6.1	[2.8-11.2]							90.5	3.4			4.1	2.0					
Spectinomycin	Broilers	1.6	[0.2-5.8]										76.4	22.0			1.6			
	Cattle	2.2	[0.3-7.6]										61.3	35.5	1.1		1.1	1.1		
	Pigs	27.0	[20.1-34.9]										42.6	27.0	3.4	10.1	9.5	7.4		
Streptomycin	Broilers	10.6	[5.7-17.4]								5.7	78.1	5.7		5.7	4.9				
	Cattle	10.8	[5.3-18.9]								65.6	21.5	2.2	4.3	5.4	1.1				
	Pigs	40.5	[32.6-48.9]								34.5	20.3	4.7	9.5	13.5	17.6				
Ciprofloxacin	Broilers	5.7	[2.3-11.4]	91.9	0.8	1.6	5.7													
	Cattle	0	[0-3.9]	98.9	1.1															
	Pigs	0.7	[0.02-3.7]	99.3			0.7													
Nalidixic acid	Broilers	7.3	[3.4-13.4]								91.1	1.6		1.6	3.3	2.4				
	Cattle	0	[0-3.9]								100									
	Pigs	0.7	[0.02-3.7]								98.7	0.7			0.7					
Colistin	Broilers	0	[0-3.0]								99.2	0.8								
	Cattle	0	[0-3.9]								100									
	Pigs	0	[0-2.5]								100									

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
a) Concentration of amoxicillin given, testet with clavulanic acid in concentration ratio 2:1

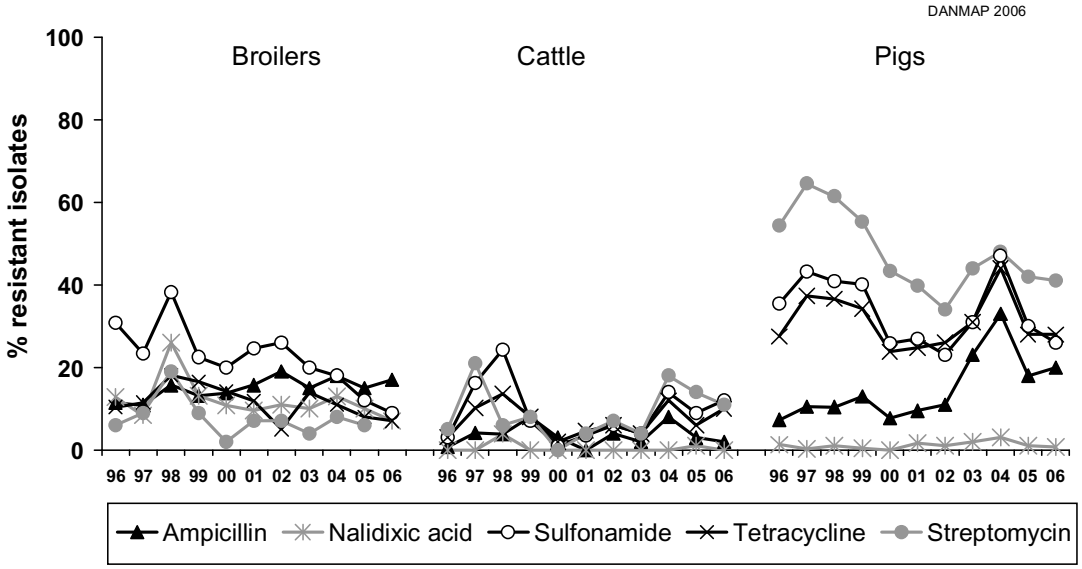

Figure 23. Trends in resistance to selected antimicrobials among Escherichia coli from food animals, Denmark

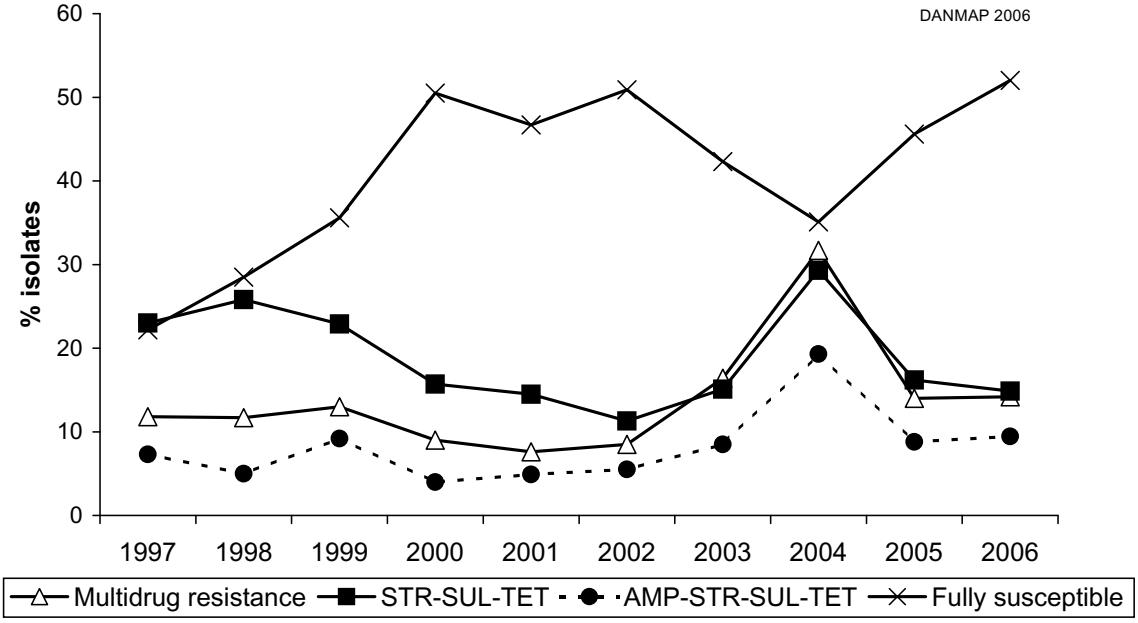

Figure 24. Trends in multidrug resistance and selected resistance patterns in indicator E. coli *from pigs, Denmark*
Multidrug resistance: is defined as isolates resistant to ≥4 of 8 antimicrobial agents (ampicillin, chloramphenicol, gentamicin, naldixic acid, streptomycin, sulfonamide, tetracycline and trimetroprim); STR-SUL-TET: at least resistant to streptomycin, sulfonamide and tetracycline; AMP-STR-SUL-TET: at least resistant to ampicillin, streptomycin, sulfonamide and tetracycline; Fully susceptible: isolates susceptible to all 8 antimicrobials

These changes in resistance patterns in *E. coli* are in contrast to the results observed in *S.* Typhimurium. In *S*. Typhimurium the proportion of fully susceptible isolates continued to decrease and the resistance pattern STR-SUL-TET and AMP-STR-SUL-TET continued to increase, although the antimicrobial consumption in pigs has decreased slightly since 2004. The reason why *E. coli* and *S.* Typhimurium respond differently to the selective pressure of antimicrobial agents is not known, however there may be a considerable diversity of resistance profiles among the *E. coli* isolates in a faecal samples, whereas the *Salmonella* isolates are likely to be more clonal.

The level of resistance was generally lower in isolates from broilers and cattle, compared to isolates from pigs. Except for a significant decrease in resistance to sulfonamide (P=0.05) in *E. coli* isolates from broilers, the occurrence of resistance in isolates from broilers and cattle remained unchanged from 2004 to 2006.

Escherichia coli from food

Tables 43 and 44 present the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates collected from broiler and turkey meat sold at wholesale and retail outlets. For further details please see „Comparison of *Escherichia coli* from animals, food and healthy human volunteers“. Pork and beef was not sampled by the Danish Veterinary and Food Administration in 2006 and therefore no *E. coli* isolates were available for the 2006 DANMAP report.

Table 43. Distribution of MICs and occurrence of resistance among Escherichia coli *from broiler meat (Danish n=534; imported n=550), Denmark*

DANMAP 2006

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Danish	11.6	[9.0-14.6]							88.0	0.2	0.2	0.8	0.4	10.5					
	Imported	49.6	[45.4-53.9]							49.3	0.7	0.4	0.4	1.1	48.2					
Chloramphenicol	Danish	0.9	[0.3-2.2]							21.0	51.9	25.5	0.8	0.2		0.8				
	Imported	8.7	[6.5-11.4]							8.0	57.5	25.1	0.7	1.3	0.7	6.7				
Florfenicol	Danish	0	[0-0.7]							23.4	60.3	16.3								
	Imported	0	[0-0.7]							10.4	66.7	22.4	0.6							
Ampicillin	Danish	15.4	[12.4-18.7]						13.5	37.5	32.0	1.7			15.4					
	Imported	45.8	[41.6-50.1]						9.6	25.5	18.6	0.6			45.8					
Amoxicillin/ clavulanic acid a)	Danish	0	[0-0.7]							36.0	47.2	16.1	0.8							
	Imported	5.5	[3.7-7.7]							24.9	32.9	32.0	4.7	3.6	1.8					
Cephalothin	Danish	2.4	[1.3-4.1]								27.0	45.3	25.3	2.3	0.2					
	Imported	11.3	[8.8-14.2]								21.1	41.5	26.2	3.1	8.2					
Ceftiofur	Danish	0.2	[0.005-1.0]					99.8					0.2							
	Imported	5.3	[3.6-7.5]					91.6	0.9	0.9	1.3	3.3	2.0							
Cefpodoxime	Danish	0.2	[0.005-1.0]			18.5	54.9	24.7	1.7			0.2								
	Imported	7.3	[5.3-9.8]			12.7	50.9	26.6	2.6			7.3								
Sulfonamide	Danish	15.7	[12.8-19.1]												83.5	0.4	0.4			15.7
	Imported	42.6	[38.4-46.8]												56.9	0.4	0.2		0.2	42.4
Trimethoprim	Danish	6.4	[4.5-8.8]								93.5	0.2	0.2		6.2					
	Imported	33.3	[29.3-37.4]								66.7				33.3					
Apramycin	Danish	0.2	[0.005-1.0]								91.6	8.1	0.2		0.2					
	Imported	0.4	[0.04-1.3]								81.1	18.0	0.6		0.4					
Gentamicin	Danish	0.2	[0.005-1.0]						99.3	0.4	0.2		0.2							
	Imported	2.2	[1.1-3.8]						96.6	1.3		0.6	0.9	0.4	0.4					
Neomycin	Danish	1.5	[0.7-2.9]							98.3	0.2			0.9	0.6					
	Imported	11.3	[8.8-14.2]							87.3	1.3	0.2	0.9	3.8	6.6					
Spectinomycin	Danish	2.1	[1.0-3.7]										93.8	2.3	1.9	1.7	0.2	0.2		
	Imported	12.2	[9.6-15.2]										69.6	15.5	2.7	3.8	4.0	4.4		
Streptomycin	Danish	12.0	[9.4-15.1]								69.5	16.9	1.7	4.3	4.5	3.2				
	Imported	33.3	[29.3-37.4]								34.4	26.2	6.2	4.2	5.5	23.6				
Ciprofloxacin	Danish	4.5	[2.9-6.6]	92.3	3.2	3.0	1.1				0.2	0.2								
	Imported	24.2	[20.7-28.0]	72.6	3.3	6.9	10.0	2.7	0.6	0.7	0.6	2.7								
Nalidixic acid	Danish	6.2	[4.3-8.6]								92.7	0.9	0.2	0.9	2.4	2.8				
	Imported	25.6	[22.0-29.5]								73.6	0.4	0.4	1.6	5.5	18.6				
Colistin	Danish	0	[0-0.7]								99.6	0.4								
	Imported	0	[0-0.7]								99.8	0.2								

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

Table 44. Distribution of MICs and occurrence of resistance among Escherichia coli from turkey meat (Danish n=9; imported n=475), Denmark

DANMAP 2006

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Danish	22.2	[2.8-60.0]							77.8					22.2					
	Imported	81.7	[77.9-85.1]							17.9		0.4		1.7	80.0					
Chloramphenicol	Danish	22.2	[2.8-60.0]							22.2	44.4	11.1		11.1		11.1				
	Imported	15.4	[12.2-18.9]							10.1	51.4	22.1	1.1	3.2	1.5	10.7				
Florfenicol	Danish	11.1	[0.3-48.3]							11.1	77.8			11.1						
	Imported	0.8	[0.2-2.1]							11.6	60.4	23.6	3.6	0.4		0.4				
Ampicillin	Danish	22.2	[2.8-60.0]						11.1	44.4	11.1		11.1		22.2					
	Imported	65.3	[60.8-69.5]						4.0	16.6	12.4	1.5	0.2		65.3					
Amoxicillin/ clavulanic acid a)	Danish	0	[0-33.6]							22.2	44.4	33.3								
	Imported	1.5	[0.6-3.0]							13.7	26.3	49.1	9.5	1.3	0.2					
Cephalothin	Danish	11.1	[0.3-48.3]								11.1	55.6	22.2		11.1					
	Imported	10.5	[7.9-13.6]								11.6	38.3	39.6	8.8	1.7					
Ceftiofur	Danish	11.1	[0.3-48.3]					88.9					11.1							
	Imported	0	[0-0.8]					98.3	1.3	0.2	0.2									
Cefpodoxime	Danish	11.1	[0.3-48.3]				66.7	22.2				11.1								
	Imported	1.3	[0.5-2.7]			7.6	53.3	33.5	4.4	0.6	0.2	0.4								
Sulfonamide	Danish	22.2	[2.8-60.0]												77.8					22.2
	Imported	45.5	[40.9-50.1]												54.3	0.2				45.5
Trimethoprim	Danish	22.2	[2.8-60.0]								66.7	11.1			22.2					
	Imported	32.0	[27.8-36.4]								67.8	0.2			32.0					
Apramycin	Danish	0	[0-33.6]								100									
	Imported	0	[0-0.8]								84.2	13.7	2.1							
Gentamicin	Danish	0	[0-33.6]						100											
	Imported	0.8	[0.2-2.1]						97.7	1.3	0.2		0.2	0.2	0.4					
Neomycin	Danish	11.1	[0.3-48.3]							88.9					11.1					
	Imported	7.4	[5.2-10.1]							91.8	0.8		0.6	2.5	4.2					
Spectinomycin	Danish	11.1	[0.3-48.3]										77.8	11.1		11.1				
	Imported	13.3	[10.3-16.7]										71.6	11.2	4.0	5.3	4.0	4.0		
Streptomycin	Danish	11.1	[0.3-48.3]								55.6	22.2	11.1			11.1				
	Imported	36.0	[31.7-40.5]								35.2	23.4	5.5	4.8	5.3	25.9				
Ciprofloxacin	Danish	22.2	[2.8-60.0]	77.8		22.2														
	Imported	21.3	[17.7-25.2]	77.3	1.5	7.0	7.0	1.9	0.8		0.6	4.0								
Nalidixic acid	Danish	11.1	[0.3-48.3]								88.9				11.1					
	Imported	20.2	[16.7-24.1]								77.1	1.5	1.3	0.6	2.1	17.5				
Colistin	Danish	0	[0-33.6]								100									
	Imported	0	[0-0.8]								100									

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

Escherichia coli from healthy human volunteers

In 2006, stool samples from 57 healthy human volunteers were collected and 48 *E. coli* isolates were subsequently isolated and susceptibility tested (Table 45). No significant changes in resistance were observed from 2005 to 2006. Nalidixic acid resistance was observed in 2 isolates (6%).

Comparison of resistance in *Escherichia coli* from animals, food and healthy human volunteers

Data on the occurrence of resistance in food animals, food and healthy human volunteers is presented in Table 46. The occurrence of resistance to tetracycline, sulfonamide and ampicillin over time in pigs, pork, and healthy humans from 1996 to 2006 is presented in Figures 25-27.

In 2006, ceftiofur resistance was for the first time detected in broiler meat of Danish and imported origin sold in Denmark. When comparing isolates from Danish and imported broiler meat, the occurrence of resistance was significantly higher in imported broiler meat than in Danish broiler meat for 15 out of 18 antimicrobial agents. From a public health perspective it is of concern that resistance to amoxicillin + clavulanic acid, ceftiofur, gentamicin, ciprofloxacin and nalidixic acid occur in *E. coli* isolates from broiler meat, and that the occurrence is significantly higher in imported broiler meat compared to Danish broiler meat. The consumption of imported poultry meat in Denmark increased from 17% in 2003 to 33% in 2006 (Data from Statistics Denmark, www.dst.dk) (Table 2). From 2004 to 2006, significant increases in the occurrence of resistance to ciprofloxacin was observed for both Danish and imported broiler meat P=0.003 and P<0.0001 respectively.

Based on preliminary data for 2006 from Statistics Denmark, close to 100% of all turkey meat consumed in Denmark was imported. Due to the small number of isolates from Danish turkey meat it is difficult to compare resistance in *E. coli* isolates from turkey meat of Danish and imported origin, however in 2006, one *E. coli* isolate from Danish turkey meat sold in Denmark was resistant to ceftiofur. Like for broiler meat it is the first time ceftiofur resistance is found in *E. coli* from turkey meat in Denmark.

The occurrence of resistance in *E. coli* from healthy human volunteers was similar to resistance levels in *E. coli* from Danish broiler and turkey meat, whereas resistance in isolates from healthy human volunteers was significantly lower than in isolates from imported broiler and turkey meat for several antimicrobial agents. Imported meat constitutes a stronger potential for introduction of resistant isolates to humans compared to Danish meat.

