DANMAP 2004

DANMAP 2004 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark

Statens Serum Institut
Danish Veterinary and Food Administration
Danish Medicines Agency
Danish Institute for Food and Veterinary Research

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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 antimirobial resistance in food animals, foods and humans in 2004. The report is produced in collaboration between the Danish Institute for Food and Veterinary Research, the Danish Veterinary and Food Administration, the Danish Medicines Agency and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Family and Consumer Affairs 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 Institute, the Danish Institute for Food and Veterinary Research and the Danish Veterinary and Food Administration. The objectives of DANMAP are:

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

The monitoring of antimicrobial resistance is based on three categories of bacteria: human and animal pathogens, zoonotic bacteria and indicator bacteria. Human and animal pathogens are included because these cause infections and they primarily reflect resistance caused by use of antimicrobials 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 antimicrobials 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
CPR	Danish Civil Registry
DFVF	Danish Institute for Food and Veterinary Research
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
PMWS	Post-weaning Multisystemic Wasting Syndrome
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/).

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. The CHR farm identity number is registered in VetStat records on all prescriptions for production animals in Denmark.

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 ADD_{kg}). 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 ADD_{kg} is the ADD per kg animal. Consumption calculated in ADD_{kg} allows summation of consumption across different age groups and animal species.

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.

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).

Antimicrobials. The term "antimicrobials" covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term "antimicrobials" 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 "antibacterials" is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only).

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
Forbruget af antibiotika til produktionsdyr steg fra 102,5 ton i 2003 til 112,5 ton i 2004. Trods denne stigning er det samlede antibiotikaforbrug til produktionsdyr stadig betydelig mindre end før ophøret med brug af antibiotiske vækstfremmere til dyr i Danmark. Stigningen i 2004 skyldes primært en 13,3% stigning i antibiotikaforbruget til svin fra 81,8 tons i 2003 til 92,7 tons i 2004. I den samme periode steg produktionen af svin med 2,9%. Makrolider, tetracykliner og tiamulin er som i tidligere år de mest anvendte antibiotika til svin.
Fluorokinolonforbruget faldt yderligere til 7 kg i 2004 mod 94 kg i 2001. Den mest sandsynlige årsag til dette fald er en lovændring fra 2002, der medførte at de regionale veterinærchefer skal give tilladelse hver gang en praktiserede dyrlæge ønsker at anvende fluorokinoloner til behandling.

En opgørelse af antibiotikaforbruget til svin fordelt på aldersgrupper viser, at forbruget til fravænningsgrise steg med 19% i 2004, mens forbruget til slagtesvin steg med 12% og til søer/smågrise med 5%. Til fravænnede grise steg især forbruget af receptordineret antibiotika til behandling af luftvejslidelser (29%) og mavetarminfektioner (17%). I 2004 blev der observeret store forskelle i antibiotikaforbruget fra amt til amt. I Jylland og på Fyn var antibiotikaforbruget til søer/smågrise og slagtesvin næsten dobbelt så stort som på Sjælland og Bornholm.

Flere faktorer kan være årsag til det stigende forbrug af antibiotika til svin. Brugen af vandmedicinering er steget mere end brugen af fodermedicinering. De enkelte vandrør i svinestaldene vil i de fleste tilfælde forsyne en langt større del af grisene end de enkelte foderrør. Ved anvendelse af vandmedicinering er sandsynligheden for også at medicinere raske grise derfor større, sammenlignet med anvendelse af fodermedicinering. De senere år er virussygdommen "Post-weaning Multisystemic Wasting Syndrome" (PMWS) etableret i den danske svinepopulation. Det har ført til en teori om at dele af det øgede forbrug af antibiotika til svin kan være relateret til forekomsten af PMWS. Et forskningsprojekt undersøger for øjeblikket hvilken betydning PMWS har for antibiotikaforbruget i svineproduktionen.

Med udgangen af 1999 ophørte brugen af antibiotiske vækstfremmere til dyr i Danmark. På trods af at dette nu er 5 år siden, bliver det med mellemrum fremført, at det stigende antibiotikaforbrug til svin skyldes vækstfremmerophøret. Andre forhold end vækstfremmerophøret synes imidlertid at have haft afgørende betydning for antibiotikaforbruget, hvilke understøttes af påvisningen af store regionale forskelle i antibiotikaforbruget. Dertil kommer den stigende brug af vandmedicinering samt den mulige effekt af PMWS, hvilket yderligere sandsynliggør at andre faktorer end vækstfremmerophøret har indflydelse på det stigende antibiotikaforbrug.

Med henblik på at stoppe de seneste års stigning i antibiotikaforbruget til svin iværksætter Fødevarestyrelsen i samarbejde med Danmarks Fødevareforskning, Danske Slagterier og Den Danske Dyrlægeforeningen en strategi som er rettet mod svineproducenter med stort antibiotikaforbrug per svin, samt deres praktiserende dyrlæger.

Både i 2003 og 2004 blev det totale antibiotikaforbrug til kvæg estimeret til 11 ton per år. I 2004 faldt produktionen af kalvekød med 10% og antallet af fedekalve som blev slagtet eller eksporteret faldt med 13%. Fra 2003 til 2004 blev fluorokinolonforbruget til kalve næsten fordoblet men er stadig på et lavt niveau.

I 2004 blev der samlet brugt 400 kg antibiotika til fjerkræ hvilket svarer til 0,4% af det totale antibiotikaforbrug til dyr. Forbruget til slagtekyllinger steg med 20% men er stadig på et meget lavt niveau. Derimod blev forbruget til opdrætflokke til konsumægs- og slagtekyllingeproduktionen næsten fordoblet. Fluorokinolonforbruget til kalkuner og opdræt steg betydelig i 2004, og ifølge de praktiserende dyrlæger var den primære årsag mavetarminfektioner og blodforgiftninger forårsaget af multiresistente *Escherichia coli*.

Antibiotikaforbruget til mennesker

I 2004 steg det totale forbrug af antibakterielle midler anvendt til behandling af mennesker med yderligere 4,1% til 30,8 mio. DDD eller 15,6 DDD/1.000 indbygger-dage sammenholdt med 2003.

I primærsektoren steg forbruget af antibakterielle midler i 2004 med 3,9%, dog uden ændringer i fordelingen af forbruget mellem de enkelte klasser. Forbruget af β-laktamase følsomme penicilliner, penicilliner med udvidet spektrum samt makrolider repræsenterer stadig knap 72% af det totale forbrug i primærsektoren. Forbruget af fluorokinoloner steg fortsat i 2004, formentligt på grund af lav pris og lav forekomst af resistens sammenlignet med andre antibakterielle midler. Det stigende forbrug har allerede ført til stigende resistens over for fluorokinoloner i *E. coli* isolater fra urinvejsinfektioner, hvilket understreger risikoen ved et fortsat stigende forbrug.

Forbruget af antibakterielle midler på danske sygehuse udtrykt i DDD/1.000 senge-dage er steget med 36,2% siden 1997, hvorimod stigningen kun var på 10,1% for den samme periode, når forbruget udtrykkes som DDD/1.000 udskrivninger. Forbruget af cephalosporiner, fluorokinoloner og carbapenemer er steget på bekostning af β-laktamase følsomme penicilliner, penicilliner med udvidet spektrum, aminoglykosider og makrolider. I 2004 udgjorde forbruget af cephalosporiner, fluorokinoloner og carbapenemer 23,7% af det totale forbrug af antibakterielle midler på sygehuse sammenholdt med 17,4% i 2000. Nye data har vist at dette skift til de nye bredspektrede antibiotika allerede har medført en øget forekomst af antibiotikaresistens på de danske sygehuse.

Resistens i zoonotiske bakterier

Resistensniveauet blandt *Salmonella* Typhimurium isolater fra svin var uændret fra 2003 til 2004, men set over en længere årrække (1999 til 2004) har der været en stigning i forekomsten af resistens overfor både tetracyklin og ampicillin. Denne stigning falder sammen med et stigende forbrug af både tetracykliner og bredspektrede penicilliner til svin.

I 2004 blev der kun fundet få *S.* Typhimurium isolater i dansk svinekød og ingen i dansk kyllingekød, hvilket gør det vanskelig at påvise eventuelle forskelle i resistensforekomsten mellem dansk og importeret kød. Sammenlignes resistensforekomsten blandt *S*. Typhimurium isolater fra danske svin og dansk svinekød, var resistensforekomsten i dansk svinekød signifikant højere for 3 af de testede antibiotika. Sammenlignes derimod resistensforekomsten blandt *S*. Typhimurium isolater fra danske svin og importeret svinekød, var resistensforekomsten i det importerede svinekød signifikant højere for 10 af de testede antibiotika. Dette indikerer, at resistensforekomsten blandt *S*. Typhimurium isolater fra importeret svinekød var højere end i isolater fra dansk svinekød. Blandt *S*. Typhimurium og *S*. Enteritidis isolater fra humane infektioner var der signifikant højere forekomst af resistens overfor ciprofloxacin og nalidixinsyre, hvis isolaterne stammede fra infektioner erhvervet i udlandet sammenlignet med isolater fra infektioner erhvervet i Danmark.

Fluorokinolonforbruget til svin har været faldende siden 2001 som følge af en lovmæssig ændring, alligevel var der signifikant højere ciprofloxacin/nalidixinsyre resistensforekomst blandt *Campylobacter coli* fra svin i 2004 (16%) sammenlignet med 2003 (3%). Årsagen til denne stigning er ukendt. Blandt *C. jejuni* fra kvæg og fjerkræ forblev resistensforekomsten uændret fra 2003 til 2004. Sammenlignes *C. coli* og *C. jejuni* isolater fra danske og importerede kødprodukter var der signifikant højere forekomst af resistens overfor tetracyklin, nalidixinsyre og ciprofloxacin i *C. coli* og *C. jejuni* fra importeret kyllingekød sammenlignet med isolater fra dansk kød. Blandt *C. jejuni* isolater fra humane infektioner erhvervet i Danmark var der fra 2003 til 2004 en signifikant stigning i forekomsten af resistens overfor nalidixinsyre.

Resistens i indikator bakterier

Blandt *Enterococcus faecium* fra svin og mennesker var der en signifikant højere forekomst af resistens overfor erythromycin i 2004 sammenholdt med 2003. I den samme periode steg forbruget af makrolider til behandling af svin betydeligt. Derimod faldt forekomsten af penicillin resistente *E. faecium* isolater fra svin på trods af et øget forbrug af penicillin til behandling af svin.

Forekomsten af resistens overfor bl.a. erythromycin og tetracyklin i *E. faecium* og *E. faecalis* fra importeret kyllingekød var signifikant højere end i isolater fra dansk kyllingekød. Der blev ligeledes observeret en signifikant højere forekomsten af resistens overfor erythromycin, tetracyklin og penicillin i *E. faecium* fra importeret oksekød sammenlignet med dansk oksekød. Tetracyklin resistente *E. faecalis* isolater forekom oftere i importeret svinekød i forhold til dansk svinekød. Forekomsten af resistens i enterokokker fra raske mennesker var generelt på samme niveau som forekomsten af resistens i enterokokker fra kødprodukter.

Dette synes at stemme overens med antagelsen om, at resistensforekomst i bakterier i kødprodukter afspejles i resistensforekomst i bakterier hos raske mennesker.

Blandt indikator *E. coli* fra svin var der fra 2003 til 2004 en signifikant stigning i forekomsten af resistens overfor bl.a. tetracyklin og ampicillin. Denne stigning er sammenfaldende med en stigning i forbruget af tetracykliner og bred-spektrede penicilliner i svineproduktionen.

Sammenlignes resistensforekomsten i *E. coli* isolater fra dansk og importeret kyllingekød var resistensforekomsten i det importerede kyllingekød signifikant højere for 8 af de testede antibiotika.

I importeret oksekød blev der i 2004 fundet et *E. coli* isolat, som var resistent overfor ceftiofur. Det er første gang, at et *E. coli* isolat med resistens overfor β-laktamaser med udvidet spektrum er blevet isoleret fra et levnedsmiddel solgt i Danmark.

Forekomsten af resistens i enterokokker fra mennesker var generelt på samme niveau som forekomsten af resistens i enterokokker isoleret fra kødprodukter. Imidlertid var forekomsten af resistente *E. coli* højere i importeret fjerkrækød i forhold til forekomsten hos raske mennesker.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Antallet af infektioner forårsaget af methicillin-resistente *Staphylococcus aureus* (MRSA) forsatte med at stige i 2004. Der blev rapporteret 411 infektioner forårsaget af MRSA, hvilket var omtrent dobbelt så mange, som der blev rapporteret i 2003. De fleste infektioner var erhvervet i Danmark (90%) og udenfor sygehusene (62%). Antallet af MRSA bakteriæmi-tilfælde steg til 19 eller 1,2% af alle *S. aureus* bakteriæmi-tilfælde i 2004, hvilket var det højest rapporterede antal siden midten af 1970erne.

Resistens overfor penicillin og makrolid var i 2004 fortsat lav i isolater af *Streptococcus pneumoniae* og *Streptococcus pyogenes* (Gruppe A streptokokker).

I primærsektoren er der sket en signifikant stigning i forekomsten af ciprofloxacin resistente *E. coli* urin-isolater, således var 2,9% af isolaterne resistente i 2004. I flere amter blev der observeret en tilsvarende stigning i forekomsten af ciprofloxacin resistente *E. coli* urin-isolater på hospitalerne. Stigningen i ciprofloxacin resistens er sket sideløbende med en stigning i forbruget af fluorokinoloner i de seneste år - både på hospitalerne og i primærsektoren. Ciprofloxacin og de øvrige fluorokinoloner er potente antibiotika, der bør reserveres til behandling af alvorlige infektioner, primært i hospitalsregi. Forekomsten af ampicillin resistens i *E. coli* blod-isolater forblev mellem 30% og 50% mens gentamicin og cefuroxim resistens i blod-isolater fra flere amter steg signifikant til henholdsvis 1,7% og 2,7%.

Summary

Antimicrobial consumption

DANMAP presents the use of antimicrobials in animals and humans. Since 2001, data on all medicines prescribed by veterinarians for use in animals have been registered at farm level by the VetStat programme. 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.