Table 45. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy humans (n=48), Denmark*

DANMAP 2006

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	14.6	[6.1-27.8]								85.4				14.6					
Chloramphenicol	2.1	[0.05-11.1]							2.1	45.8	50.0				2.1				
Florfenicol	0	[0-7.4]							4.2	64.6	31.3								
Ampicillin	18.8	[9.0-32.6]						10.4	47.9	22.9				18.8					
Amoxicillin/clavulanic acid a)	0	[0-7.4]							12.5	58.3	27.1	2.1							
Cephalothin	6.3	[1.3-17.2]								25.0	50.0	18.8	4.2	2.1					
Ceftiofur	0	[0-7.4]					100												
Cefpodoxime	0	[0-7.4]			6.3	66.7	22.9	4.2											
Sulfonamide	20.8	[10.5-35.0]												79.2					20.8
Trimethoprim	14.6	[6.1-27.8]								85.4				14.6					
Apramycin	0	[0-7.4]								77.1	20.8	2.1							
Gentamicin	2.1	[0.05-11.1]						95.8	2.1					2.1					
Neomycin	2.1	[0.05-11.1]							93.8	4.2			2.1						
Spectinomycin	2.1	[0.05-11.1]										87.5	4.2	6.3	2.1				
Streptomycin	18.8	[9.0-32.6]								35.4	41.7	4.2		4.2	14.6				
Ciprofloxacin	6.3	[1.3-17.2]	91.7	2.1	2.1						4.2								
Nalidixic acid	6.3	[1.3-17.2]									93.8			2.1	4.2				
Colistin	0	[0-7.4]								100									

Lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

Table 46. Occurrence of resistance (%) among Escherichia coli *from food animals, food of Danish and imported origin and healthy humans, Denmark*

DANMAP 2006

Compound	Broilers	Cattle	Pigs	Broiler meat		Turkey meat		Healthy humans
	Danish	Danish	Danish	Danish	Imported	Danish	Imported	
	%	%	%	%	%	%	%	%
Tetracycline	7	10	28	12	50	22	82	15
Chloramphenicol	0	0	1	<1	9	22	15	2
Florfenicol	0	0	<1	0	0	11	<1	0
Ampicillin	17	2	20	15	46	22	65	19
Amoxicillin/clavulanic acid	0	0	0	0	5	0	1	0
Cephalothin	3	2	3	2	11	11	11	6
Cefpodoxime	0	0	<1	<1	7	11	1	0
Ceftiofur	0	0	0	<1	5	11	0	0
Sulfonamide	9	12	26	16	43	22	45	21
Trimethoprim	2	3	14	6	33	22	32	14
Apramycin	0	0	<1	<1	<1	0	0	0
Gentamicin	0	0	<1	<1	2	0	<1	2
Neomycin	2	0	6	2	11	11	7	2
Spectinomycin	2	2	27	2	12	11	13	2
Streptomycin	11	11	41	12	33	11	36	19
Ciprofloxacin	7	0	<1	4	24	22	21	6
Nalidixic acid	7	0	<1	6	26	11	20	6
Colistin	0	0	0	0	0	0	0	0
Number of isolates	123	93	148	534	550	9	475	48

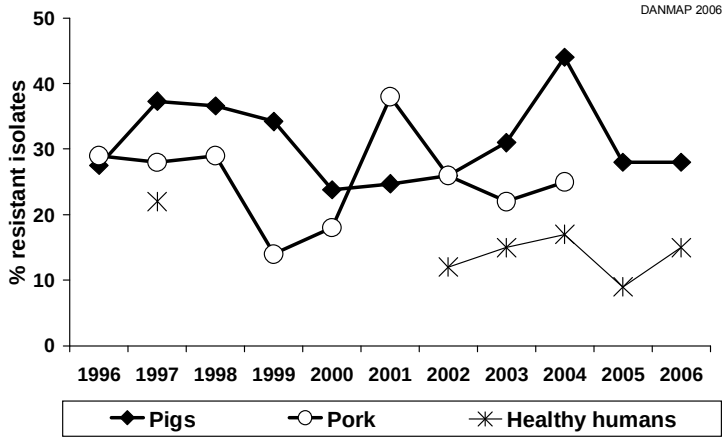

Figure 25. Trends in tetracycline resistance among Escherichia coli *from pigs, pork, and healthy humans in the community, Denmark*

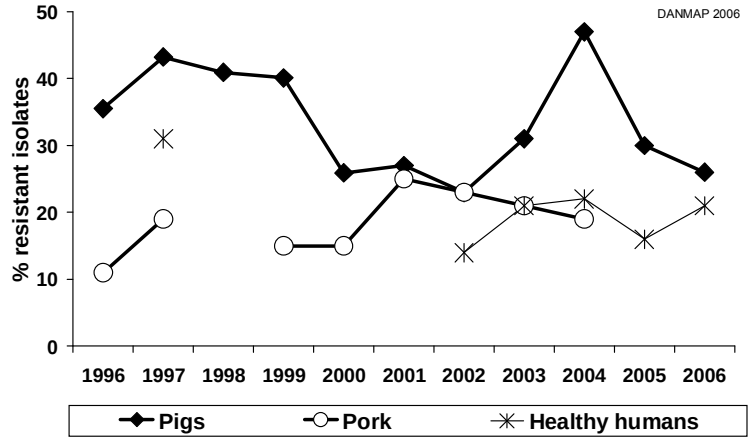

Figure 26. Trends in sulfonamide resistance among Escherichia coli *from pigs, pork, and healthy humans in the community, Denmark*

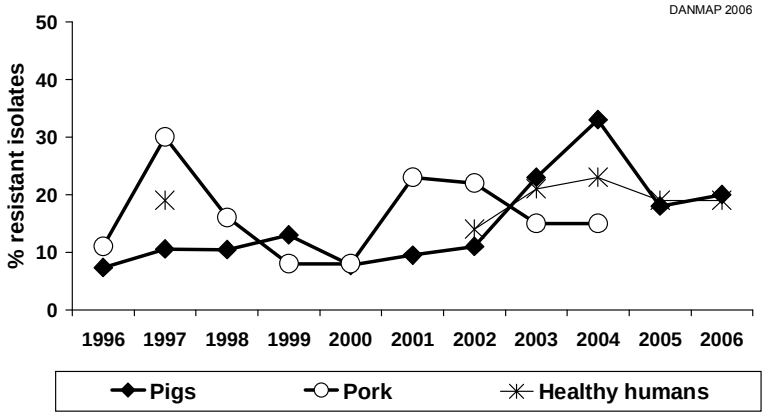

Figure 27. Trends in ampicillin resistance among Escherichia coli *from pigs, pork, and healthy humans in the community, Denmark*

Antimicrobial resistance in indicator bacteria isolated from Danish dogs

Consumption data on antimicrobial usage in companion animals has been reported in DANMAP for several years. A considerable fraction of antimicrobial agents used in animals, in particular broad-spectrum antimicrobials such as cephalosporins and fluoroquinolones, is prescribed for companion animals [Heuer *et al*. 2005. Emerg. Infect. Dis.11: 344-5]. Selection of resistant bacteria may pose a threat to humans, considering the shared environment of humans and companion animals. Indeed, various studies have documented transfer of resistant bacteria and resistance determinants between humans and dogs [Guardabassi *et al.* 2004. J. Antimicrob. Chemother. 54:321-332].

Data on antimicrobial resistance in indicator bacteria from companion animals has not previously been reported in the DANMAP report. The present study was launched in April 2006 to monitor the levels of antimicrobial resistance in indicator *E. coli* and enterococci from dogs. Staff in twelve small animal practices, located in the Danish region Zealand, collected a total of 118 faecal swabs from healthy dogs. These and additional 25 faecal samples from dogs in Copenhagen were streaked directly on MacConkey and Slanetz-Bartley agar plates and grown at 37°C for 24 and 48 hours, respectively. One colony was recovered from each plate when possible and isolates were identified as *E. coli* (n=130), *E. faecium* (n=14) or *E. faecalis* (n=56) using PCR and phenotypic tests. Antimicrobial susceptibility testing was performed by the disk diffusion method (Oxoid) according to the CLSI breakpoints.

Resistance levels of canine *E. coli* isolates were comparable to or lower than those of *E. coli* from humans and production animals (Table 1). Resistance to third generation cephalosporins and fluoroquinolones was not detected. However, *E. coli* from dogs treated with antimicrobial agents within 6 months prior to sampling were significantly more resistant to one or more antimicrobial agents than *E. coli* from untreated dogs (P=0.0036). Like for *E. coli*, overall resistance levels in *Enterococcus* isolates were generally comparable to those reported in production animals (data not shown). Interestingly, two dogs were found to carry amoxicillin-resistant *E. faecium*. Resistance to penicillins in enterococci is a serious problem due to the importance of these antibiotics in the treatment of enterococcal infections. The two amoxicillin-resistant isolates belonged to multi-locus sequence types (ST 192 and 266) previously associated with vancomycin-resistant *E. faecium* isolates from hospital patients in Europe and Asia [www.mlst.net].

Detection of amoxicillin-resistant *Enterococcus* clones previously isolated from hospital patients is of concern and requires further investigation on the frequency of these bacteria in the dog population. This finding confirms that dogs can act as reservoirs of clinically-relevant resistant bacteria. Accordingly, surveillance of antimicrobial resistance in these animals should primarily focus on resistance phenotypes of clinical relevance and employ selective isolation methods to enhance their detection.

In addition, in DANMAP 2006 data on antimicrobial resistance among *E. coli* isolates from diagnostic submissions from dogs are reported for the first time (see table 48, page 66 and [Pedersen *et al*. 2007. J. Antimicrob. Chemother. published online).

Table 1. Occurrence of resistance (%) among Escherichia coli *from dogs, food animals, and healthy humans, Denmark*

DANMAP 2006

Compound	Dogs %	Broilers Danish %	Cattle Danish %	Pigs Danish %	Healthy humans %
Tetracycline	6	7	10	28	15
Florfenicol	0	0	0	<1	0
Ampicillin	9	17	2	20	19
Amoxicillin/clavulanic acid	0	0	0	0	0
Cephalothin	2	3	2	3	6
Ceftiofur	0	0	0	0	0
Sulfonamide	8	9	12	26	21
Trimethoprim	5	2	3	14	14
Gentamicin	0	0	0	<1	2
Streptomycin	10	11	11	41	19
Ciprofloxacin	0	7	0	<1	6
Nalidixic acid	0	7	0	<1	6
Number of isolates	130	123	93	148	48

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Resistance in bacteria from diagnostic submissions

Bacteria from animals

The DANMAP programme monitors antimicrobial resistance in *Escherichia coli* and *Staphylococcus hyicus* from diagnostic submissions from animals. Most isolates from diagnostic submissions originate from animals in antimicrobial therapy, or animals with a history of previous 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 2006 data were available for *Escherichia coli* and *Staphylococcus hyicus* from pigs, and *E. coli* isolates from dogs.

Escherichia coli

The MIC distribution and the occurrence of resistance in *E. coli* O149 isolates from pigs are presented in Table 47. Figure 28 presents trends in resistance to selected antimicrobial agents in *E. coli* isolates from pigs (1996-2005) and from cattle (1996-2005). A significant decrease (*P*=0.023) in resistance to neomycin was observed in *E. coli* isolates from diagnostic submissions from pigs from 2005 to 2006. The first two ESBL-producing *E. coli* from production animals in Denmark were isolated in 2005 from diagnostic submissions from pigs. In 2006, this increased to 10 ESBL-producing pathogenic *E.coli* isolates from pigs and cattle. All ESBL isolates from pigs in 2006 were non-O149 and data from cattle are not shown.

In Table 48, the occurrence of resistance in *E. coli* isolates from diagnostic submissions from dogs from 2000 to 2005 is presented. This is the first reporting of resistance in bacteria from diagnostic submissions from dogs in the DANMAP report. Data was obtained from Pedersen *et al*. 2007 [J. Antimicrob. Chemother. published online]. The highest occurrence of resistance was observed for tetracycline, ampicillin, sulfonamide, trimethoprim and streptomycin, whereas relatively low levels of resistance to amoxicillin with clavulanic acid, gentamicin, fluoroquinolones, cephalosporins and several other antimicrobial agents were observed. Some variation in the occurrence of resistance was observed, depending on the site of isolation. Resistance tended to be higher among non-haemolytic isolates compared to haemolytic isolates.

Staphylococci

Staphylococcus hyicus isolates originated from skin infections in pigs. The MIC distributions and the occurrence of resistance among *S. hyicus* from pigs are presented in Table 49. Resistance to penicillin among *S. hyicus* isolates from pigs decreased significantly from 86% in 2003 to 71% in 2006. For all other antimicrobials in the test panel, the frequency of resistance remained unchanged from 2005 to 2006 (Figure 29). Methicillin resistance was not observed.

Table 47. Distribution of MICs and occurrence of resistance among Escherichia coli *from diagnostic submissions from pigs (n=118), Denmark*

DANMAP 2006

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	72.0	[63.0-79.9]							26.3	1.7		2.5	3.4	66.1					
Chloramphenicol	22.9	[15.7-31.5]							3.4	58.5	11.0	4.2	0.9	6.8	15.3				
Florfenicol	1.7	[0.2-6.0]							11.0	67.0	17.0	3.4	0.9		0.9				
Ampicillin	34.7	[26.2-44.1]						5.9	47.5	10.2		1.7	0.9	33.9					
Amoxicillin/clavulanic acid a)	0	[0-3.1]							44.9	28.0	22.9	4.2							
Cephalothin	3.4	[0.9-8.5]								18.6	62.7	15.3	0.9	2.5					
Cefpodoxime	4.2	[1.4-9.6]				69.5	22.0	4.2	2.5		1.7								
Ceftiofur	0	[0-3.1]					100												
Sulfonamide	64.4	[55.1-73.0]												35.6				0.9	63.6
Trimethoprim	32.2	[23.9-41.4]								67.8				32.2					
Apramycin	11.9	[6.6-19.1]								86.4	1.7			11.9					
Gentamicin	11.9	[6.6-19.1]						85.6	0.9	1.7	1.7	7.6	0.9	1.7					
Neomycin	22.9	[15.7-31.5]							74.6	1.7	0.9	0.9	5.1	17.0					
Spectinomycin	57.6	[48.2-66.7]										33.1	5.1	4.2	11.0	18.6	28.0		
Streptomycin	62.7	[53.3-71.4]								17.0	7.6	12.7	20.3	11.9	30.5				
Ciprofloxacin	12.7	[7.3-20.1]	84.8	2.5	1.7	10.2					0.9								
Nalidixic acid	12.7	[7.3-20.1]								83.9	3.4			0.9	11.9				
Colistin	0	[0-3.1]								100									

The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

a) Concentration of amoxicillin given, testet with clavulanic acid in concentration ratio 2:1

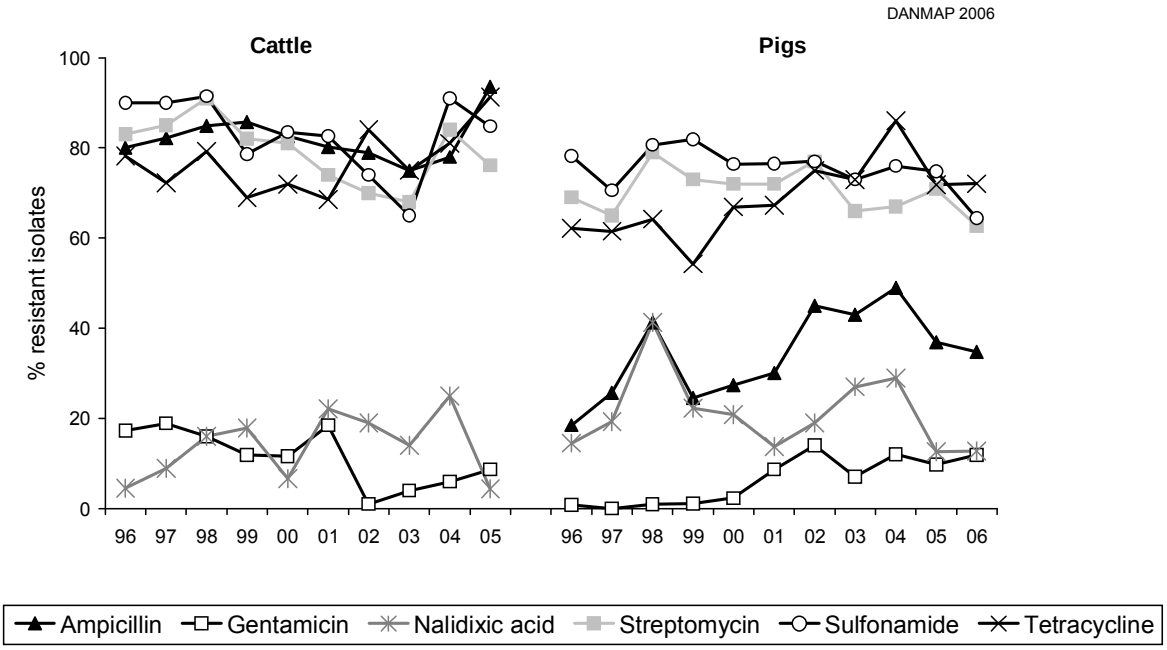

Figure 28. Trends in resistance to selected antimicrobials among Escherichia coli *from diagnostic submissions from animals, Denmark*