Antimicrobial consumption in animals

In 2004, antimicrobial consumption in animals increased by 9.7% from 102.5 to 112.5 tonnes, however the total consumption of antimicrobials in animals in Denmark is still considerably lower than before the termination of antimicrobial growth promoters (AGPs). The increase in 2004 was due to a 13.3% increase in antimicrobial consumption in pigs, from 81.8 tonnes in 2003 to 92.7 tonnes in 2004. In the same period the production of pigs increased by 2.9%. As in previous years, macrolides, tetracyclines and tiamulin were the most commonly used antimicrobials for treatment of diseases in pigs. The consumption of fluoroquinolones in pigs decreased further to 7 kg in 2004, as compared to 94 kg in 2001. This decrease was most likely due to the legal restrictions of usage of fluoroquinolones in food animals, implemented in 2002.

Antimicrobial consumption varied depending on the age of pigs. The increase was mainly observed in weaners (19%), and less in finishers (12%) and sows/piglets (5%). In weaners, prescriptions for treatment of respiratory and gastrointestinal (GI) diseases increased by 29% and 17%, respectively in 2004.

Large regional differences in antimicrobial consumption were observed. In the western regions of Denmark (Funen and Jutland) the consumption of antimicrobials in sows/piglets and finishers was almost 50% higher than in the eastern regions (Zealand and Bornholm).

A number of factors may have contributed to the increase in antimicrobial consumption in pigs. The use of water medication has increased in recent years and individual water pipes often supply more pens than individual feed pipes. Therefore in addition to the diseased pigs a relatively larger number of healthy pigs will be medicated when using water medication compared to feed medication. In this way an increased amount of antimicrobials are needed for medication. The occurrence of Post-weaning Multisystemic Wasting Syndrome (PMWS) in the Danish pig population may explain part of the increase in antimicrobial consumption. A research project is currently being conducted to investigate the relative importance of PMWS on antimicrobial consumption.

It has been suggested that the recent increase in the consumption of prescribed antimicrobials in animals could be the result of AGP withdrawal in the late 1990s. However, AGPs were withdrawn more than 5 years ago and the effect would have been seen earlier. Furthermore, large regional differences of antimicrobial consumption were observed suggesting that withdrawal of AGPs is not the cause of increased antimicrobial consumption. The increase in water medication and the possible significance of PMWS are factors supporting the assumption that the increase in antimicrobial consumption is not related to the termination of AGP use.

The Danish Veterinary and Food Administration in collaboration with the Danish Institute for Food and Veterinary Research, Danish Bacon and Meat Council, and Danish Veterinary Association have initiated a strategy directed towards veterinary swine practitioners and pig producers in order to reduce antimicrobial consumption in pigs.

Antimicrobial consumption in cattle was estimated at 11 tonnes in 2004, the same amount as in 2003. In 2004, the production of veal decreased by 10%, and the number of fattening calves slaughtered or exported decreased by 13%. From 2003 to 2004, fluoroquinolone consumption in calves almost doubled, but remained at a very low level.

In 2004, 400 kg active compound or 0.4% of the total veterinary antimicrobial consumption was prescribed for poultry production. The consumption of prescribed antimicrobials in broilers increased by 20%, but the total consumption in broilers remains at a very low level. In rearing hens antimicrobial consumption more than doubled. The consumption of fluoroquinolones increased significantly in both rearing hens and turkeys. According to veterinary practitioners, fluoroquinolones were prescribed primarily for intestinal infections and septicaemia caused by multiresistant *Escherichia coli.*

Antimicrobial consumption in humans

In 2004, the overall consumption of antibacterials for systemic use in humans in Denmark increased 4.1% to 30.8 million DDDs or 15.6 DDD/1,000 inhabitant-days in 2004.

In the primary health care sector, consumption of antibacterials increased by 3.9% in 2004, but without significant changes in the distribution of the antibacterials used. Consumption of β-lactamase sensitive penicillins, penicillins with extended spectrum and macrolides represented almost 72% of the total consumption. Consumption of fluoroquinolones increased further in 2004, this was likely due to low price and low level of resistance as compared to other antibacterial agents. However, the increase in consumption has already resulted in increased resistance to fluoroquinolones in *E. coli* isolates from urinary tract infections. This illustrates the risk of a continued increase in fluoroquinolone consumption.

Consumption of antibacterials in Danish hospitals expressed as DDD/1,000 bed-days has increased by 36.2% since 1997, whereas this increase was only 10.1% for the same period when presented as DDD/1,000 discharged patients. However, as in previous years, a change in the distribution of antibacterials used - with an increasing consumption of cephalosporins, fluoroquinolones and carbapenems on behalf of β-lactamase sensitive penicillins, broad spectrum penicillins, aminoglycosides and macrolides – was observed. In 2004, cephalosporins, fluoroquinolones and carbapenems represented 23.7% of hospital antibacterial consumption compared to 17.4% in 2000. Recent data suggest that this shift towards newer, broad-spectrum antimicrobials is already resulting in increased resistance in hospitals.

Resistance in zoonotic bacteria

From 2003 to 2004, no significant changes in resistance were observed among *Salmonella* Typhimurium isolates from pigs. However, during the five year period, 1999 to 2004, resistance to tetracycline and ampicillin significantly increased. This increase coincided with increased consumption of tetracycline and broad spectrum penicillins in pigs in the same period.

In 2004, few *S*. Typhimurium isolates were obtained from Danish pork and none from Danish broiler meat, which makes it difficult to detect differences in the occurrence of resistance among *S*. Typhimurium isolates from Danish and imported meat products. When comparing resistance in *S*. Typhimurium isolates from Danish pigs and Danish pork, resistance to three antimicrobial agents was significantly higher among *S*. Typhimurium isolates from Danish pork as compared to Danish pigs. When comparing resistance in *S*. Typhimurium isolates from Danish pigs and imported pork, resistance to ten antimicrobial agents (including nalidixic acid and ciprofloxacin) was significantly higher among *S*. Typhimurium isolates from imported pork as compared to Danish pigs. This indicates that, in 2004, antimicrobial resistance in *S*. Typhimurium was more prevalent in isolates from imported pork than in isolates from Danish pork.

In human isolates of *S*. Enteritidis and *S*. Typhimurium resistance to ciprofloxacin and nalidixic acid was significantly higher in infections acquired abroad than from infections acquired in Denmark in 2004.

Fluoroquinolone consumption has decreased since 2001 due to legislative changes, even though ciprofloxacin/nalidixic acid resistance in *Campylobacter coli* isolates from pigs increased significantly from 3% in 2003 to 16% in 2004. The reason for the increase in resistance remains unknown. No significant changes in resistance were observed in *C. jejuni* isolates from broilers and cattle. In *C. coli* and *C. jejuni* isolates obtained from Danish and imported meat products, resistance to tetracycline, nalidixic acid and ciprofloxacin was significantly higher in *C. coli* and *C. jejuni* from imported broiler meat compared with isolates from Danish meats. In isolates of *C. jejuni* from humans, that did not report recent travel abroad, a significant increase in resistance to nalidixic acid was observed in 2004 as compared to 2003.

Resistance in indicator bacteria

From 2003 to 2004, erythromycin resistance in *Enterococcus faecium* from pigs and healthy humans significantly increased. This coincided with a substantial increase in macrolide consumption in pigs in the same period. In contrast, penicillin resistance among *E. faecium* from pigs decreased significantly from 2003 to 2004, although penicillin consumption in pigs increased during the same period.

The occurrence of resistance in *E. faecium* and *E. faecalis* isolates from imported broiler meat was significantly higher than in isolates from Danish broiler meat for a number of antimicrobials (including erythromycin and tetracycline). In *E. faecium* isolates from imported beef the occurrence of resistance was significantly higher for erythromycin, tetracycline and penicillin compared with isolates from Danish beef. The occurrence of resistance in *E. faecalis* isolates from Danish and imported pork was similar with the exception of significantly higher resistance to tetracycline in isolates from imported pork. In general, resistance levels in *E. faecium* and *E. faecalis* from healthy humans were similar to the levels observed in isolates from Danish and imported meat products. These observations are consistent with the assumption that resistance levels found in isolates from meat

products are reflected in the occurrence of resistance in the normal faecal flora of healthy humans.

Among indicator *Escherichia coli* isolates from pigs resistance to five of the tested antimicrobial agents, including tetracycline and ampicillin, increased significantly from 2003 to 2004. This coincided with an increased consumption of tetracyclines and broad-spectrum penicillins in pigs. However, this increase in resistance among indicator *E. coli* from pigs was not reflected in the occurrence of resistance in *E. coli* from Danish pork.

Resistance to eight antimicrobial agents was significantly higher in isolates from imported broiler meat compared to isolates from Danish broiler meat.

Ceftiofur resistance was observed in one food isolate from imported beef. This is the first *E. coli* isolate with extended β-lactamase resistance ever found in foodstuff sold in Denmark.

For most antimicrobials, resistance levels in *E. coli* isolates from healthy humans were similar to the levels observed in isolates from Danish meat products, imported pork and imported beef products. However, *E. coli* isolates from imported broiler meat were significantly more resistant to a number of antimicrobials compared to isolates from healthy humans.

Resistance in bacteria from diagnostic submissions

The number of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in Denmark continued to increase. In 2004, 411 cases were reported, approximately twice the number reported in 2003. Most infections were acquired in Denmark (90%) and had a community onset (62%). The number of MRSA bacteraemia cases increased to 19, or 1.2% of all *S. aureus* bacteraemia cases in 2004, the highest reported number since the mid -1970s.

Resistance to penicillins and macrolides in *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Group A Streptococci) remained low in 2004.

Among *E. coli* urine isolates from primary health care, resistance to ciprofloxacin increased significantly reaching 2.9% in 2004. In *E. coli* urine isolates from hospitals in several counties, ciprofloxacin resistance also increased significantly to 3.1%. These increases in ciprofloxacin resistance were consistent with parallel increases in consumption of fluoroquinolones (including ciprofloxacin) observed in recent years, in primary health care and in hospitals. Ciprofloxacin, as well as other fluoroquinolones, are potent antimicrobials, which should be reserved for treatment of serious infections, primarily in hospitals. Among *E. coli* blood isolates, ampicillin resistance remained between 30% and 50%. Gentamicin and cefuroxime resistance in blood isolates from several reporting counties increased significantly to 1.7% and 2.7%, respectively.

Demographic data

Demographic data

Demographic information is presented in order to show the magnitude of animal and human populations in which antimicrobials were used during 2004. Figure 1 shows counties in Denmark.

Table 1 shows the production of food animals (including animals for live export), meat, and the population of dairy cattle. From 2003 to 2004, the production of broilers, cattle and pigs increased by 0.6%, 1.1% and 2.9%, respectively. Forty-seven percent of all beef consumed was imported, compared with an estimated 50% for poultry and 15-20% for pork. Table 2 provides information on distribution of the human population in Denmark and the Danish health care system by county.

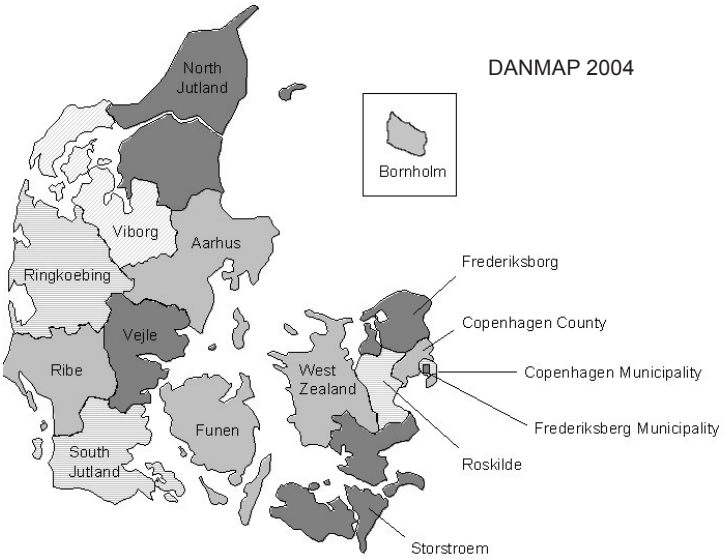

Figure 1. Counties in Denmark

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

DANMAP 2004

Year	Broilers		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 milk	1,000 heads	mill. kg	mill. kg	mill. kg
1990	94,560	116	789	219	753	4,542	16,425	1,260		
1992	107,188	137	862	236	712	4,405	18,442	1,442	35	7
1994	116,036	152	813	210	700	4,442	20,651	1,604	35	7
1996	107,895	149	789	198	701	4,494	20,424	1,592	32	8
1998	126,063	168	732	179	669	4,468	22,738	1,770	32	7
2000	133,987	181	691	171	636	4,520	22,414	1,748	32	7
2001	136,603	192	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	625	161	596	4,540	24,434	1,898	29	8
2004	130,674	181	632	165	563	4,434	25,141	1,965	28	7

Source: Statistics Denmark

Table 2. Distribution of the human population and health care structure by county, Denmark

DANMAP 2004

County name	No. inhabitants (01/01/2004)	No. inh./km2 (2004)	No. inh./GP c) (2003)	No. bed-days d) (2003 provisional)	No. hospitals (2004)
Copenhagen Municipality a)	501,664	5,684	1,571	944,618	4
Frederiksberg Municipality a)	91,721	10,459	1,576	e)	1
Copenhagen County b)	618,407	1,176	1,572	590,178	3
Frederiksborg	373,668	277	1,551	307,483	4
Roskilde	237,089	266	1,651	224,631	2
West Zealand	302,479	102	1,591	250,795	4
Storstroem	261,884	77	1,583	259,705	5
Bornholm	43,673	73	1,332	39,097	1
Funen	475,082	136	1,594	505,011	5
South Jutland	252,936	64	1,480	201,171	4
Ribe	224,595	72	1,547	175,908	3
Vejle	355,691	119	1,563	330,758	6
Ringkoebing	274,830	57	1,545	229,586	5
Aarhus	653,472	143	1,542	657,968	8
Viborg	234,659	57	1,563	229,969	5
North Jutland	495,669	80	1,594	468,902	7
Denmark	5,397,519	125	1,565	5,415,780	67

a) Inner Copenhagen
b) Outer Copenhagen
c) GP, general practitioner
d) Excluding psychiatry, private hospitals and seven rehabilitation centers
e) Public hospitals in Copenhagen and Frederiksberg municipalities (inner Copenhagen) represent one single administrative authority and include the national referral hospital

Antimicrobial consumption

Antimicrobial consumption in animals

In Denmark, all antimicrobials, except those approved by the EU as feed additives, are prescription only medicines, and must either be purchased through a pharmacy (99% of the consumption) or through a feed mill (1% of the consumption). Antimicrobial drugs purchased at pharmacies are either administered by veterinary practitioners (13% of the consumption), or administered by farmers according to veterinary prescription (87% of the consumption).