Table 48. Occurrence of resistance (%) among Escherichia coli *from diagnostic submissions from dogs (2000 - 2005), Denmark*

DANMAP 2006

Compound	Haemolytic isolates			Non-haemolytic isolates		
	Faeces %	Intstine %	Urogenital %	Faeces %	Intstine %	Urogenital %
Tetracycline	9	12	19	20	31	26
Chloramphenicol	0	0	8	6	11	4
Florfenicol	0	0	4	1	0	0
Ampicillin	17	39	15	26	40	22
Amoxicillin/clavulanic acid	0	0	4	4	11	4
Cephalothin	7	0	5	4	11	6
Ceftiofur	1	0	4	1	2	4
Sulfonamide	19	23	19	28	38	19
Trimethoprim	7	19	12	25	16	19
Gentamicin	1	0	0	4	2	4
Neomycin	6	0	4	6	7	4
Spectinomycin	3	4	12	9	16	4
Streptomycin	22	27	19	28	33	19
Ciprofloxacin	0	0	0	5	0	7
Nalidixic acid	7	0	8	16	11	19
Colistin	4	0	4	5	2	0
Number of isolates	69	26	26	246	55	27

Table 49. Distribution of MICs and occurrence of resistance among Staphylococcus hyicus *from pigs (n=42), Denmark*

DANMAP 2006

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs														
			0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline	42.9	[27.7-59.0]				57.1					14.3	19.0	9.5				
Chloramphenicol	0	[0-8.4]							90.5	9.5							
Florfenicol	0	[0-8.4]					2.4	95.2	2.4								
Penicillin	71.4	[55.4-84.3]	28.6			4.8	7.1	11.9	11.9	16.7	11.9	7.1					
Ceftiofur	0	[0-8.4]				35.7	64.3										
Sulfonamide	0	[0-8.4]								4.8	45.2	42.9	4.8		2.4		
Trimethoprim	21.4	[10.3-36.8]					31.0	40.5	7.1				21.4				
Erythromycin	21.4	[10.3-36.8]			73.8	4.8						21.4					
Spectinomycin	11.9	[4.0-25.6]									2.4	45.2	40.5			11.9	
Streptomycin	40.5	[25.6-56.7]						11.9	47.6					2.4	38.1		
Ciprofloxacin	0	[0-8.4]		71.4	23.8	4.8											
Tiamulin	21.4	[10.3-36.8]			16.7	52.4	4.8				4.8		21.4				

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

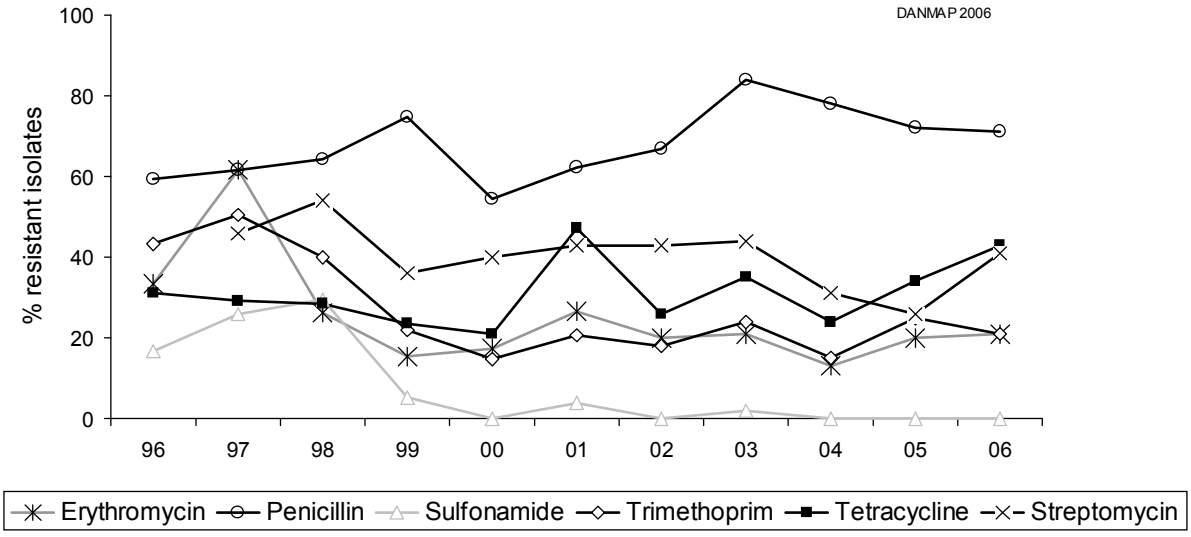

Figure 29. Trends in resistance to some selected antimicrobials among Staphylococcus hyicus *from diagnostic submissions from pigs, Denmark*

Occurrence of Extended-Spectrum Beta-lactamase (ESBL)-producing *E. coli* from food animals in Denmark

Extended-spectrum beta-lactamase (ESBL)-mediated resistance is a cause of increasing concern worldwide because of the widespread dissemination of bacterial pathogens harboring ESBLs in human clinical settings, which can lead to antimicrobial treatment failure. ESBLs have been widely reported in *E. coli* isolated from clinical infections in humans, but to a lesser extend from bacteria isolated from food animals. The first ESBL-producing *E. coli* from the Danish primary production were isolated in 2005 when two *E. coli* isolates carrying the $bla_{CTX\text{-}M\text{-}1}$ gene were isolated from diseased Danish pigs as part of the routine diagnostic performed in Denmark [Aarestrup *et al*. 2006. J.Antimicrob.Chemother. 57:1258-1259]. In 2006, this increased to 10 ESBL-producing pathogenic *E. coli* isolated from diseased Danish pigs and cattle as well as the first ESBL-producing *Salmonella* Typhimurium isolated from a healthy pig in Denmark. All these isolates carried versions of the $bla_{CTX\text{-}M}$ gene, either $bla_{CTX\text{-}M\text{-}1}$ $bla_{CTX\text{-}M\text{-}2}$ or $bla_{CTX\text{-}M\text{-}9}$ gene. All isolates were from farms, which had used cephalosporins previously.

Also in 2006, a survey on the occurrence of cefotaxime resistant *E. coli* in the normal flora was performed in 10 Danish pig farms using ceftiofur and 10 control farms not using ceftiofur. A total of 72 isolates with reduced sensitivity to cefotaxime were detected at five farms using ceftiofur and one control farm. Nineteen *E. coli* isolates carrying $bla_{CTX\text{-}M\text{-}1}$ were found from two pig farms with a history of ceftiofur usage, and the remaining 53 isolates were confirmed to be *ampC* promoter mutants. The study showed a significant association between ceftiofur treatment and occurrence of *E. coli* with reduced sensitivity to cefotaxime. Due to the limited number of farms statistically significant association could not be concluded between ceftiofur use and the occurrence of ESBL-producing *E. coli*. [Jørgensen *et al*.2007. J.Antimicrob.Chemother. accepted].

Another survey was conducted at Danish slaughterhouses, where a total of 137 fecal samples were collected at slaughterhouses from healthy pigs and examined for the presence of cefotaxime resistant *E. coli*. Four *E. coli* isolates with a reduced susceptibility to cefotaxime were obtained from pigs originating from four different farms. One of the isolates carried both $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}1b}$. The remaining three isolates were verified as promoter mutants of *ampC* at position of -42(C $\rightarrow$T) and -18(G $\rightarrow$A). According to the data on cephalosporin consumption obtained from VetStat, the ESBL-producing *E. coli* was not from a pig that originated from a farm using cephalosporins.

The Danish studies conducted at farm-level suggest that there is an association between cephalosporin usage and the presence of ESBL-producing *E. coli*. The number of isolates obtained from farms and from healthy animals at slaughter (where cross-contamination between animals might have taken place), is still too low to make firm conclusions. However, the results highlight the indisputable emergence of ESBLs in sick and healthy animals and underline the potential possibilities of ESBL-mediated resistance to spread through the food chain in Denmark.

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Extended-spectrum Beta-lactamase (ESBL) producing *Enterobacteriaceae* isolated from human clinical samples in Denmark

Since the beginning of the 1980s, *Enterobacteriaceae* resistant to 3rd-generation cephalosporins due to the production of extended-spectrum beta-lactamases (ESBLs) have emerged worldwide and many different ESBL genes have been identified. Until the mid-1990s, the predominant ESBLs were derivatives of the TEM and SHV enzymes; however, CTX-M enzymes are now the most widespread ESBLs. Spread of CTX-M producing *E. coli* isolates occurs not only in hospitals, but also in the community, and has reached epidemic proportion in many countries. There is only limited data about ESBL producing *Enterobacteriaceae* in Denmark.

The first two ESBL-producing isolates from humans in Denmark were reported in 1994. Both isolates were *Klebsiella pneumoniae* producing SHV-2 and SHV-5 ESBLs, respectively. One of the isolates was recovered from a patient transferred from Turkey after having been hospitalized in an intensive care unit following a car accident [Hansen DS *et al.* 1998. Ugeskr Laeger 160: 2261-2]. Indeed, transfer from a foreign hospital was then a common feature of patients with ESBL producing *Enterobacteriaceae*.

In a retrospective study, we found that 7% of 200 gentamicin resistant *E. coli* and *Klebsiella* spp., primarily from urine samples, isolated at the Department of Clinical Microbiology, H:S Hvidovre Hospital during the period 1998 to 2003, produced an ESBL. Of the 14 ESBL positive isolates, 8 produced an SHV enzyme and 6 produced a CTX-M enzyme [Kjerulf A *et al.*, submitted]. In March 2003, a prospective study at the same hospital showed that only 3 (0.8%) of 380 consecutive *E. coli* and *Klebsiella* spp. isolates from urine (n = 360), sputum (n = 11) and aspirates from primarily sterile sites (n = 9) were ESBL positive; two isolates producing CTX-M group 9 and the last isolate producing SHV-2 [Kjerulf A *et al.*, submitted].

During the period January to June 2006, we conducted a multicenter prospective study on suspected ESBL producing *Enterobacteriaceae*. Preliminary results from the first 99 isolates reported to us show that 79% produced an ESBL, 4% produced AmpC, 7% produced both types of enzymes, and 5% were *K. oxytoca* with presumed hyper-production of the K1 chromosomal beta-lactamase. Of the ESBL producing isolates, 95% produced a CTX-M enzyme, predominantly of CTX-M group 1.

Prevalence of ESBL producing *Enterobacteriaceae* seems to remain low in Denmark. However, our perception is that they are much more prevalent than for just few years ago and that the present epidemic of CTX-M producing *Enterobacteriaceae* has now reached Denmark. Many of these isolates are co-resistant to other antimicrobials such as ciprofloxacin and the recent increase in fluoroquinolone – mainly ciprofloxacin – consumption in Denmark may select for ESBL producing isolates. Further studies are needed to estimate the incidence of ESBL producing *Enterobacteriaceae* and the risk factors for infection and colonisation with these bacteria in Denmark.

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Bacteria from humans

Data on resistance levels in *Streptococcus pneumoniae* isolates, (see page 74) cover all 16 counties in Denmark. Data on resistance levels in *Staphylococcus aureus* isolates cover 15 counties. For *E. coli* and coagulase-negative staphylococci, this report includes data from clinical microbiology laboratories of 14 counties, namely Copenhagen and Frederiksberg municipalities (which also have the status of counties) and the counties of Copenhagen, Frederiksborg, Roskilde, West Zealand, Storstroem, Funen, Ribe, Vejle, Ringkøbing, Aarhus, Viborg and North Jutland, representing 95% of the Danish population. Demographic data is presented in Table 3**,** page 16.

Escherichia coli

Results from blood and urine isolates of *E. coli* in hospitals were obtained from 14 counties. Additionally, 13 counties contributed data on urine isolates in primary health care. The results for the period 2000-2006 are presented for Denmark in Figures 30a and b, Figures 31a and b, Figures 32a and b and for each county for the period 1995-2006 in Figures A1, A2 and A3 in appendix 3, showing resistance to selected antimicrobial agents in *E. coli* blood and urine isolates.

Escherichia coli blood isolates obtained from hospital patients

Data on resistance in *E. coli* blood isolates from hospitals in Denmark are presented in Figures 30a and b (Figure A1 in appendix 3 show each individual county). In *E. coli* blood isolates, the generally high level of ampicillin resistance remained between 30 and 50% with an average of 41.3% (95% CI: 39.7-43.0). It was thus unchanged compared to 2005, although Rigshospitalet, the national referral hospital, reported a resistance level of 51.4%. Gentamicin resistance in *E. coli* blood isolates generally increased between 2004 and 2006. Overall, it significantly increased from 1.6% in 2004 to 2.5% in 2006 (P=0.004). Cefuroxime resistance in *E. coli* blood isolates remained at a low level with an average of 3.5% (95% CI: 2.9-4.2) and was unchanged compared to 2005. As in 2005, mecillinam resistance in *E. coli* blood isolates was reported from 10 counties in 2006, representing 70% of the Danish population. Overall, it was at an average 3.5% (95% CI: 2.9-4.3) decreasing from 4.2% in 2005. In one county (Roskilde county), there was a significant decrease in mecillinam resistance in *E. coli* blood isolates from 7.4% in 2005 to 1.8% in 2006 (P=0.03). In an other county (North Jutland county), there was a significant increase in mecillinam resistance in *E. coli* blood isolates from 4.1% in 2005 to 7.6% in 2006 (P=0.04). Tetracycline resistance in *E. coli* blood isolates was reported from three counties as well as Rigshospitalet, representing around 25% of the Danish population. Overall, it was at an average 23.1% (95% CI: 20.3-26.0) and unchanged compared to 2005. In Funen county, there was a significant decrease in tetracycline resistance in *E. coli* blood isolates from 25.2% in 2005 to 18.0% in 2006 (P=0.03).

Escherichia coli urine isolates obtained from hospital patients

Data on resistance in *E. coli* urine isolates from hospitals in Denmark are presented in Figures 31a and b (Figure A3 in appendix 3 show each individual county). Overall, ampicillin resistance in *E. coli* urine isolates from hospitals was at an average 39.0% in 2006 and unchanged compared to 2005. At Rigshospitalet, a significant increase in ampicillin resistance in *E. coli* urine isolates from hospitals was observed, from 41.0% in 2005 to 46.1% in 2006 (P=0.005). No significant variations in ampicillin resistance in *E. coli* urine isolates from hospitals were observed in the counties. Sulfonamide resistance increased significantly from 32.7% in 2005 to 33.8% in 2006 (P=0.0009). A significant increase in sulfonamide resistance was observed in Ringkøbing county, from 34.8% in 2005 to 36.3% in 2006 (P<0.0001), and at Rigshospitalet, from 34.0% in 2005 to 50.1% in 2006 (P<0.0001). No significant variations in sulfonamide resistance in hospital *E. coli* urine isolates were observed in other counties. Data on ciprofloxacin resistance in *E. coli* urine isolates from hospitals in 2006 were available from eight counties as well as from Rigshospitalet (see appendix 3, Figure A3), representing 55% of the Danish population. A significant increase in resistance to ciprofloxacin was observed: from 5.1% in 2005 to 6.3% in 2006 (P<0.0001). This increase was also highly significant (P<0.0001) when considering only the seven counties, as well as Rigshospitalet, that reported ciprofloxacin resistance data in both 2005 and 2006. Among the counties that tested for ciprofloxacin resistance, four counties showed a significant increase in ciprofloxacin resistance in hospitals, as compared to 2005 (Copenhagen county, P=0.03; West Zealand county, P<0.0001; Vejle county, P=0.01; North Jutland county, P=0.0003). Data on nalidixic acid resistance in *E. coli* urine isolates from hospitals in 2006 were available from seven counties, representing 48% of the Danish population. Overall, it was at an average of 8.6% and increased significantly from 6.6% in 2005 to 8.6% in 2006 (P<0.0001). This increase was also highly significant (P<0.0001) when considering only the four counties, that reported nalidixic acid resistance data in both 2005 and 2006. The increase in

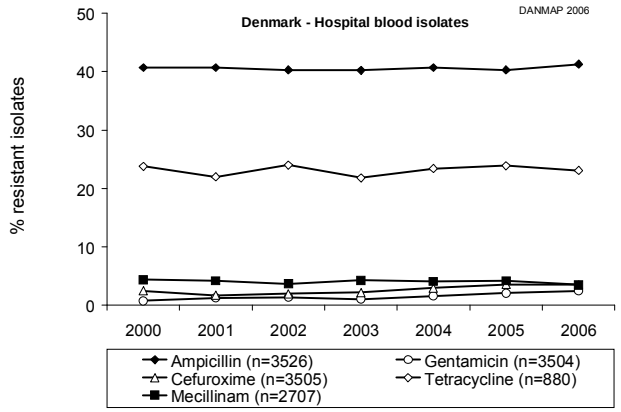

Figure 30a. Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam and tetracycline in Escherichia coli *blood isolates from humans, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