From 2001, the Danish register of veterinary medicines, VetStat, has collected detailed data on antimicrobial consumption in animals from pharmacies, veterinary practitioners, and feed mills. (See Appendix 1 for further details on the VetStat programme).

Consumption of prescribed antimicrobials (kg active compound)

Table 3 shows the trends from 1990 to 2004 in the consumption of prescribed antimicrobials in food animals. From 1996 to 2001, the veterinary consumption of prescribed antimicrobials almost doubled from 48.0 tonnes in 1996 to 94.7 tonnes in 2001. From 2001 to 2002, the antimicrobial consumption remained unchanged with a yearly increase in animal production exceeding the increase in antimicrobial consumption. Finally, in 2003 and 2004, the yearly antimicrobial consumption in food animals increased again by 6.9% and 9.7%, respectively, and reached a total of 112.5 tonnes active compound in 2004 (Table 3). This was due to increased consumption in pigs.

Figure 2 shows the trends in consumption of prescribed antimicrobials and growth promoters in food animals compared to antimicrobials prescribed for humans. The antimicrobial consumption in food animals is still low compared to the total consumption prior to the termination of growth promoter use.

Table 4 shows the total veterinary consumption of prescribed antimicrobial drugs in 2004 in kg active compound by animal species and age group, including consumption in companion animals. A total of 14.6 tonnes of antimicrobials were used or re-sold by veterinary practitioners. An estimated 9 tonnes of these were used in cattle practice, approx. 2 tonnes were used in small animal practice and approx. 3 tonnes were used in the other animal species.

Antimicrobial consumption in pigs

From 2003 to 2004, the antimicrobial consumption in pigs increased by 13% from 81.8 to 92.7 tonnes (Table 4). The production of pigs increased by only 3% in 2004 (Table 1). The increase in consumption was mainly within the following therapeutic groups: macrolides (1.8 tonnes), tiamulin (1.4 tonnes), β-lactamase sensitive penicillins (1.9 tonnes), other penicillins (1.9 tonnes) and tetracyclines (2.2 tonnes).

Taking production figures from 2004 into account (Table 1), the consumption of 92.7 tonnes antimicrobials in pigs equals 47mg antimicrobial/kg meat produced. This represents a 10% increase compared to 2003. When measured in ADD_{kg}.

Table 3. Trends in the estimated total consumption (kg active compound a)) of prescribed antimicrobials for food animals, Denmark

DANMAP 2004

ATC_{vet} group b)	Therapeutic group	1990	1992	1994	1996	1998	1999	2000	2001	2002 c)	2003 c)	2004
QJ01AA	Tetracyclines	9,300	22,000	36,500	12,900	12,100	16,200	24,000	28,500	24,500	27,300	29,500
QJ01CE	Penicillins, β−lactamase sensitive	5,000	6,700	9,400	7,200	14,300	14,700	15,100	16,400	17,400	19,000	20,900
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	6,600	7,300	8,800	9,900	11,100	12,900
QJ01EW	Sulfonamides + trimethoprim d)	3,800	7,900	9,500	4,800	7,700	6,800	7,000	9,200	10,600	10,600	11,500
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	1,000	950	900	850	850
QJ01F/QJ01XX	Macrolides, lincosamides, tiamulin	10,900	12,900	11,400	7,600	7,100	8,700	15,600	18,400	19,200	20,700	24,200
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	7,500	10,400	11,600	11,700	11,700	11,600
	Others d)	6,700	6,800	4,400	600	650	350	300	900	1,600	1,500	1,000
Total		53,400	73,200	89,900	48,000	57,300	61,900	80,700	94,700	95,900	102,500	112,500

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 2001-2004: 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. Topical (dermal) formulations are not included

a) Kg active compound rounded to nearest 50 or 100

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

c) Data for 2002-2003 has been corrected due to delayed reporting from two pharmacies

d) Consumption in aquaculture was not included before 2001

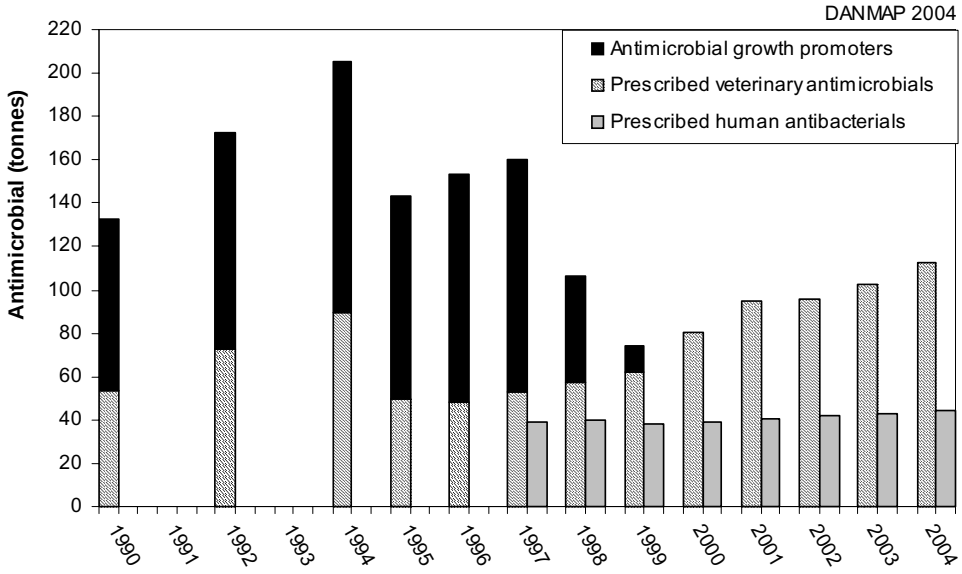

Figure 2. Consumption of prescribed antimicrobials and growth promoters in animal production and prescribed antibacterials in humans, Denmark

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). 2001-2004: Data from VetStat.

Table 4. Antimicrobials sold (kg active compound) from pharmacies and feedmills by animal species and age group a), Denmark

DANMAP 2004

Therapeutic group	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macro	Tiamul	Pen-β-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATC_{vet} groups b)	QJ01B	QJ01G	QJ01DA	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01XX	QJ01CE	QJ01CA	QJ01E	QJ01AA	QJ01X c)	
Pigs														
Sows and piglets	28	2,568	62	1	0	779	1,124	1,270	8,043	3,436	4,532	2,892	24	24,758
Weaners	32	6,247	11	1	0	1,041	5,979	2,725	1,389	3,663	2,217	11,387	226	34,918
Finishers	32	828	7	<1	0	1,359	4,141	3,627	5,011	2,603	289	12,371	4	30,272
Age not given	5	237	1	<1	0	99	402	347	449	247	157	793	5	2,742
Cattle														
Cows and bulls	2	13	5	<1	0	4	26	5	124	22	41	37	<1	280
Calves <12 months	76	229	3	1	0	8	24	1	318	262	326	343	2	1,594
Heifers and steers	2	2	<1	<1	0	<1	2	1	6	10	2	6	<1	29
Age not given	4	12	1	<1	0	2	5	8	25	14	21	26	<1	118
Poultry														
Broilers	0	6	0	8	0	<1	<1	0	0	59	4	<1	<1	76
Layers	0	0	0	1	0		<1	0	0	10	<1	<1	0	11
Rearing flocks	0	1	0	1	0	<1	6	<1	0	21	22	2	<1	53
Turkeys	0	1	0	8	0	<1	1	0	0	19	3	0	0	33
Other poultry	0	3	0	2	0	0	7	1	0	15	25	7	0	60
No production category	0	0	0	1	0	0	<1	0	0	19	9	3	<1	31
Small ruminants	<1	3	0	0	0	1	1	2	4	20	9	6	<1	46
Mink	0	285	<1	<1	0	43	116	<1	<1	457	126	39	<1	1,066
Aquaculture	80	<1	0	0	284	<1	0	0	<1	4	2,003	7	0	2,379
Other production animals	1	16	1	<1	0	4	4	3	22	19	16	13	<1	99
Horses	<1	5	<1	1	0	<1	2	1	23	3	126	2	<1	161
Pet animals	<1	8	76	5	0	13	8	3	19	85	78	23	11	328
Species not given														
- Farm identified d)	<1	36	<1	<1	0	9	28	21	35	59	16	46	1	250
- For use in vet. practice e)	54	1,175	346	27	26	144	812	115	5,482	1,998	2,753	1,595	30	14,557
Total	317	11,675	513	58	309	3,507	12,689	8,128	20,950	13,044	12,776	29,599	304	113,870

Amcol=amphenicols, Amglc=aminoglycosides, Ceph=cephalosporins, FQ=fluoroquinolones, Quinol=other quinolones, Linco=lincosamides, Macro=macrolides, Tiamul=tiamulin, Pen-β-sens=β-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) Consumption is given in kg active compound, rounded to nearest kg

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

c) Not including tiamulin and valnemulin

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

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

Table 5 shows the antimicrobial consumption in pigs by route of administration. From 2003 to 2004, the consumption of antimicrobials for water medication increased by 16% while feed medication increased by 11%. The increases in oral treatments were mainly in tiamulin, macrolides, tetracyclines and penicillins with extended spectrum. The use of antimicrobials for parenteral administration (injectables) increased by 12%. This increase was mainly in β-lactamase sensitive penicillins.

Figure 3 presents the development in ADD_{kg} per pig slaughtered, for the most important antimicrobials used for treatment of pigs. As in previous years, macrolides, tetracyclines and tiamulin were the most commonly used antimicrobials for treatment of pigs. The increase in tetracyclines, tiamulin, β-lactamase sensitive penicillins, other penicillins, and sulfonamide/

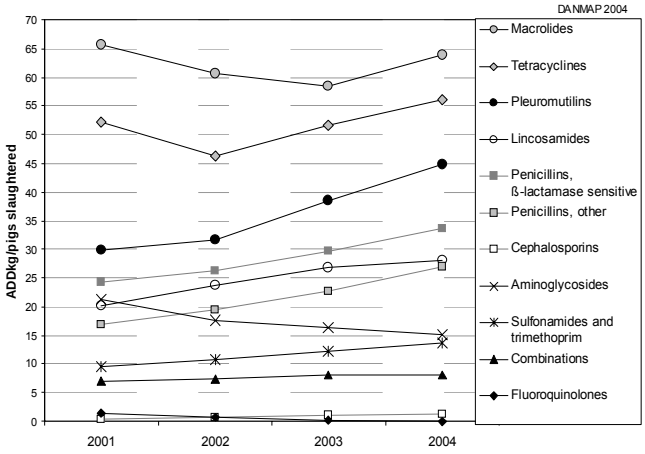

Figure 3. Trends in antimicrobial consumption (in ADD_{kg}) in pigs, 2001-2004, Denmark

Antimicrobial groups refer to ATC_{vet} groups (see Table 6) Amphenicols, colistin, intramammaries and gynecologicals are not included in the figure. Data from veterinary practice are not included (amounts to <2%)

Table 5. Consumption of antimicrobials (ADD_{kg}) for pigs by administration route a), Denmark

DANMAP2004

		Oral, in water			Oral, in feed			Parenteral		
		2002	2003	2004	2002	2003	2004	2002	2003	2004
ATC_{vet} group b)	Therapeutic group c)	ADD_{kg} (1,000s)								
QJ01A	Tetracyclines	101,635	145,110	217,562	843,271	912,969	965,224	185,633	204,984	230,782
QJ01B	Amphenicols	0	5	0	0	0	0	172	3,823	4,831
QJ01CE	Penicillins, β-lactamase sensitive	0	0	0	1	3	1	643,268	725,553	848,491
QJ01CA/CR	Penicillins, other	290,096	309,571	383,178	3,969	31,794	65,598	183,379	211,803	228,504
QJ01DA	Cephalosporins	0	0	0	0	0	0	16,342	26,886	29,938
QJ01E/QP51	Sulfonamides, trimethoprim	46	21	19	82,891	79,088	105,085	187,056	218,506	239,246
QJ01FA	Macrolides	716,133	692,800	773,111	742,546	703,436	796,183	26,356	32,232	38,661
QJ01FF	Lincosamides	202,866	238,086	263,920	243,564	267,841	280,700	135,908	147,226	161,490
QJ01XX	Tiamulin	391,000	479,552	613,985	372,512	440,834	489,885	15,996	21,094	23,833
QJ01G/QA07A	Aminoglycosides	389,069	363,878	349,399	41,086	34,165	31,314	0	0	0
QJ01MA	Fluoroquinolones	0	0	0	353	1	1	16,356	4,553	919
QJ01 d)	Other antimicrobials	36,519	50,391	51,651	0	0	0	182,150	199,639	202,412
Total		2,127,364	2,279,414	2,652,825	2,330,194	2,470,132	2,733,991	1,592,615	1,796,300	2,009,108

a) Based on VetStat data from pharmacies and feedmills, excluding use in practice (1-2% of the consumption in pigs)
b) For each compound, only the ATC group for the more important antimicrobial group is shown
c) Formulations for intramammary, intra-uterine, and topical administration are not included
d) Includes colistin for oral use and penicillin/streptomycin combinations for parenteral use

Table 6. Consumption of antimicrobials in pigs given as Defined Animal Daily Doses (ADDs) from 2001 to 2004, Denmark