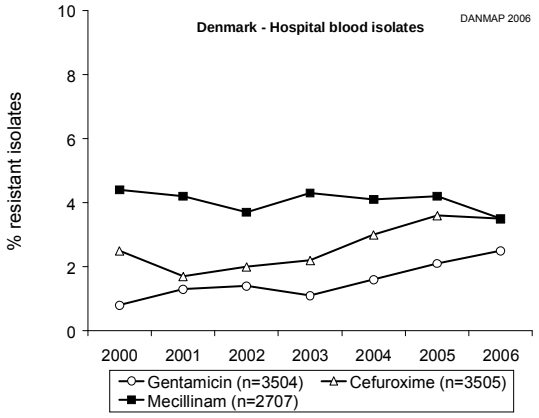

Figure 30b. Resistance (%) to gentamicin, cefuroxime and mecillinam in Escherichia coli *blood isolates from humans, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

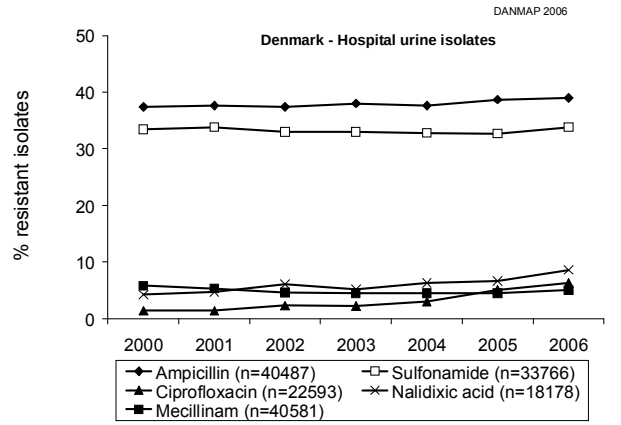

Figure 31a. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in hospitals, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

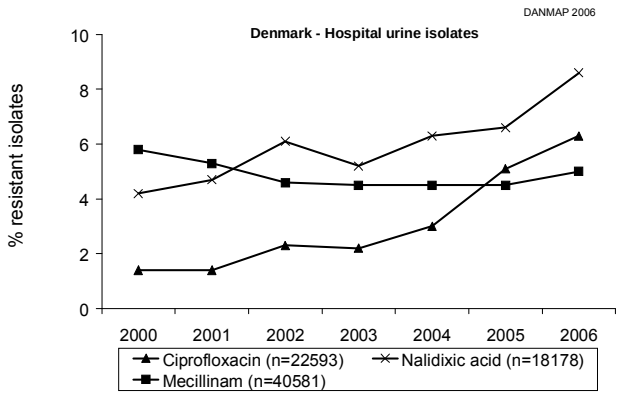

Figure 31b. Resistance (%) to ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in hospitals, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

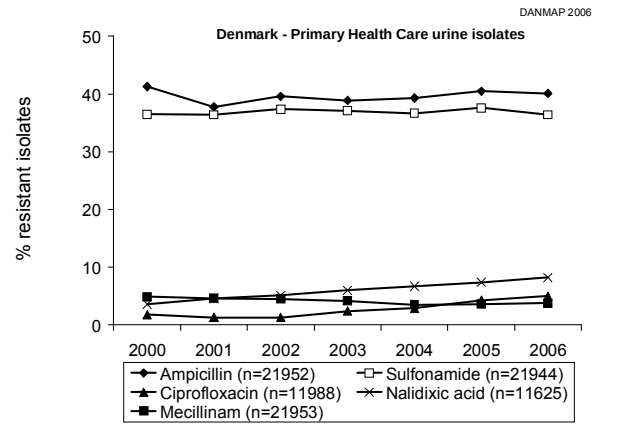

Figure 32a. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

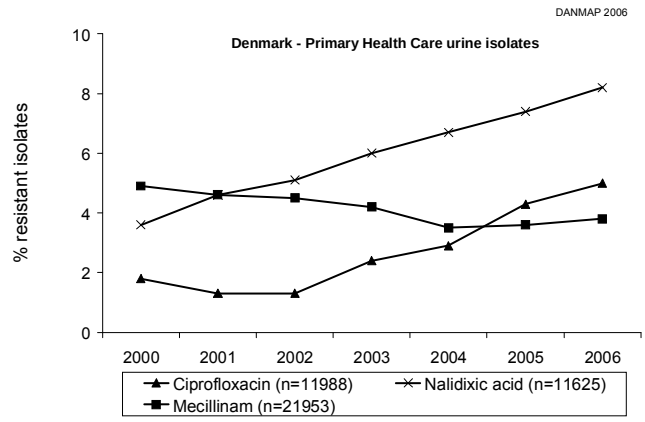

Figure 32b. Resistance (%) to ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans, Denmark.*
The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006.

ciprofloxacin resistance was concomitant to the steady increase in the consumption of fluoroquinolones reported from both hospitals and primary health care in recent years (see Tables 10 and 12). All 14 participating counties reported data on mecillinam resistance in *E. coli* urine isolates from hospitals. Overall, a significant increase was observed, from 4.5% in 2005 to 5.0% in 2006 (P=0.001). In Copenhagen county, a significant increase in mecillinam resistance in *E. coli* urine isolates from hospitals was observed, from 4.5% in 2005 to 5.7% in 2006 (P=0.04).

Escherichia coli obtained from urine isolates from primary health care

Data on resistance in *E. coli* urine isolates from primary health care in Denmark are presented in Figures 32a and b (Figure A2 in appendix 3 show each individual county). In *E. coli* urine isolates from primary health care, the generally high level of ampicillin resistance remained between 30 and 50% with an average of 40.1% (95% CI: 39.5-40.8) and was unchanged compared to 2005. Sulfonamide resistance in *E. coli* urine isolates from primary health care decreased significantly from 37.6% in 2005 to 36.4% in 2006 (P=0.01). Sulfonamide resistance in *E. coli* urine isolates from primary health care decreased significantly in North Jutland county, from 41.3% in 2005 to 38.1% in 2006 (P=0.01), and in Copenhagen and Frederiksberg municipalities, from 35.3% in 2005 to 32.9% in 2006 (P=0.01). The high level of resistance to ampicillin and sulfonamides in *E. coli* from urine makes 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. A study performed in 1997-1999 showed that ampicillin and sulfonamide resistance in *E. coli* isolates from uncomplicated urinary tract infections in primary health care in Denmark was at only 20% and 22%, respectively, compared to 34% and 39%, respectively, in complicated urinary tract infections [Kerrn *et al.* 2002. J. Antimicrob. Chemother. 50: 513-6]. Data on ciprofloxacin resistance in *E. coli* urine isolates from primary health care in 2006 were available from eight counties (see appendix Figure A2), representing 57% of the Danish population. A significant increase in resistance to ciprofloxacin was observed: from 4.3% in 2005 to 5.0% in 2006 (P=0.004). Between 2005 and 2006, consumption of fluoroquinolones in primary health care increased from 0.32 to 0.37 DDD per 1,000 inhabitant-days. For the eight counties reporting resistance data reasons for the increase in fluoroquinolone consumption are discussed in the section on antimicrobial consumption in humans (see page 23). West Zealand county showed a significant increase in ciprofloxacin resistance in *E. coli* urine isolates from primary health care from 5.2% in 2005 to 10.6% in 2006 (P<0.0001). Data on nalidixic acid resistance in *E. coli* urine isolates from primary health care in 2006 were available from six counties, representing 43% of the Danish population. Overall, it was at an average of 8.2% (95% CI: 7.7-8.7) and unchanged compared to 2005. However, when considering only the three counties that reported nalidixic acid resistance data each year since 2004, a significant increase in resistance to nalidixic acid was observed: from 6.7% in 2004 to 8.3% in 2006 (P=0.0009). All 14 participating counties reported data on mecillinam resistance in *E. coli* urine isolates from primary health care. Overall, it was at an average of 3.8% (95% CI: 3.6-4.1) and unchanged compared to 2005. Vejle county showed a significant decrease in mecillinam resistance in *E. coli* urine isolates from primary health care from 6.8% in 2005 to 4.2% in 2006 (P=0.02).

First detection of transmissible quinolone resistance genes in *Escherichia coli* isolates from humans in Denmark

In 2006, a study was performed to characterize and compare quinolone resistance mechanisms present in *E. coli* isolates from humans and pigs in Denmark, including chromosomal mutations, efflux pump mechanisms and also screening for transmissible mechanisms.

Until recently, chromosomal mutations in genes involved in DNA-transcription and replication were considered the main mechanisms of quinolone resistance in *Enterobacteriaceae*. A new transferable mechanism (*qnrA* gene) was described in 1998 in a *Klebsiella pneumoniae* isolate from a patient in 1994 in Alabama, USA [Martinez-Martinez *et al*., 1998. Lancet. 351:797-9]. Since then two other *qnr*-genes (*qnrS* and *qnrB*) have been identified [Hata *et al*. 2005. Antimicrob Agents Chemother. 49: 801-803; Jacoby *et al*. 2006. Antimicrob Agents Chemother. 50:1178-82]. These encode Qnr proteins able to protect topoisomerases and reduce their susceptibility to fluoroquinolones and increase the likelihood of selection of mutations. Plasmid mediated quinolone resistance seems to have spread rapidly and is now found in the USA, Africa, Asia and also in Europe and is often located on transferable plasmids together with other resistance genes especially genes encoding resistance to beta-lactams [Robicsek *et al*. 2006. Lancet Infect Dis. 6: 629-40].

In 2006 another mechanism of transferable quinolone resistance was reported. The *cr* variant of *aac(6')Ib* encodes an aminoglycoside acetyltransferase that confers resistance to ciprofloxacin by N-acetylation of its piperazinyl amine. This variant was first described in *E. coli* isolates from Shanghai [Robicsek *et al*. 2006. Nat Med. 12:83-8], but has since been found with a high prevalence in the USA [Park *et al*. 2006. Antimicrob Agents Chemother. 50: 3953-5]. It has, furthermore, been described in Portugal, Nigeria and in the United Kingdom and as for *qnr* it is frequently located on multiple resistance plasmids which commonly also encode cephalosporin resistance [Karisik *et al*. 2006. J Antimicrob Chemother. 58:665-8; Machado *et al*. 2006. Antimicrob Agents Chemother.50:3220-1; Robicsek *et al*. 2006. The Lancet Infect Dis.6:629-40; Soge *et al*. 2006. J Antimicrob Chemother. 58: 1048-53].
In our study, in an *E. coli* strain collection including 83 nalidixic acid resistant and 5 susceptible isolates from humans and 39 nalidixic acid resistant and 3 susceptible isolated from pigs, screening by PCR for *qnr* genes showed two isolates that harboured, *qnrS* and *qnrA* genes, respectively. These isolates showed reduced susceptibility to ciprofloxacin (MICs= 0.5 µg/ml), but were susceptible to nalidixic acid (MICs= 8 and 4 µg/ml) and were both isolated at Hvidovre Hospital. The first was isolated from a urine sample of an 88 year-old woman with pneumonia and dysuria and the second from a blood culture of a 72 year-old man with colon cancer and clinical sepsis. There were no indications of acquisition of the strains outside Denmark. [Cavaco *et al*. 2007. J Antimicrob Chemother. Published online].

Furthermore, screening by PCR showed that, among the 88 human isolates, 14 harboured the *aac (6')Ib* gene (15.9%). Sequencing revealed the the *aac (6')Ib–cr* variant in 12 isolates (14.1%). All *aac(6')Ib-cr* positive isolates were resistant to nalidixic acid and ciprofloxacin (MIC≥ 256 µg/ml and MIC≥ 4 µg/ml, respectively) and harboured chromosomal mutations in *gyrA,* and *parC* (data not shown).

Presence of transferable quinolone resistance genes does not necessarily confer full resistance to nalidixic acid and might only confer low-level resistance to fluoroquinolones. Thus, these genes might not be detected by normal screening procedures, especially when using diffusion testing. It is recommended that MIC testing and low-level break points for fluoroquinolones (MIC≤ 0.125 µg/ml for ciprofloxacin) are used for the detection of resistance.

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Streptococcus pneumoniae

The national reference centre at Statens Serum Institut performs typing and susceptibility testing on *S. pneumoniae* isolates referred by the clinical microbiology laboratories in Denmark. In 2006, susceptibility testing was performed on 891 non-duplicate isolates from blood or cerebrospinal fluid samples. The percentage of *S. pneumoniae* isolates that were not susceptible (resistant plus intermediate) to penicillin was 3.2% in 2004, 4.2% in 2005 and 3.4% in 2006 (Figure 33). This level of resistance is much lower than reported in many other European countries [http://www.rivm.nl/earss/database/].

Macrolide resistance in *S. pneumoniae* isolates from blood and cerebrospinal fluid has been around 5% since 2000. The percentage of macrolide resistant *S. pneumoniae* was 4.6% in 2004, 5.5% in 2005 and 5.5% in 2006 **(**Figure 33)**.**

Streptococcus pyogenes (group A Streptococcus, GAS)

In 2006, data were reported on 6,341 non-invasive Group A streptococci (GAS) isolates from clinical samples in 12 counties. Resistance to macrolides (erythromycin) in GAS isolates was 1.6% in 2006 and unchanged as compared to 2005. County-to-county variations ranged from 0.0% to 4.0%. In 2006, data on 131 GAS isolates from normally sterile body sites were reported to the national reference centre at Statens Serum Institut. Resistance to macrolides in isolates from normally sterile body sites was 0.8% in 2006 and unchanged compared to 2005. As in previous years, no penicillin-resistant GAS isolate was detected in 2006.

Group B, C and G Streptococci

In addition to GAS isolates, 245 other haemolytic streptococcal isolates from normally sterile body sites were reported to the national reference centre at Statens Serum Institut, in 2006. In 2006, 88 *Streptococcus agalactiae* (group B streptococci, GBS) isolates from normally sterile body sites were reported, of which 4.6% were macrolide (erythromycin) resistant. There were 27 group C streptococci from normally sterile body sites, of which 3.7% were resistant to macrolides. The remaining were 130 group G streptococci isolates from normally sterile body sites, of which 2.3% were resistant to macrolides. No resistance to penicillin in group B, C or G streptococci isolates from normally sterile body sites was detected in 2006.

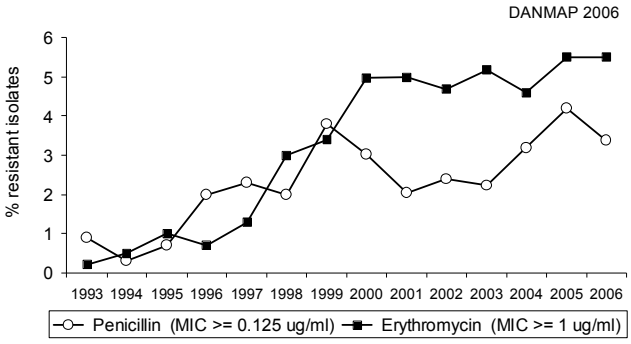

Figure 33. Resistance (%) to penicillin and macrolides in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

Coagulase-negative staphylococci

In 2006, data were reported on 3,941 coagulase-negative staphylococci blood isolates from hospitals. The average level of penicillin resistance was at an average 80% among counties (min. 56% - max. 92%). Since 1996, penicillin resistance in coagulase-negative staphylococci blood isolates only showed small variations. Overall, resistance to erythromycin increased from 36.5 in 2005 to 40.5% in 2006 (*P*=0.002), although it largely varied among counties (min. 13% - max. 66%). In the 13 counties reporting methicillin resistance data in 2005 and 2006, resistance was at an average of 52% although it largely varied among counties (min. 23% - max. 85%). As stated in previous reports, it is however possible that the large variability in resistance is a consequence of the procedure for selection of isolates that are submitted for susceptibility testing. Caution is therefore warranted when making comparisons of resistance levels between counties.

Staphylococcus aureus

Surveillance of bacteraemia: In 2006, a total of 1,351 *S. aureus* bacteraemia cases were reported from the 15 participating Danish counties/municipalities, covering 95% of the Danish population. This corresponded to an incidence of 23.5 per 100,000 inhabitants, which is a slight decrease compared to 2004 and 2005. A total of 19 (1.4%) of the bacteraemia cases were caused by methicillin resistant *S. aureus* (MRSA), which is similar to 2005 (23 cases (1.5%)). Table 50 presents occurrence of antimicrobial resistance among *S. aureus* bacteraemia and MRSA in 2006. A more detailed description of the *S. aureus* bacteraemia cases is published in the Annual *S. aureus* bacteraemia report [available at http://www.ssi.dk/sw3425.asp].

Surveillance of Methicillin Resistant *S. aureus*:
In 2006 a total of 706 new MRSA-positive cases were reported (13.0 per 100,000 inhabitants), this is a decrease from the 851 new cases detected in 2005 (Figure 34). The decrease was primarily due to a reduction of cases from Vejle County, where a prolonged outbreak has been ongoing at the hospitals since 2002. In Vejle county the reduction from 311 cases in 2005 to 145 cases in 2006 was achieved by a massive search and destroy effort including admission screening of all patients (Figure 35).
In the rest of the country, 571 new cases were observed in 2006 compared to 540 in 2005, indicating stagnation in the rapid yearly increase observed since 2002. Large regional variations in the incidence of MRSA cases were observed. The highest incidence was reported in the Greater Copenhagen area (Copenhagen and Frederiksberg Municipalities and Copenhagen County), with an incidence of 26 cases per 100,000 inhabitants. For the rest of Denmark the incidence was between 2-10 cases per 100.000 inhabitants.
The place of detection, possible risk association, and the type of infection or carriage was obtained from

Table 50. Comparison of resistance (%) among Staphylococcus aureus *bacteraemia and methicillin resistant Staphylococcus aureus (MRSA) isolates, Denmark*

DANMAP 2006

Compound	*S. aureus* Bacteraemia isolates a) %	MRSA All body sites %
Penicillin	79	100
Cefoxitin	1	1
Erythromycin	5	43
Clindamycin	4	35
Tetracycline	3	22
Fluoroquinolones	5	44
Rifampin	1	6
Fusidic acid	11	27
Kanamycin	2	35
Streptomycin	1	18
Vancomycin	0	0
Numbers of isolates	1351	707

a) Includes 19 bacteraemia MRSA

DANMAP 2006

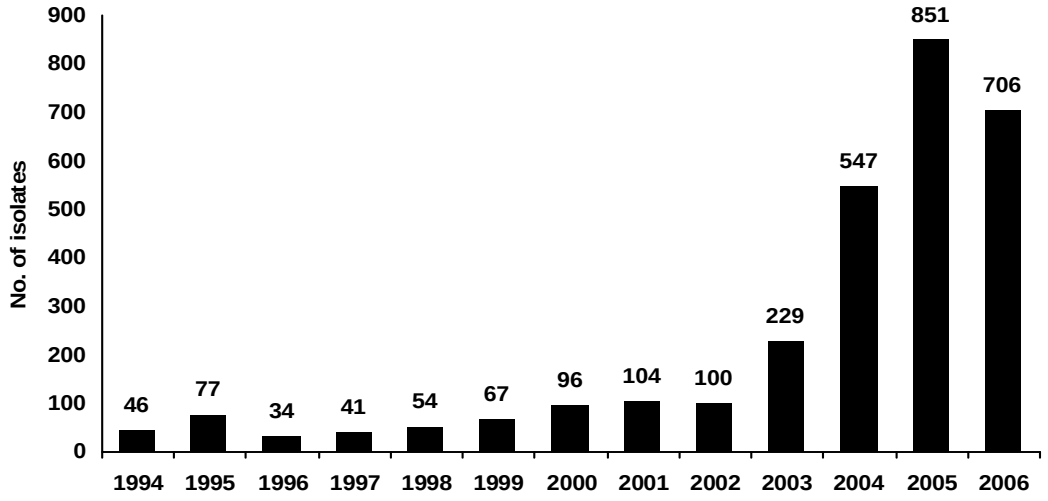

Figure 34. Number of reported cases of methicillin resistant Staphylococcus aureus *(MRSA),1994-2006, Denmark*

Repetitative MRSA isolates from the same patient/person were excluded.

discharge summaries and notes from general practitioners. In 2006, 453 (64%) of the cases were obtained in patients with infections and 253 (36%) cases were detected by screening (Figure 36). For infectious cases the majority (87%) was acquired in Denmark and most of these was detected in primary health care (community onset, CO, n = 323). Among these 323 cases only 136 (42%) reported contact to hospitals or nursing homes within the last 12 months, which traditionally has been the main risk factor for acquisition for MRSA. Additionally 14% had a known risk exposition for MRSA outside hospitals (CO with community risk, CO-CR) e.g. a spouse or sibling, but in 143 (44%) cases no risk exposition for MRSA was noted. This indicates that MRSA now is occurring and spreading in the community without any relation to health care. This is a major problem as this inevitably will lead to increased introduction into hospitals with further possibility of spread. As a consequence eradication treatment of CO MRSA is an important feature of the newly implemented National MRSA guideline (se below)

Skin and soft tissue infections were by far the most frequent type of infection 326 (72%), followed by respiratory tract infections 30 (7%), and post operative infections 28 (6%).

Susceptibility testing showed that resistance to beta-lactams only was seen in 17% of the isolates, which is an increase from 13% in 2005. This is in concordance with the high number of CO cases. However, the majority (57%) of the MRSA isolates were multi-resistant, i.e. resistant to three or more classes of antimicrobial agents. A significant increased level of resistance compared to 2005 was observed for the following antimicrobial agents: tetracycline, (18% to 22%), fusidic acid (17% to 27%), kanamycin (25% to 35%), streptomycin (14% to 18%) and rifampicin (4% to 6%). All MRSA isolates from 2006 were susceptible to glycopeptides. Only 3 isolates (0.4%) was resistant to mupirocin, the drug used for eradication therapy of carriage.

DANMAP 2006

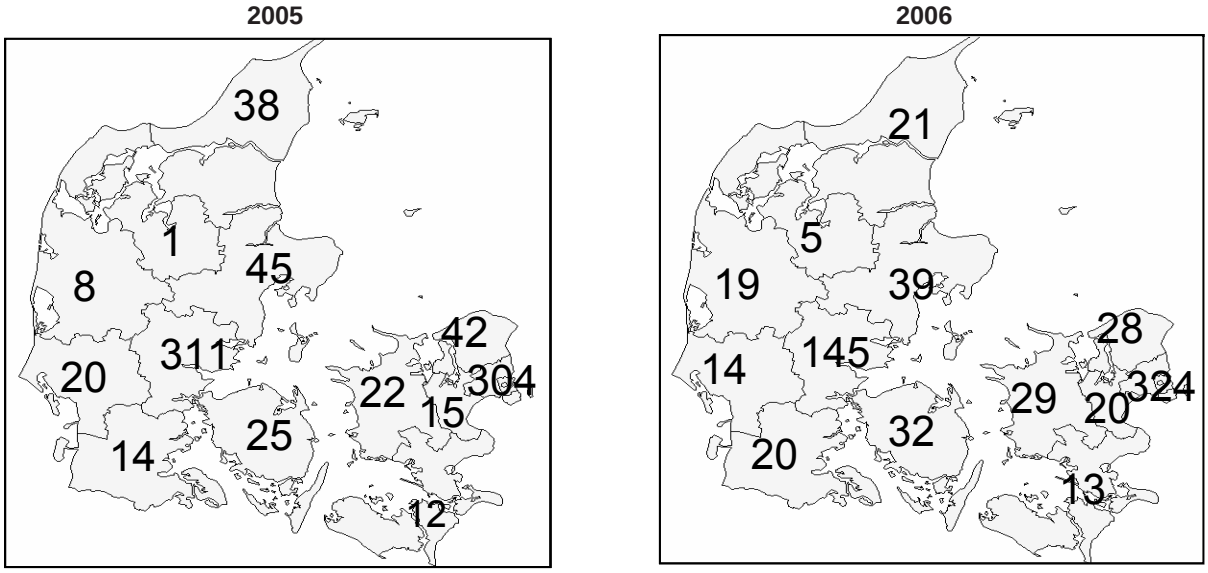

Figure 35. Number of reported cases of methicillin resistant Staphylococcus aureus (MRSA) in 2005 and 2006 per county, Denmark

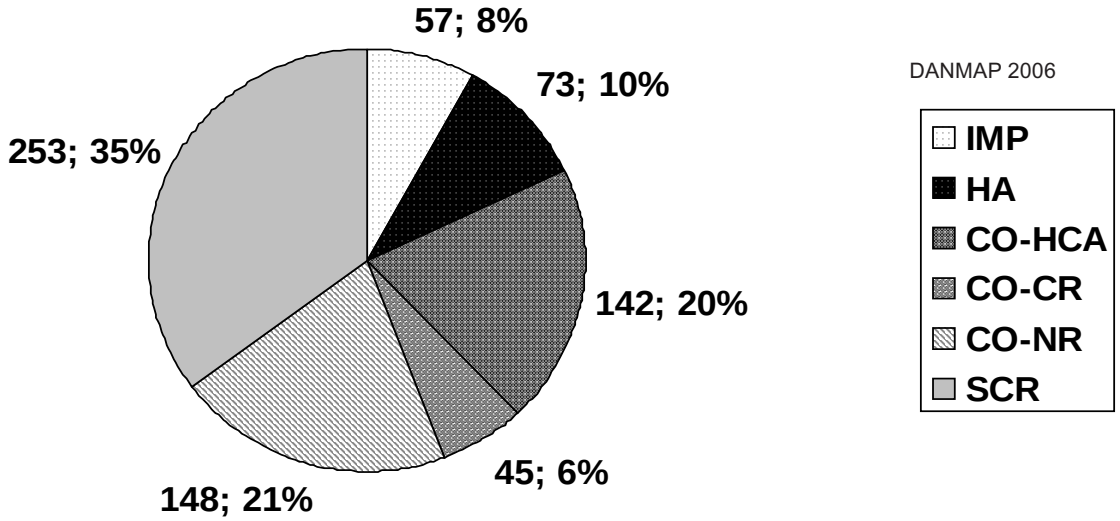

Figure 36. Distribution of methicillin resistant Staphylococcus aureus *(MRSA) cases in 2006 according to screening or origin of infection, Denmark*
IMP (Imported infection); HA (hospital acquired infection); CO-HCA (community onset infection, health care risk identified); CO-CR (community onset infection, community risk identified); CO-NR (community onset infection, no identified risk); SCR (Screening).

Molecular typing showed that 93% of the MRSA isolated in Denmark in 2006 belonged to seven clonal complexes (CC): CC8 (31%), CC22 (18%), CC5 (16%), CC80 (12%), CC30 (11%), CC45 (3%) and CC1 (3%). The major CC groups found in Denmark are also among the most frequently isolated worldwide.
Within CC8, 28 isolates were identified as USA300. In Denmark isolates of this type has been encountered since 2000. However, in contrary to US this clone has not spread epidemically and the cases instead in most cases represent single cases often related to travel [Larsen A *et al*. Euro Surveill. 2007:12].
Six cases of MRSA CC398 were detected in 2006, a recently identified *S. aureus* type that has been associated with animal husbandry, especially swine (see textbox page 78).

Intensified efforts, such as those described in the new national MRSA guidelines issued by the Danish National Board of Health on November 1st 2006, are probably the explanation for halting the 8-fold increase of new MRSA cases observed from 2002 to 2005. In 2006, MRSA was furthermore made reportable both for infections and carriage only.
The guidelines include recommendations for preventing the spread of MRSA in primary health care, where the majority of the increase has been seen previously. Eradication of MRSA carriage in the community is an important part of the guidelines. For such cases it is recommended that the whole household of an MRSA positive person is treated simultaneously regardless of carrier status.
The data from 2006 strongly indicates that control of MRSA is possible both in hospital outbreak situations as well as in the community.

Methicillin-resistant *Staphylococcus aureus* in animals

From being almost exclusively a nosocomial pathogen Methicillin-resistant *Staphylococcus aureus* (MRSA) have during the last two decades emerged into the community and have recently also caused infections in and colonized pets and production animals. MRSA have been detected in cattle, chickens, horses, pigs, dogs, rabbits, seals, birds and cats. The colonization in animals has in several cases been implicated in infections in humans and MRSA should today be considered a zoonosis. It is, however, important to distinguish between the epidemiology of MRSA in relation to production animals, where a new clone (ST 398) is emerging, and pet animals, that most often are colonized or infected with classical human variants of MRSA.

MRSA ST 398 was first observed in 2004 in the Netherlands among persons in contact with farm animals, mainly pigs [Voss *et al.* Emerg. Inf. Dis, 2005, 111: 965-66]. The clone has since been found widespread among pigs, cattle and latest also broilers as well as in farmers in the Netherlands. The same clone (ST 398) has also been found in several other countries and from both production animals and humans especially in farmers but also veterinarians have been shown to be at risk.

In Denmark a total of 32 human cases with ST 398 have been identified since 2003 and the first case in pigs was detected in 2006. To identify potential sources a case-control investigation was initiated in 2007. Compared with the controls cases had more frequently worked on or been in contact with farm animals (odds ratio (OR) 35.4 for healthy controls and OR 14.5 for MRSA controls). Thirteen cases, but no controls had been in contact with pigs. From contact farms a total of 23 out of 50 pigs from five farms were MRSA ST 398 positive. This case-control investigation provides epidemiological and microbiological evidence for MRSA ST 398 as a zoonosis in Denmark and people living or working in close contact with farm animals mainly pigs could be at increased risk for infections with MRSA ST 398. MRSA has not been found in other animals than pigs in Denmark.

MRSA ST 398 thus seems to be transmitted from production animals to humans; its origin is still unknown. It is remarkable that this type has only been known for a few years yet it is quite diverse with several different pattern of resistance, at least 4 different SCC*mec* cassettes including two possible new variants, and at least 5 different *spa* types. The reason for the colonization of MRSA ST 398 in pigs and other production animals or the epidemiology of this clone is currently not known. It can be speculated that the use of cephalosporins, tetracycline and other antibiotics have provided a niche for this clone, but until further studies are carried out this is merely speculation.

MRSA ST 398 in humans behave like other community acquired MRSA strains predominantly causing skin and soft tissue infections in healthy people, but has also been causing severe infections i.e. endocarditis. The importance for human health and the possibilities for infection control are currently unclear, but a true animal reservoir of MRSA represents a potential threat to the control of MRSA especially in low prevalence countries. The route of transmission is probably direct or indirect contact to colonized animals, whereas there is no risk of eating meat from pigs. In the Netherlands the recommendations have been changed such that pig breeders, veterinarians and slaughterhouse personnel are put into precautionary isolation until surveillance cultures are proven negative if admitted to a hospital. For cattle breeders screening without isolation on admission to a hospital is sufficient. In Denmark, The Danish National Board of Health is considering which actions to take.

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Appendix 1

Materials and Methods

Materials and methods

Demographics

Hospitals in Denmark

The reported number of hospitals in each county in Denmark corresponds to the number of geographically 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. It is larger than the official number of hospitals in Denmark since reorganisation of the hospital sector has resulted in regrouping hospitals that are distant geographically under the same administration and therefore the same name.

Additionally, certain categories of hospitals were excluded. This year, data from five private hospitals and clinics, nine psychiatric hospitals, seven specialised non-acute care clinics, seven rehabilitation centres and two hospices were excluded from DANMAP.

Data on consumption of antimicrobials

Consumption of antimicrobial agents in animals

Consumption data presented in this report were obtained from VetStat. In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals and the consumption of coccidiostatics and antimicrobial growth promoters. The VetStat programme was initiated in 2001. Before 2001, data on antimicrobial consumption in animals were based on sales figures reported by the pharmaceutical industry.

All prescription medicines are sold through a pharmacy (approximately 97%). The only exception is premix used in medicated feed, which is sold through feed mills. The pharmacy either sells the medicines to veterinarians for use in practice or for re-sale to farmers, or sells directly to the animal owner on presentation of a prescription. By law, the profit that veterinarians can make on the sale of medicines is very limited.

The monitoring programme VetStat contains detailed information about source and consumption for each prescription item, the data comprise: date of sale, source (pharmacy, feed mill, veterinarian), drug identity and amount, animal species, age-group, disease category and code for farm-identity (CHR – Danish Central Husbandry Register). Knowledge of the target animal species enables the presentation of consumption data in Defined Animal Daily Doses (ADD). The ADD system is a veterinary national equivalent to the international Defined Daily Doses (DDD) system applied in the human field.

Data on all sales of veterinary prescription medicine from the pharmacies are sent electronically to VetStat. Veterinarians are required by law to report to VetStat the use of all prescription medicines in production animals. 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. Feed mills report all sales of medicated feed directly to VetStat.

Antimicrobials used in humans / animals are presented in Table A1.

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 antimicrobials 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 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 antibacterials for systemic use, or group J01 of the 2005 update of ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antimicrobials in primary health care is expressed as a number of DDD per 1,000 inhabitants and per day (DDD/1,000 inhabitant-days). Consumption in primary health care is also reported as a number of packages per 1,000 inhabitants. Consumption of antimicrobials in hospitals is expressed as a number of DDD per 1,000 occupied beds and per day (DDD/1,000 occupied bed-days). Since antimicrobial consumption expressed as DDD/1,000 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DDD/ 1,000 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

Isolates from animals

Bacterial isolates included in the monitoring programme are collected from animals at slaughter (*E. coli*, enterococci and *Campylobacter spp.*), as well as from diagnostic submissions (*Staphylococcus hyicus* from pigs, *E. coli* from diarrhoea in cattle and pigs, and *E. coli* from septicaemia in poultry). Finally, *Salmonella* isolates from sub-clinical infections as well as from cases of clinical salmonellosis are included.

The samples from animals at slaughter are collected by meat inspection staff or company personnel and sent for examination to the National Food Institute, DTU or, when poultry, to the National Veterinary Institute, DTU. The number of samples taken at the slaughter plants is proportional to the number of animals slaughtered at each plant per year. Each sample represents one herd or flock. Samples are collected once a month (once weekly for broilers) in the period January-November.