DANMAP 2004

	Pharmacies and feed mills															
Age group	Sows/piglets				Weaners				Finishers				Age not given			
Animal standard weight	200 kg				15 kg				50 kg				50 kg			
	2001	2002	2003	2004	2001	2002	2003	2004	2001	2002	2003	2004	2001	2002 a)	2003 a)	2004
Therapeutic group	ADD (1,000s)															
Tetracyclines	873	878	915	927	36,766	32,089	32,367	38,207	9,060	8,721	11,138	12,212	769	192	754	888
Amphenicols	<1	0	6	7	<1	3	84	105	0	0	22	32	0	7	4	5
Penicillins, β–lact. sen. b)	1,558	1,779	2,015	2,230	2,224	2,519	2,903	3,969	4,098	4,577	5,121	6,323	375	81	461	538
Penicillins, other	820	1,024	1,118	1,201	8,500	9,965	12,720	16,348	1,813	2,144	2,420	3,500	293	322	357	337
Cephalosporins	38	60	99	113	59	147	254	263	16	36	56	60	6	417	8	9
Sulfonam./trimeth.	788	949	1,084	1,217	3,478	4,018	4,146	5,454	168	205	174	232	175	321	197	151
Macrolides	818	809	746	773	50,579	46,467	41,173	52,157	11,376	11,588	12,255	12,274	1,102	130	977	1,148
Lincosamides	437	567	580	588	14,000	17,472	19,821	22,411	3,225	3,817	4,412	4,622	365	63	383	425
Aminoglycosides	292	254	237	220	27,423	23,915	22,231	21,469	261	220	194	124	366	33	148	170
Colistin (local GI)	1	17	23	24	50	2,108	2,910	3,017	1	15	18	14	0	935	24	19
Fluoroquinolones	91	49	21	3	521	182	11	7	124	67	5	3	70	1	0	3
Combinations	592	642	703	669	1,905	2,145	2,211	3,075	291	351	423	380	105	752	94	70
Pleuromutilins c)	504	497	946	987	15,109	18,234	19,759	24,811	6,991	7,517	8,478	10,177	499	0	641	986
Intramammaries	8	6	4	2	1	1	1	0	1	1	<1	1	1	0	0	<1
Gynecologic (local)	<1	<1	0	<1	<1	<1	0	0	0	0	0	0	0	615	0	0
Total	6,821	7,531	8,498	8,958	160,615	159,264	160,590	191,295	37,424	39,257	44,717	49,956	4,125	3,869	4,049	4,749

a) Consumption in veterinary practice comprises an estimated 1-2% (not included)
b) β-lactamase sensitive penicillins
c) Mainly tiamulin

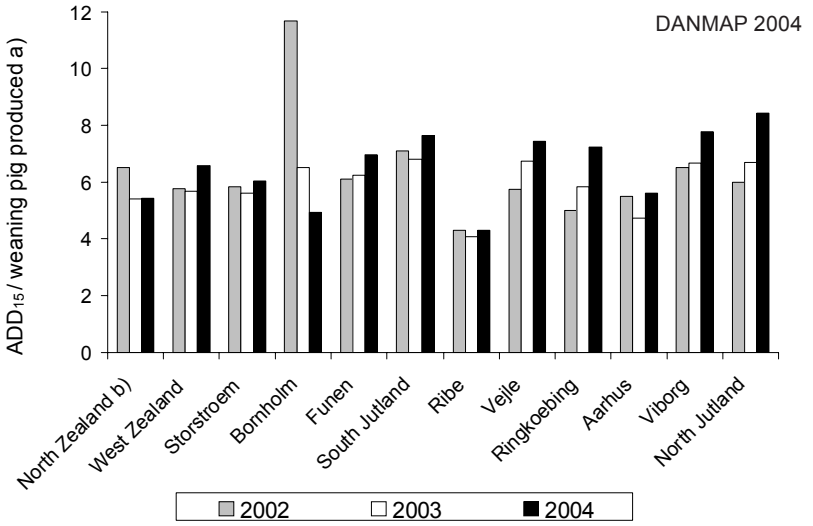

Figure 4. Antimicrobial consumption in weaning pig herds by county from 2002-2004, Denmark
a) ADD_{15} = ADD for 15 kg pig
b) North Zealand comprises Frederiksborg and Roskilde Counties

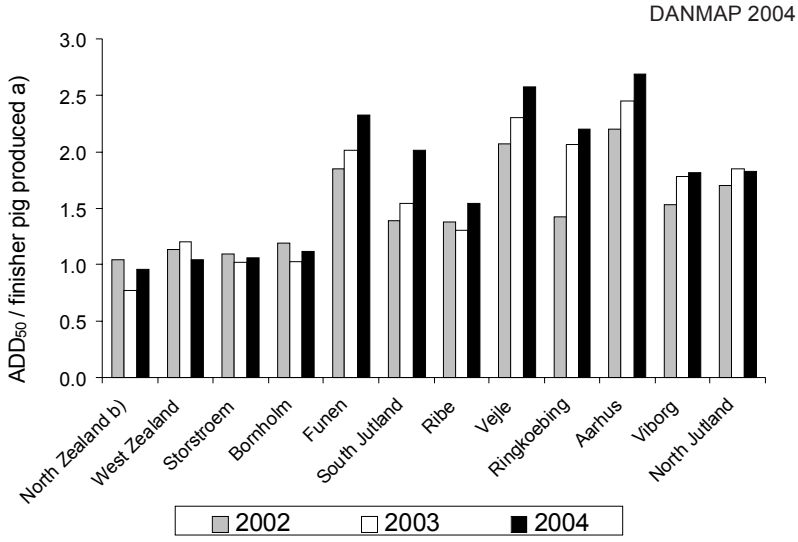

Figure 5. Antimicrobial consumption in finisher herds by county from 2002-2004, Denmark
a) ADD_{50} = ADD for 50 kg pig
b) North Zealand comprises Frederiksborg and Roskilde Counties

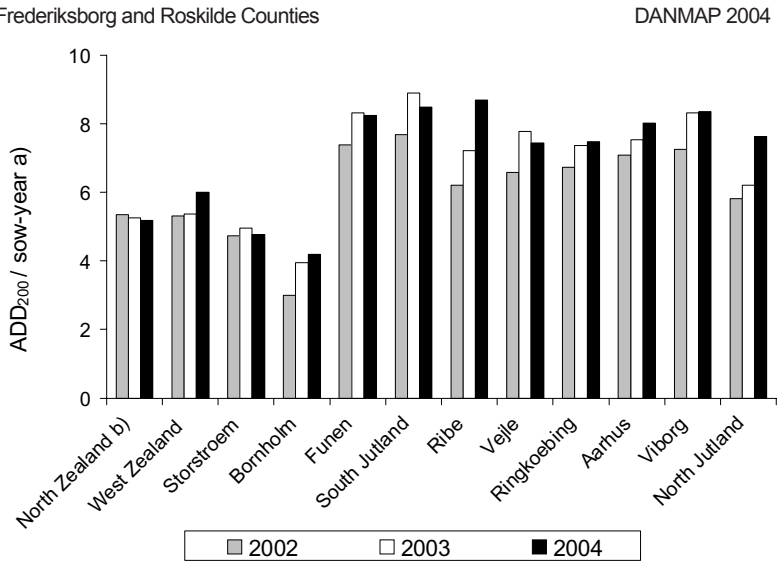

Figure 6. Antimicrobial consumption in sow herds by county from 2002-2004, Denmark
a) ADD_{200} = ADD for 200 kg pig
b) North Zealand comprises Frederiksborg and Roskilde Counties

trimethoprim observed from 2003 to 2004 was a continuation of the trends from 2002 to 2003. The consumption of fluoroquinolones in pigs decreased further to 7 kg in 2004, as compared to 94 kg in 2001. This decrease was most likely due to the legal restrictions in consumption of fluoroquinolones in food animals, implemented in 2002.