Table A1. Antibacterials used in humans and/or in animals in Denmark a)

Antibacterials, which are only used in animals are mentioned in italics (animal growth promoters used before 1999 are mentioned in parentheses).
Antibacterials, which are used both in humans and animals are underlined

DANMAP 2006

ATC/ATCvet codes	Therapeutic group	Names of antibacterials in group
J01AA/*QJ01AA/QJ51AA*	Tetracyclines	Doxycycline, *chlortetracycline*, lymecycline, oxytetracycline, tetracycline, tigecycline
J01BA/*QJ01BA*	Amphenicols	*Florfenicol*
J01CA/*QJ01CA*	Penicillins with extended spectrum	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE/*QJ01CE*	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, *procaine penicillin*, *penethamate hydroiodide*
J01CF/*QJ51CF*	Beta-lactamase resistant penicillins	Dicloxacillin, *cloxacillin*, flucloxacillin, *nafcillin*
J01CR/*QJ01CR*	Comb. of penicillins, incl. beta-lactamase inhibitors	Amoxicillin/clavulanate, piperacillin/tazobactam
J01DB/*QJ01DB/QJ51DA*	First-generation cephalosporins	Cefalexin, *cefadroxil*, *cefapirin, cephalothin*
J01DC	Second-generation cephalosporins	Cefuroxime
J01DD/*QJ01DD/QJ51DA*	Third-generation cephalosporins	Cefotaxime, ceftazidime, ceftriaxone, *cefoperazone, ceftiofur, cefpodoxime*
J01DE/*QJ51DA*	Fourth-generation cephalosporins	Cefepime, *cefquinome*
J01DF	Monobactams	Aztreonam
J01DH	Carbapenems	Meropenem, imipenem/cilastatin, ertapenem
J01EA	Trimethoprim and derivatives	Trimethoprim
J01EB/*QJ01EQ/QJ51R*	Short-acting sulfonamides	Sulfamethizole, *sulfadimidine, sulfathiazole*
J01EE/*QJ01EW*	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfamethoxazole/trimethoprim, *sulfadiazine/trimethoprim*, *sulfadoxine/trimethoprim*
J01FA/*QJ01FA*	Macrolides	Erythromycin, *spiramycin*, roxithromycin, clarithromycin, azithromycin, *tylosin*, *tilmicosin, acetylisovaleryltylosin, tulathromycin*
J01FF/*QJ01FF*	Lincosamides	Clindamycin, *lincomycin*
J01FG/*QJ01XX*	Streptogramins	*(Virginiamycin) b)*
J01G/A07AA/*QJ01G/QA07AA c)*	Aminoglycosides	*Streptomycin*, *dihydrostreptomycin*, tobramycin, gentamicin, *neomycin*, netilmicin, *apramycin*
J01MA/*QJ01MA*	Fluoroquinolones	Ofloxacin, ciprofloxacin, moxifloxacin, *enrofloxacin*, *danofloxacin*, *marbofloxacin, difloxacin*
QJ01MB	Other quinolones	*Oxolinic acid*
QJ01MQ	Quinoxalines	*(Carbadox, olaquindox)*
J01XA	Glycopeptides	Vancomycin, teicoplanin, *(avoparcin)*
J01XB/A07AA/*QA07AA c)*	Polypeptides (incl. polymyxins)	Colistin, *(bacitracin)*
J01XC	Steroid antibacterials	Fusidic acid
J01XD/P01AB/*QJ01XD c)*	Imidazole derivatives	Metronidazole
J01XE/*QJ01XE*	Nitrofurane derivatives	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) Antibiotics for intrammamary use in animals are included. Antibiotics only used topically in humans or in animals are not included
b) Pristinamycin and quinupristin/dalfopristin (for humans) are not used in Denmark
c) Although intestinal antiinfectives (A07AA) and nitroimidazole derivatives for protozoal diseases (P01AB) are used to treat human patients in Denmark, their consumption is not reported by DANMAP

The broiler, cattle and pig slaughter plants included in the surveillance programme account for 95%, 90% and 95%, respectively, of the total number of animals slaughtered in Denmark per year. 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.

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, DTU and for poultry, at the National Veterinary Institute, DTU, one isolate per serotype per farm is selected for the DANMAP report.

Bacterial isolates from diagnostic submissions are selected by a pseudo-random process that also includes isolates from submissions to the National Food Institute, DTU from the Laboratory of Swine Diseases, Danish Meat Association, Kjellerup. Accordingly, the programme achieves nationwide coverage for these pathogens.

Isolates from food

All food samples were 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 surveillance programme. The collected material consisted of both Danish and imported foods. The food samples were 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].

Isolates from humans

***Salmonella* spp. and *Campylobacter* spp.**
Antimicrobial susceptibility was performed on a sample of isolates grown from diagnostic faecal specimens submitted to the Unit of Gastrointestinal Infections at Statens Serum Institut (SSI). Exact figures of the proportion tested and the sampling strategy for the different species can be found in the corresponding chapters of this report.

***E. faecium*, *E. faecalis*, vancomycin-resistant** enterococci and *E. coli* (NorMat study). To monitor the level of resistance among healthy individuals an ongoing surveillance comprising of approximately 200 stool samples per year was initiated in 2002. The subjects for participation in the surveillance were selected through the Danish Civil Registry system (CPR), which is a continuously updated register of all residents in Denmark. In total, 252 individuals were invited to participate in the study in 2006. A selection algorithm was used to generate birthdays and gender of the individuals to be invited for the study. In order to have a representative study population the selection algorithm was based on the age and gender distribution of the total Danish population. A letter including information on the study together with a consent form was mailed to the selected individuals. They were asked to confirm their willingness to participate by returning the signed form. Faecal test tubes were mailed to the SSI. The study protocol has the approval of the scientific ethics committee for Copenhagen and Frederiksberg municipalities.

***Staphylococcus aureus*.** All blood isolates from 15 of the 16 counties in Denmark and all methicillin-resistant *Staphylococcus aureus* (MRSA) nationwide are referred to the Staphylococcus reference laboratory at SSI for confirmation of susceptibility results and phage typing. MRSA isolates are further confirmed by the EVIGENE™ Detection kit (SSI) and is subjected to pulsed-field gel electrophoresis (PFGE) typing.

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

***Escherichia coli*, coagulase-negative staphylococci and *Streptococcus pyogenes*.** Data was provided on all isolates recorded from either blood samples (*E. coli*, coagulase-negative staphylococci), urine samples (*E. coli*) or all clinical samples (*S. pyogenes*) submitted for susceptibility testing to the participating laboratories serving the municipalities of Copenhagen and Frederiksberg, and the counties of Copenhagen, Frederiksborg, Roskilde, West Zealand, Storstrøm, Funen, Ribe, Vejle, Ringkøbing, Aarhus, Viborg, and North Jutland.

Isolation and identification of bacteria

Isolates from animals

***Salmonella* spp.** Examination of samples was done by non-selective pre-enrichment of 22-25 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 on the agar as 3 drops and in addition for cattle samples 1.0 ml BPW was inoculated in 9 ml selenite cystein broth. After enrichment overnight at 41.5°C material from MSRV swarming zones and 0.01 ml broth were inoculated onto Brilliant Green Agar (for samples from cattle and pigs) or onto Rambach Agar (for samples from poultry). Overnight incubation at 37°C was followed by serotyping of suspect colonies by slide agglutination.

***Campylobacter* spp.** The samples were examined by direct inoculation of selective agar (samples from pigs and poultry) or by selective enrichment (samples from cattle). The selective agar (mCCD) was incubated in micro-aerophilic atmosphere for 1-5 days at 42°C. Selective enrichment was done by inoculation of Preston broth at a ratio of 1:10, followed by incubation in microaerophilic atmosphere for 24 h at 42°C. Ten µl of the enrichment culture was inoculated onto mCCD agar and incubated 1-5 days at 42°C. Campylobacter-like colonies were identified by phase-contrast microscopy, by catalase activity and the ability to hydrolyse hippurate and indoxyl acetate. For isolates from cattle and pigs, oxidase activity was also tested.

***Escherichia coli* from healthy animals (indicator *E. coli*).** The material was inoculated directly onto Drigalski agar and incubated at 37°C overnight. For poultry, yellow colonies that were catalase positive and oxidase negative were identified according to the following criteria: indole, citrate, methyl red and Voges-Proskauer reaction. 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.

Enterococci from 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. Up to three colonies showing a morphology typical of *E. faecalis* and *E. faecium* were sub-cultivated onto blood agar. White colonies were identified by the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose.

Enterococci from broilers. Cloacal swabs were incubated overnight at 42°C in Enterococcus Selective Broth, prepared with a composition identical to that of Enterococcosel broth. Cultures were streaked on Slanetz-Bartley agar and incubated for 48 h at 37°C. Colonies that morphologically resembled *E. faecium* and *E. faecalis* were identified to species level using standard biochemical and physiological tests as described above.

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

Isolates from food

***Salmonella* spp.** was isolated according to the guidelines for microbiological examination of foods from the Danish Veterinary and Food Administration [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 Danish Veterinary and Food Administration. [NMKL No. 119, 2nd ed., 1990]. Subsequently, due to outsourcing, the isolates were sent to Eurofins A/S for further identification. No data available at present.

Indicator *E. coli* was isolated by the RVFCA and subsequently, due to outsourcing, sent to Eurofins A/S for verification of species identification. *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. Presumptive *E. coli* were identified as *E. coli* using API 20E test (BioMérieux, France).

Enterococci was isolated by the RFCA and subsequently, due to outsourcing, sent to Eurofins A/S for verification of species identification. 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 the plates were examined for growth, and colonies typical of *E. faecium* and *E. faecalis* were sub-cultured on blood agar. Species identification was performed by PCR according to Dutka-Malen S *et al.* J. Clin.Microbiol.,1995; 33:24-27.

Isolates from humans

***Salmonella* spp.** were isolated from faecal samples using the SSI Enteric Medium (SSI Diagnostika, Copenhagen, Denmark) including enrichment using 0.6% selenite medium (SSI Diagnostika).

***Campylobacter* spp.** were isolated from faecal samples using modified CCDA (SSI Diagnostika). Species identification was performed using a species

specific PCR assay [Persson S and Olsen KE, J. Med. Microbiol. 2005; 54: 1043-1047].

Enterococci. Enterococci from healthy humans in the community (NorMat study) were isolated and identified by the following procedure. One spoonful of faeces suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz agar and incubated for 2 days at 35°C. Ten µl of the faeces suspension was furthermore added to 5 ml Enterococcosel broth incubated overnight. Cultures were spread on Slanetz agar and incubated for 2 days at 35°C. Colonies showing morphology typical of *E. faecalis* or *E. faecium* were sub-cultivated on 5% blood agar plates. The isolates were identified as *E. faecalis* or *E. faecium* using API api20 strep tests (BioMérieux, France) and PCR according to Poulsen et al. and Dutka-Malen et al. [Poulsen RL *et al.*, APMIS 1999; 107: 404-412 and Dutka-Malen S *et al.*, J. Clin. Microbiol. 1995; 33: 24-27].

Vancomycin-resistant enterococci. A selective method for isolation of vancomycin-resistant enterococci from healthy humans in the community was used in the NorMat study. Ten µl of the faeces suspension was added to 5 ml Enterococcosel broth incubated overnight. Cultures were spread on Bile Aesculin agar with 16 µg/ml vancomycin and incubated for 2 days at 35°C. Colonies showing morphology typical of *Enterococcus* spp. were sub-cultivated on 5% blood agar plates. The isolates were identified as enterococci using API api20 strep tests (BioMérieux, France) and PCR according to Poulsen *et al.* and Dutka-Malen et al. [Poulsen RL *et al.,* APMIS 1999; 107: 404-412 and Dutka-Malen S *et al.*, J. Clin. Microbiol., 1995; 33: 24-27].

Escherichia coli. *E. coli* from healthy humans in the community (NorMat study) were isolated and identified by the following procedure.One spoonful of faeces suspended in 2 ml sodium chloride (0.9%) was spread on the SSI Enteric Medium. Presumptive *E. coli* isolates were sub-cultured on 5% blood agar plates. The isolates were identified as *E. coli* using API 20E test (BioMérieux, France).

***Staphylococcus aureus*.** All blood isolates from 15 of the 16 counties in Denmark and all methicillin-resistant *Staphylococcus aureus* (MRSA) nationwide (one per person) are referred to the Staphylococcus reference laboratory at SSI for confirmation of susceptibility results and typing. All isolates were phage typed according to Blair & Williams using the present set of international phages at 100xRTD (routine test dilution) concentration. Presence of the *mecA* gene was confirmed by PCR for MRSA isolates. All MRSA isolates were further subjected to pulsed-field gel electrophoresis (PFGE) using the Harmony protocol and SCC*mec* typing. Selected isolates were typed using sequence typing i.e. *spa* and/or MLST typing. Based on the PFGE patterns /spa and or MLST typing each isolate were assigned to a clonal complex group (CC).
For each case, the discharge summary of the patient was retrospectively collected from the general practitioner or the hospital.

Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* spp., *Campylobacter* spp., indicator *E. coli, Enterococcus* spp., and *Staphylococcus hyicus* was performed with a commercially available MIC technique using dehydrated antimicrobials in microtitre wells (Sensititre, Trek Diagnostic Systems Ltd., UK). The wells were inoculated and incubated according to the CLSI guidelines. The MIC was defined as the lowest concentration of antimicrobial with no visible growth. The breakpoints used are shown in Table A2, and the following strains were used for quality control: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.

Isolates of animal origin were susceptibility tested at the National Food Institute, DTU and for poultry, at the National Veterinary Institute, DTU. The *Salmonella* spp., *Campylobacter* spp.*,* indicator *E. coli* and *Enterococcus* spp. of human origin were susceptibility tested at the SSI. Isolates from food were susceptibility tested at Eurofins A/S, except for *Salmonella* spp. that were susceptibility tested at the National Food Institute, DTU or Eurofins A/S. Susceptibility data on *Campylobacter* spp. from food was not submitted and therefore not available for the DANMAP 2006 report.

One isolate per bacterial species per herd, or per food sample, or per patient was tested for antimicrobial susceptibility.

Additional information on animal isolates

Isolates of *Staphylococcus hyicus* were screened for *mec*A-mediated resistance (methicillin-resistance) by PCR.

Table A2. Breakpoints for resistance and range of dilutions used for MIC-determination by Sensititre for bacteria from animals, food and humans, Denmark DANMAP 2006

Antimicrobial agent	*E. coli / Salmonella*		*Staphylococcus hyicus*		Enterococci		*Campylobacter*	
	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range
Amoxicillin/clavulanic acid a)	>16	2-32						
Ampicillin	>16	1-32			>8	2-64		
Apramycin	>16	4-32						
Avilamycin					>8	2-16		
Cefpodoxime	>1	0.125-4						
Ceftiofur	>4	0.5-8	>4	0.125-16				
Cephalothin	>16	4-32						
Chloramphenicol	>16	2-64	>16	2-64	>16	2-64	>16	2-32
Ciprofloxacin	>0.06	0.03-4	>2	0.125-8			>2	0.03-4
Colistin	>8	4-16						
Daptomycin					>4	0.125-16		
Erythromycin			>4	0.125-16	>4	0.5-32	>16	0.5-32
Flavomycin					>8	4-32		
Florfenicol	>16	2-64	>16	1-64	>16	4-32		
Gentamicin	>4	1-32			>512	128-2,048	>8	0.125-16
Kanamycin					>1,024	128-2,048		
Linezolid					>4	1-8		
Nalidixic acid	>16	4-64					>32	2-64
Neomycin	>8	2-32						
Penicillin			>0.125	0.06-16				
Salinomycin					>8	2-16		
Spectinomycin	>64	16-256	>64	8-256				
Streptomycin	>16	4-64	>16	2-128	>1,024	128-2,048	>8	2-16
Sulfonamide	>256	64-1,024	>256	8-512				
Quinupristin/dalfopristin b)					>4	0.5-16		
Tetracycline	>8	2-32	>8	0.5-32	>8	1-32	>8	0.25-16
Tiamulin			>16	0.25-32				
Tigecycline					>0.25	0.015-2		
Trimethoprim	>8	4-32	>8	1-32				
Vancomycin					>16	2-32		

a) Concentration of amoxicillin presented. Amoxicillin was tested with clavulanic acid in concentration ratio 2/1

b) The trade name is Synercid. Breakpoint evaluated and changed according to textbox, page 49-50

Additional information on human isolates

***Salmonella* spp.** Besides susceptibility testing with Sensititre, *Salmonella* spp. isolates were screened for mecillinam and fosfomycin resistance using a 33 mg mecillinam tablet and a 70 + 40 mg fosfomycin tablet, respectively, (Neo-Sensitabs®, A/S Rosco) on Mueller-Hinton agar (SSI Diagnostika). The breakpoints used are those defined by the CLSI.
Etests, testing resistance towards cefotaxime and ceftazidime with and without clavulanic acid (Etest technical guide 3B, Gram-negative aerobic specific EAS 004, from AB BIODISK, Solna, Sweden) were carried out on *Salmonella* isolates that were resistant to both cefpodoxime and ceftiofur to asses extended-spectrum beta-lactamase (ESBL) status.

***Staphylococcus aureus*.** Susceptibility testing was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco, Denmark) on Danish Blood Agar (Resistensplade, SSI Diagnostika, Denmark) towards: penicillin, cefoxitin, streptomycin, kanamycin, erythromycin, clindamycin (only when isolate was resistant to erythromycin), tetracycline, fusidic acid, norfloxacin and linezolid. A cefoxitin 60 µg tablet was used for screening for methicillin susceptibility. Isolates with an inhibition zone <32 mm were further tested for the presence of the *mec*A gene by PCR. MRSA isolates were further tested for susceptibility towards glycopeptides by using the Etest® (AB Biodisk, Sweden) macro screen method on Brain-Heart infusion agar (Becton Dickinson, Germany).

***Streptococcus pneumoniae*.** The *Neisseria* and Streptococcus Reference laboratory at SSI screens for penicillin-resistant *S. pneumoniae* using a 1 µg oxacillin disk (Oxoid, Greve, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), 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). Penicillin and erythromycin MIC's are determined using the E-test (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.

Invasive *Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. The Neisseria and Streptococcus Reference laboratory at SSI screens for penicillin-resistant streptococci using a 1µg 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 MIC's are determined using the E-test (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 intermediate resistant isolates.

***Escherichia coli*, coagulase-negative staphylococci, and *Streptococcus pyogenes*.** In 2006, the clinical microbiology laboratories serving Roskilde, Storstrøm, Funen, Vejle and Viborg counties, and Rigshospitalet, which is the national referral hospital and serves part of the municipality of Copenhagen, 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, when testing isolates of coagulase-negative staphylococci Vejle county used the Neo-Sensitabs® on Müeller-Hinton II agar (SSI Diagnostica), and Funen county 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 clinical microbiology laboratory serving Ribe county used the above described method for testing isolates of *Streptococcus pyogenes*. However, Müeller-Hinton II agar (SSI Diagnostika) was used when testing *E. coli* and coagulase-negative staphylococci. The clinical microbiology laboratory serving North Jutland county 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. The only material exception from SRGA was that the wildtype population of *E.coli* was deemed susceptible for ampicillin (and not intermediately susceptible).

In 2006, the clinical microbiology laboratories serving the Copenhagen and Frederiksberg Municipalities, Copenhagen county, Ringkøbing county and Aarhus county used the disk diffusion method (Oxoid, Basingstoke, UK) on Iso-Sensitest (ISA) medium (Oxoid). The clinical microbiology laboratories serving Frederiksborg county and West Zealand county used the same disks on Iso-Sensitest (ISA) medium with 5% horse blood (Oxoid). 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/).

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

Fluoroquinolone breakpoint

The current CLSI breakpoint for resistance to the fluoroquinolone ciprofloxacin is >2 µg/ml. Because of compelling evidence that the treatment efficacy of fluoroquinolones is reduced in humans infected with strains of *Salmonella enterica* with decreased susceptibility to fluoroquinolones, it has been recommended that for *Salmonella* a breakpoint of >0.06 µg/ml for fluoroquinolones should be used [Aarestrup *et al.* 2003. Antimicrob. Agents Chemother. 47: 827-9]. Since 2004, a breakpoint of >0.06 µg/ml was used in DANMAP for both *Salmonella* spp. and indicator *E. coli,* and for 2006, the breakpoint of >0.06 µg/ml is further applied to pathogenic *E. coli*.

Gentamicin and apramycin breakpoints

Since 2004, the *E. coli* and *Salmonella* spp. breakpoints for resistance to gentamicin and apramycin have been changed from >8 to >4 µg/ml and from >8 to >16 µg/ml, respectively.

Quinupristin/dalfopristin breakpoint

Since 2006, the *E. faecium* breakpoint for resistance to quinupristin/dalfopristin has been changed from >2 µg/ml to >4 µg/ml. Please see textbox, page 49-50 for more details.

Performance test

A performance test was carried out similar to previous years in order to ascertain the comparability of susceptibility tests of the laboratories involved in the presentation of data. The laboratory in Department of Gastrointestinal Infections, SSI, the National Center for Antimicrobials and Infection Control, SSI, the Section for Antimicrobial Resistance at the National Food Institute, DTU, and Section of Poultry at the National

Veterinary Institute, DTU as well as Eurofins A/S received 5 *E. coli* strains, 5 *Salmonella* spp., 5 *Enterococcus* spp. and 9 *Campylobacter* spp. All antimicrobial susceptibility testing was performed with a commercially available MIC technique using dehydrated antimicrobials in microtitre wells (Sensititre, Trek Diagnostic Systems Ltd., UK), and all laboratories inoculated and incubated according to the CLSI guidelines. The *E. coli* strains were tested in all five laboratories, whereas the *Salmonella* spp., *Enterococcus* spp. were tested in four laboratories and *Campylobacter* spp. in three laboratories. A total of 1264 antibiotic-bacterium susceptibility tests were performed and the overall results were 0.48% failures. The detailed results are shown in Table A3.

Data handling

Data on 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®. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant breakpoint. 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.

Data on food isolates

Results from the analysis of food samples were reported via the database administrated by the Danish Veterinary and Food Administration. For each bacterial isolate information is available on the type of food sample, bacterial species, date of sampling, date of examination of the sample, 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, information about the country of origin was recorded whenever possible.

Data on human isolates

***Salmonella* spp. and *Campylobacter* spp.** Data on *Salmonella* spp. and *Campylobacter* spp. infections were exported from the Danish Registry of Enteric Pathogens (Microsoft®Access) maintained by the Unit of Gastrointestinal Infections, SSI. This register

Table A3. Results of performance testing (Correct result/number of tests performed) among laboratories participating in DANMAP 2006, Denmark

DANMAP 2006

Antimicrobial agent	*E. coli*		*Salmonella* spp.		*Enterococcus* spp.		*Campylobacter* spp.	
	S + I [a]	R[b]	S + I [a]	R[b]	S + I [a]	R[b]	S + I [a]	R[b]
Penicillin	-	-	-	-	-	-	-	-
Ampicillin	10/10	15/15	12/12	8/8	20/20	-	-	-
Amoxicillin/clavulanic acid	25/25	-	20/20	-	-	-	-	-
Cephalothin	20/20	5/5	20/20	-	-	-	-	-
Ceftiofur	20/20	4/5	20/20	-	-	-	-	-
Cefpodoxime	20/20	5/5	20/20	-	-	-	-	-
Erythromycin	-	-	-	-	7/8	12/12	12/12	9/9
Tetracycline	20/20	5/5	12/12	8/8	4/4	16/16	9/9	12/12
Tigecycline	-	-	-	-	20/20	-	-	-
Chloramfenicol	25/25	-	12/12	8/8	16/16	4/4	21/21	-
Vancomycin	-	-	-	-	20/20	-	-	-
Daptomycin	-	-	-	-	20/20	-	-	-
Linezolid	-	-	-	-	20/20	-	-	-
Quinopristin/dalfopristin	-	-	-	-	8/8	12/12	-	-
Nalidixic acid	20/20	5/5	12/12	8/8	-	-	9/9	12/12
Ciprofloxacin	15/15	10/10	12/12	8/8	-	-	12/12	9/9
Neomycin	25/25	-	20/20	-	-	-	-	-
Streptomycin	15/15	10/10	12/12	8/8	12/12	8/8	12/12	8/9
Apramycin	25/25	-	15/16	4/4	-	-	-	-
Kanamycin	-	-	-	-	16/16	4/4	-	-
Gentamicin	15/15	10/10	15/16	4/4	16/16	3/4	18/18	3/3
Spectinomycin	25/25	-	12/12	8/8	-	-	-	-
Colistin	25/25	-	20/20	-	-	-	-	-
Sulfamethoxazole	15/15	10/10	12/12	8/8	-	-	-	-
Trimethoprim	15/15	10/10	19/20	4/4	-	-	-	-
Florfenicol	25/25	-	20/20	4/4	20/20	-	-	-
Avilamycin	-	-	-	-	20/20	-	-	-
Flavomycin	-	-	-	-	8/8	12/12	-	-
Salinomycin	-	-	-	-	20/20	-	-	-
Total	335/335	89/90	286/288	84/84	247/248	71/72	93/93	53/54
	100%	98.9%	99.3%	100%	99.5%	98.6%	100%	98.1%

a) S + I: susceptible and intermediate
b) R: resistant

includes only one isolate per patient within a window of six months. Data on susceptibility testing of gastrointestinal pathogens are stored as MIC values (µg/ml) for *Salmonella* isolates in a Microsoft®Access database. Using the isolate identification number, the Danish Registry of Enteric Pathogens was merged with the database containing the results of susceptibility testing. Additionally, for *Campylobacter* spp. infections the dataset containing the results of the species identification was linked to this merged database. Data were analysed using EpiInfo™ 2000.

***Staphylococcus aureus*.** For MRSA, data on the characteristics of the MRSA isolates and the clinical/ epidemiological information were exported from the Danish MRSA registry (Microsoft® Excel) maintained by the Staphylococcus Laboratory at SSI. In this database, patients are only registered the first time they are diagnosed with MRSA, regardless of whether it was colonisation or infection. MRSA cases were classified as 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 (HA-MRSA) or infection diagnosed outside hospitals (community onset). Cases of community onset MRSA infection were further classified according to risk factors in the discharge summary as: community onset – no risk identified (CO-NR), community onset - community risk identified (CO- CR), community onset - health care risk identified within the last 12 months (CO-HCA).

***Streptococcus pneumoniae, Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci.** Data on susceptibility testing of isolates are stored as MIC's in a Microsoft® Access database at the Neisseria and Streptococcus Reference laboratory 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* and coagulase-negative staphylococci.** Fourteen clinical microbiology laboratories provided aggregated data on resistance levels in *E. coli* blood and urine isolates and coagulase-negative staphylococci blood isolates. Data were extracted from the following laboratory information systems:

- ADBakt (Autonik AB, Skoldinge, Sweden) for Copenhagen and Frederiksberg Municipalities (Hvidovre Hospital), Copenhagen county (Herlev Hospital), West Zealand county (Slagelse Hospital), and North Jutland county (Ålborg Hospital);
- MADS (Clinical Microbiology Laboratory, Skejby Sygehus, Aarhus, Denmark) for Copenhagen Municipality (Rigshospitalet), Storstrøm county (Næstved Hospital), Funen county (Odense University Hospital), Ribe county (Esbjerg Hospital), Vejle county (Vejle Hospital), Ringkøbing county (Herning Hospital), Aarhus county (Skejby Sygehus) and Viborg county (Viborg Hospital);
- SafirLIS Microbiology (Profdoc Lab AB, Borlänge, Sweden) for Frederiksborg County (Hillerød Hospital);

For Roskilde county, resistance data on *E. coli* from blood samples were obtained from the laboratory information system at the SSI, and resistance data on *E. coli* from hospital urine samples from the chemical laboratory at Roskilde County Hospital.
Laboratories were asked to provide data on the number of isolates tested and the percentage found to be resistant to selected antimicrobials. Although all laboratories were asked to remove duplicate isolates from the same patient within a window of 30 days, half of them were able to comply with this rule. The other half removed duplicates so only one isolate was reported from each patient. In cases of urine samples, data on ciprofloxacin and nalidixic acid resistance in *E. coli* were excluded if susceptibility to this antimicrobial 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 StatCalc in EpiInfo™ v. 6. Yates continuity correction or 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 $P<0.0001$.

Appendix 2

DANMAP publications

DANMAP publications

2005

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Appendix 3

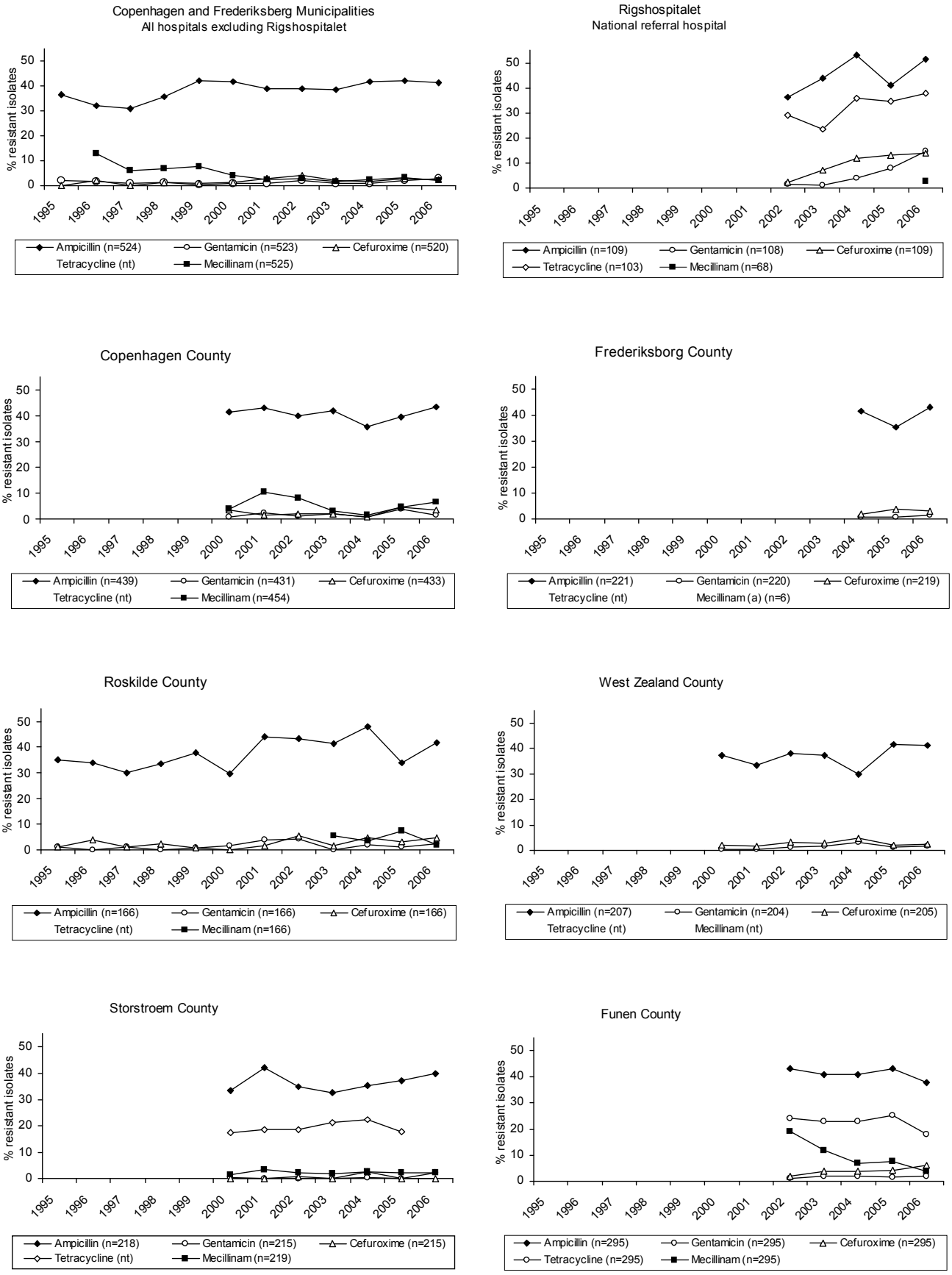

Figure A1. Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam and tetracycline in Escherichia coli *blood isolates from humans presented by county, Denmark*

Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006

(a) Data on mecillinam is not shown where tests were carried out on selected isolates only

(nt) = not tested

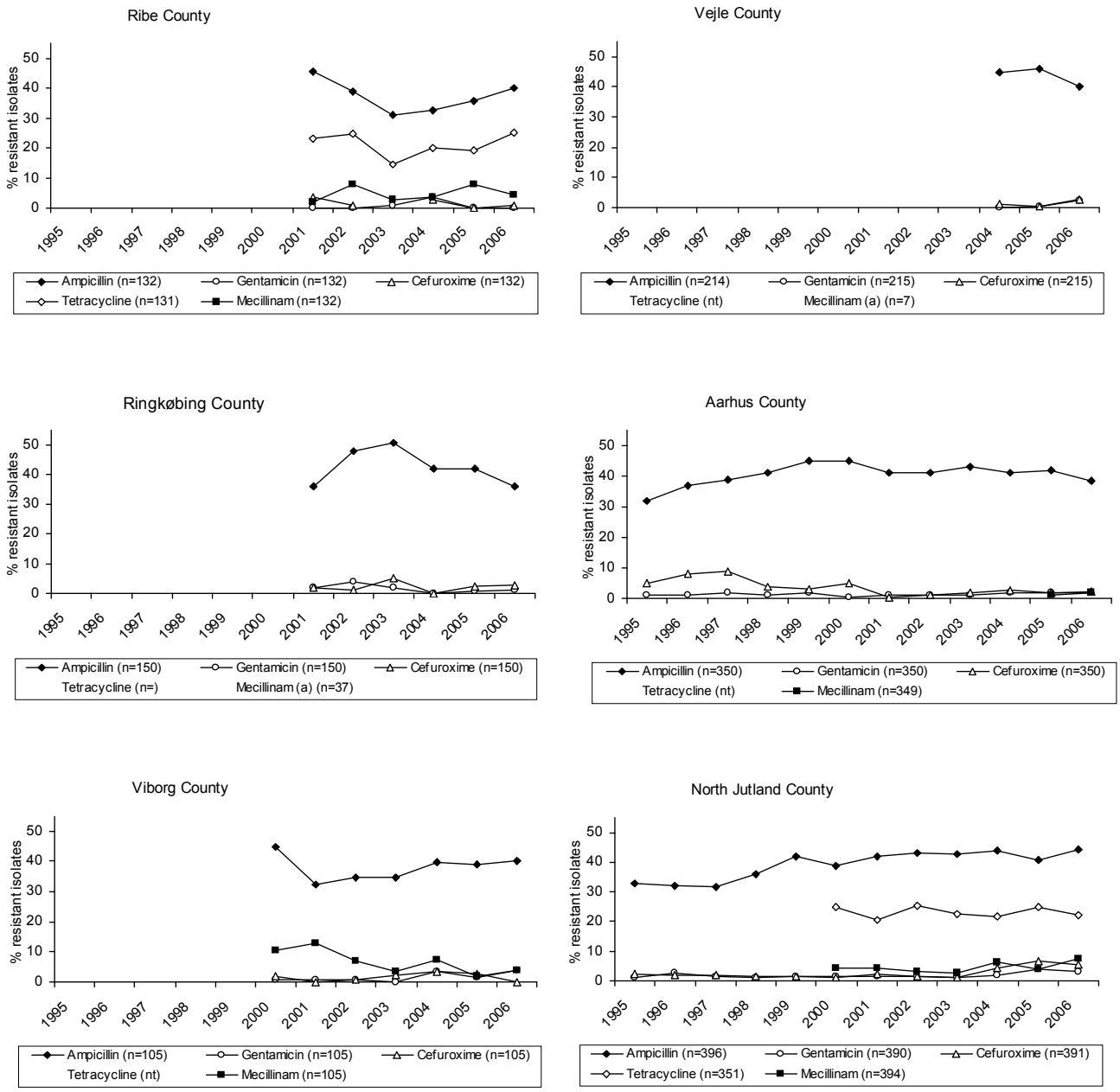

Figure A1 (Continued). Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam and tetracycline in Escherichia coli *blood isolates from humans presented by county, Denmark*

Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006

(a) Data on mecillinam is not shown where tests were carried out on selected isolates only

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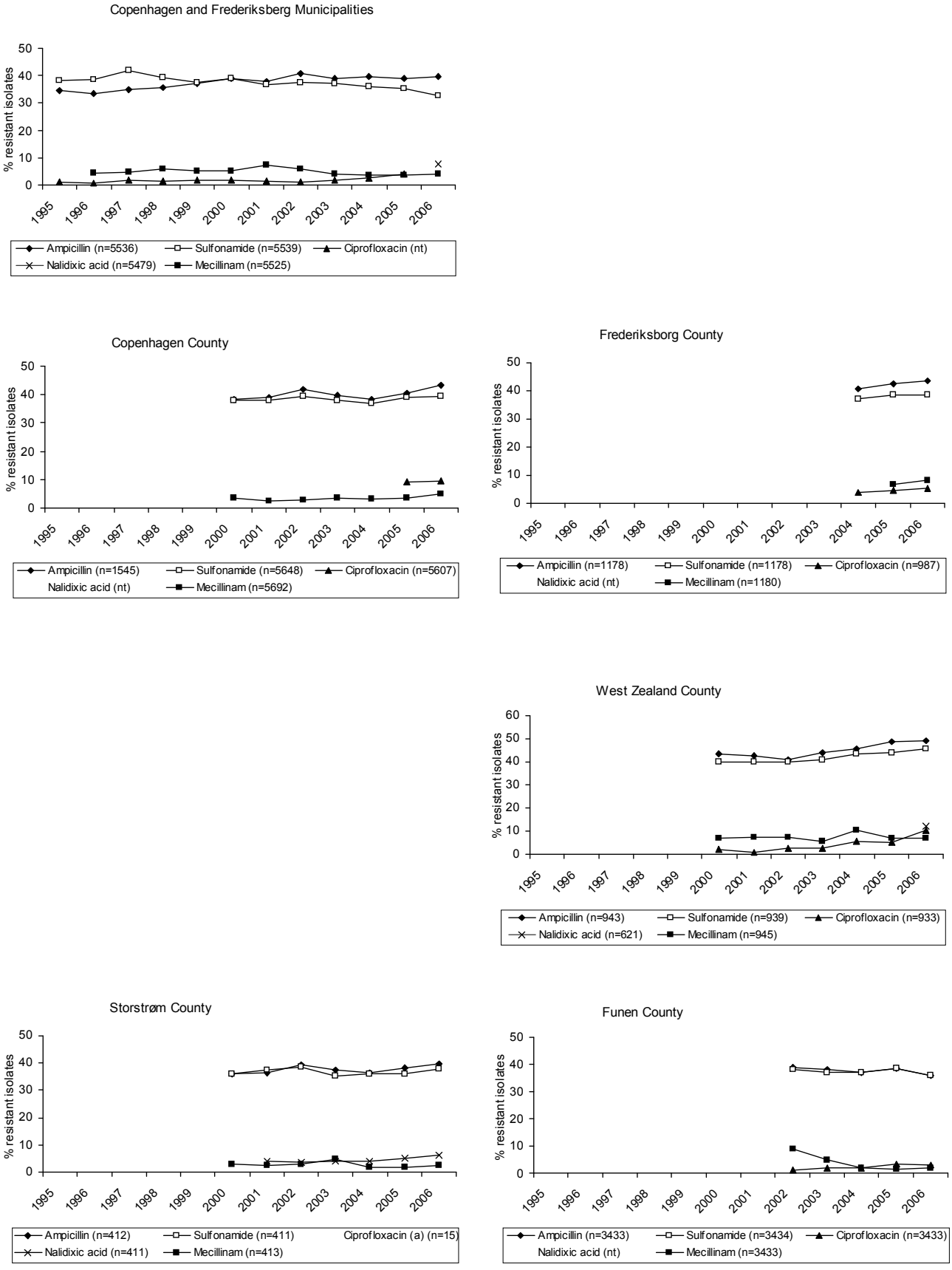

Figure A2. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in primary health care by county, Denmark*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006

(a) Data on ciprofloxacin and nalidixic acid is not shown where tests were carried out on selected isolates only

(nt) = not tested

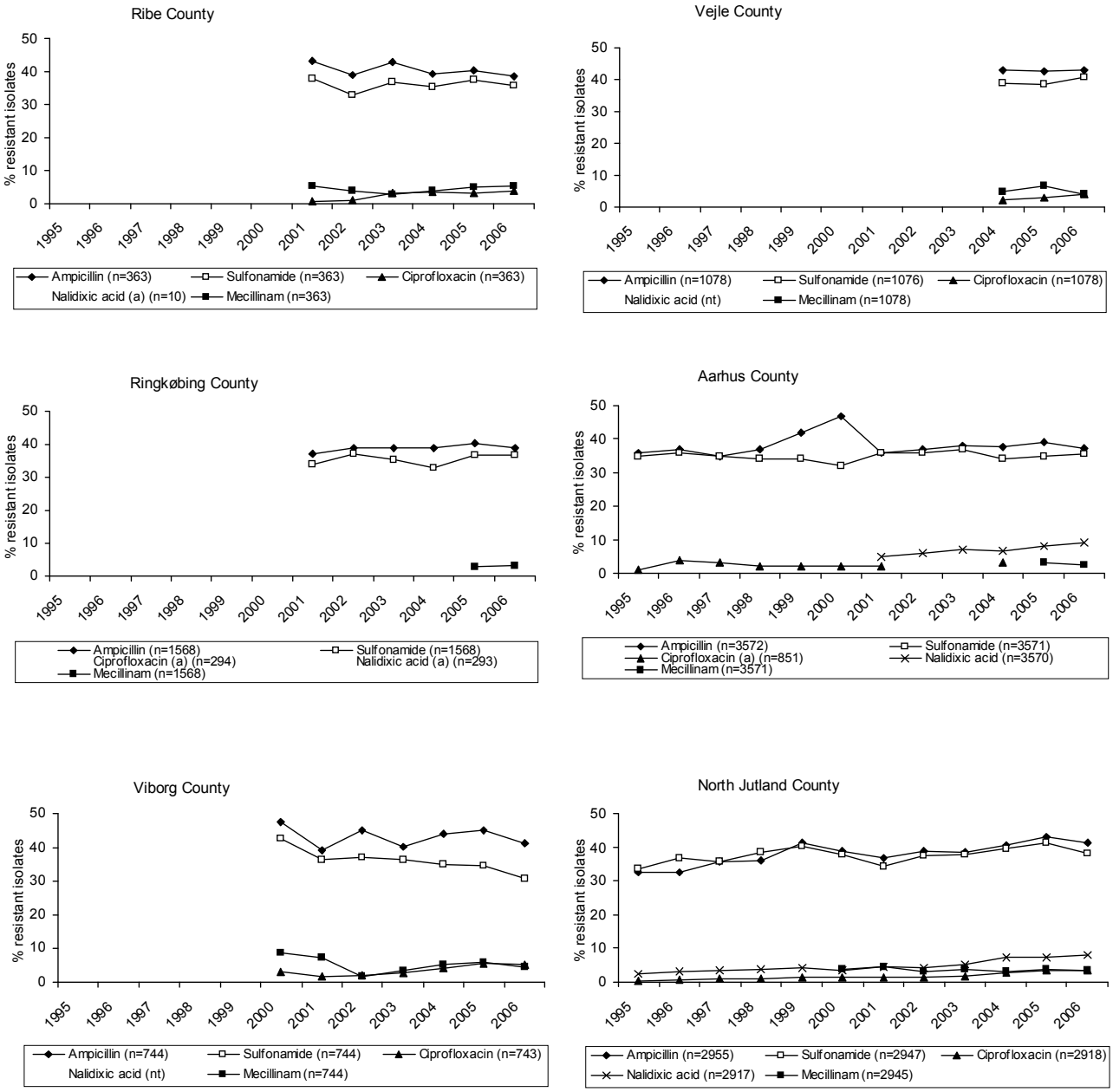

Figure A2 (Continued). Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in primary health care by county, Denmark*

The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006
(a) Data on ciprofloxacin and nalidixic acid is not shown where tests were carried out on selected isolates only
(nt) = not tested

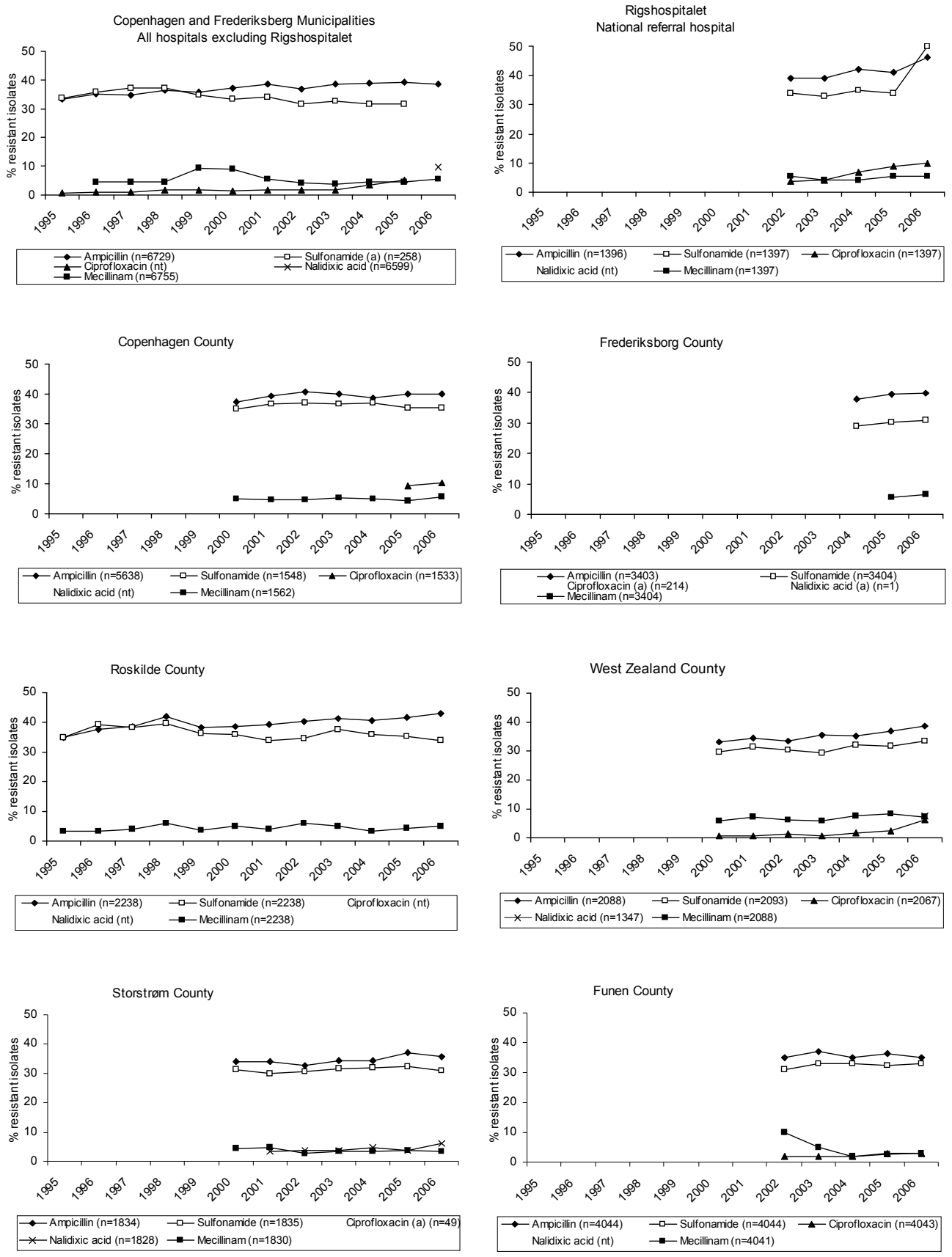

Figure A3. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in hospitals by county, Denmark*

Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006
(a) Data on ciprofloxacin, nalidixic acid and sulfonamide is not shown where tests were carried out on selected isolates only
(nt) = not tested

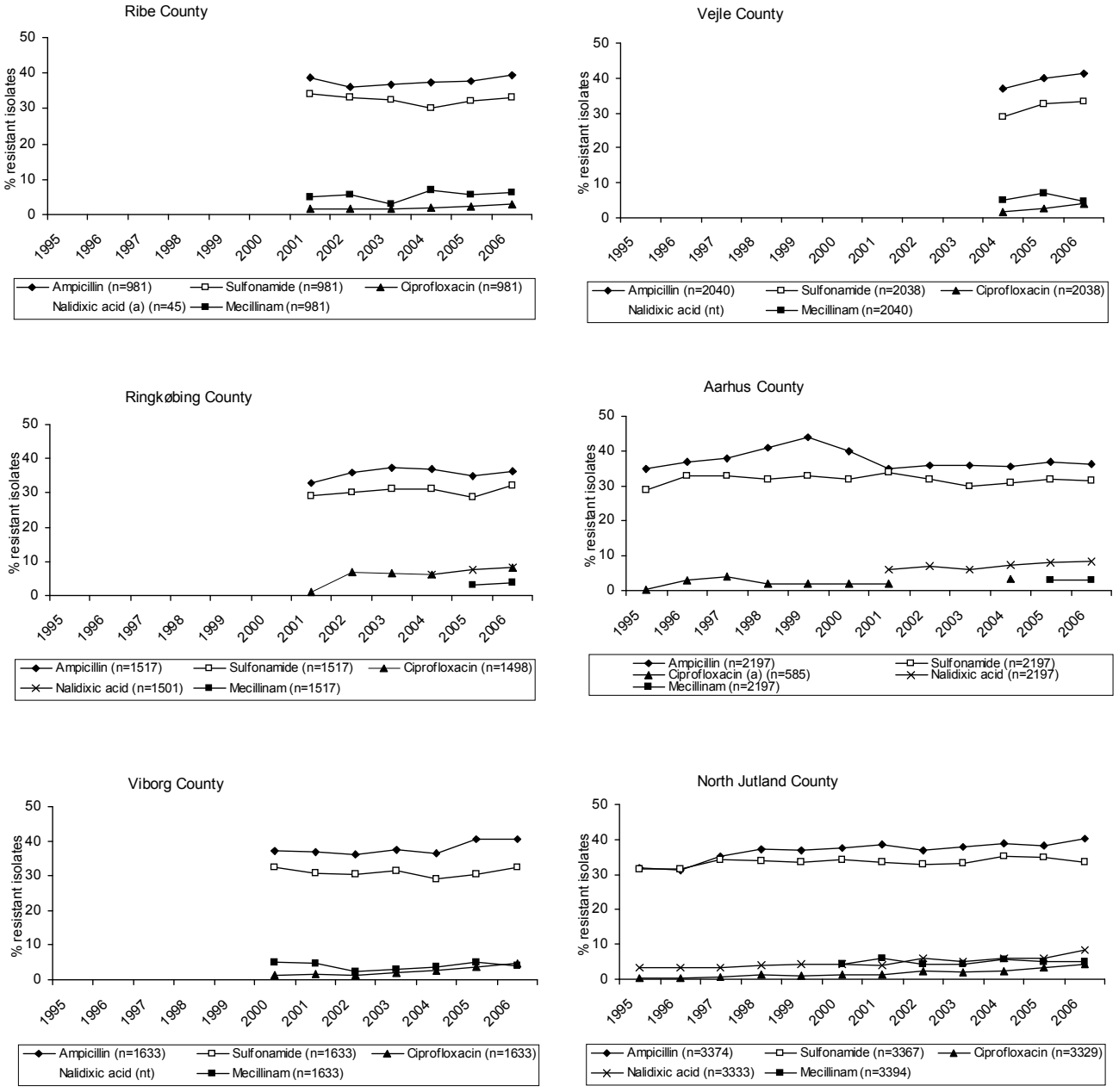

Figure A3 (Continued). Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in hospitals by county, Denmark*

Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2006
(a) Data on ciprofloxacin, nalidixic acid and sulfonamide is not shown where tests were carried out on selected isolates only
(nt) = not tested