Table 6 shows the trends in antimicrobial consumption in pigs by age group from 2001 to 2004. The increase in antimicrobial consumption from 2002 to 2003 was mainly observed in finishers (14%) and in sows/piglets (12%). From 2003 to 2004, the increase was mainly observed in weaners (19%) and less in finishers (12%) and sows/piglets (5%). Calculating ADD per pig produced the consumption in weaners was 7.1 ADD/pig[1] and in finishers 2.0 ADD/pig in 2004, as compared to 6.2 ADD/pig[1] in weaners and 1.8 ADD/pig in finishers in 2003.

Dividing the antimicrobial consumption by indication groups, showed that prescriptions for treatment of respiratory diseases in weaners, increased by 29% from 2003 to 2004. Respiratory disease accounted for 12% of the total antimicrobial consumption in 2004. Penicillins were most frequently prescribed followed by tetracyclines, macrolides and tiamulin.

In weaners, prescriptions for gastrointestinal (GI) diseases accounted for 78% of the antimicrobial consumption in 2004. Prescriptions for GI diseases increased by 17%, as compared to 2003, and accounts for 72% of the total increase in antimicrobial consumption in weaners in 2004. For treatment of GI diseases, macrolides were most frequently prescribed and to a lesser extent tetracyclines, tiamulin, and lincosamide. The increases in prescriptions for GI and respiratory diseases were nationwide. The steep decrease in antimicrobial consumption in weaners on Bornholm was due to cease of routine use of in-feed medication by two veterinarians in 2003 (Figure 4).

In finishers, GI diseases, respiratory diseases and disease of the skin/locomotor system accounted for 60%, 20% and 17%, respectively, of the antimicrobial consumption in 2004. The increase from 44.7 mill. ADDs in 2003 to 50.0 mill. ADDs in 2004 (Table 6) was distributed almost equally in these three disease groups.

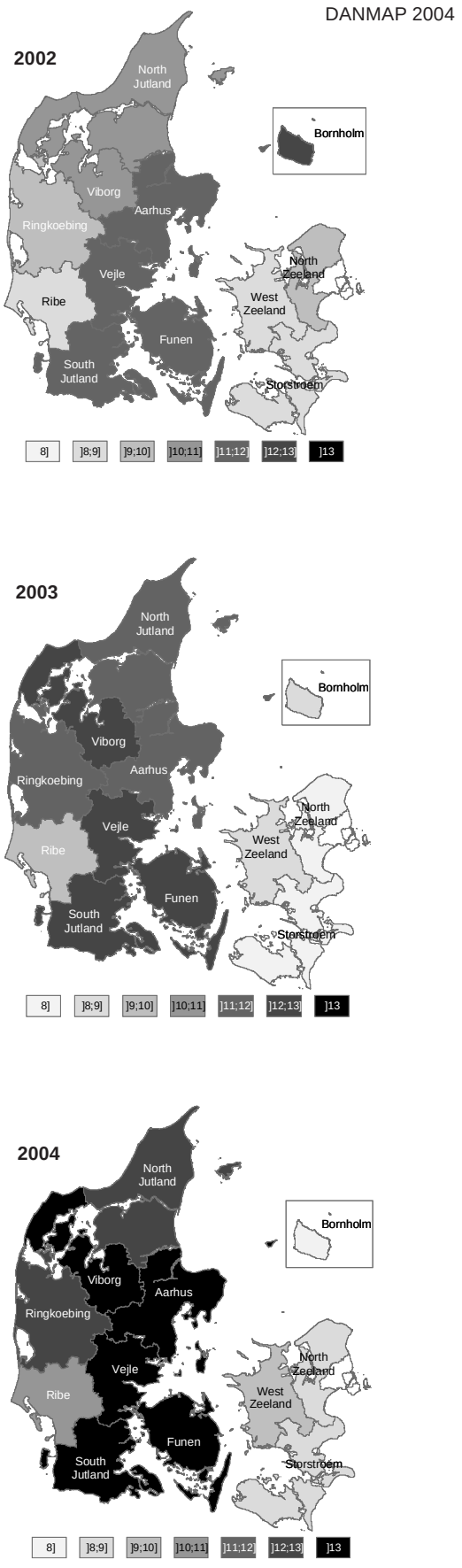

Figure 7. Antimicrobial consumption (ADD_{kg}) per kg-live-pig by county from 2002 to 2004, Denmark

Number of pigs per county (source: Statistics Denmark, 2005)
Estimated kg-live-pig assuming average live weights at treatment.
weaners 15 kg; finishers 50 kg; sows (incl. piglets) 200 kg.
North Zealand comprises Frederiksborg and Roskilde Counties.

[1] The calculation includes weaners produced for export.

In sow herds, 31% of antimicrobials were prescribed for diseases in the limbs, joints, CNS or skin in 2004. Twenty-five percent were prescribed for disease in the reproductive organs, and 8% were prescribed for respiratory disease.

Large regional differences were observed in antimicrobial consumption (Figure 5-7). In the western regions of Denmark an almost 50% higher consumption of antimicrobials in sows/piglets and finishers was seen, as compared to the eastern regions.

A number of factors may have caused the increase in antimicrobial consumption in pigs. The use of water medication has increased more than feed medication the past years (Table 5). Water medication has been recommended because diseased pigs in a pen are more likely to be sufficiently medicated by use of water medication compared to feed medication. Individual water pipes often supply more pens than individual feed pipes, therefore it is more likely that healthy pigs are medicated when using water medication compared to feed medication.

The prevalence of Post-weaning Multisystemic Wasting Syndrome (PMWS) has been increasing in the Danish pig population. The first 5 cases were diagnosed in 2001, and in 2002-2003 outbreaks were seen in all parts of Jutland and Funen (68 and 276 diagnosed cases in 2002 and 2003, respectively) and 3 cases in Zealand. The overall increase in antimicrobrial consumption in 2002 to 2003 was restricted to the western part of Denmark, the same regions with many farms affected by PMWS. This led to the theory that PMWS could be the reason for the increase in antimicrobial consumption in 2003, due to empirical treatments or because of secondary infections. In 2005, the Danish Institute of Food and Veterinary Research initiated a research project to investigate the relative importance of PMWS on antimicrobial consumption. However, PMWS alone cannot explain the regional difference seen in 2002 (Figure 6 and Figure 7). Nor can the occurrence of PMWS explain the increase in prescriptions for locomotor/skin diseases in finishers and sow herds from 2002-2004.

The increase in prescriptions for diseases in the locomotor system was nationwide. This may reflect increased attention to diseases compromising animal welfare due to introduction of higher penalties for delivering sows with severe shoulder ulcers for slaughter.

It is often claimed that the antimicrobial growth promoters (AGPs) withdrawal in 1998 and 1999 has resulted in the observed increase in the consumption of prescribed antimicrobials. However, AGPs were withdrawn more than 5 years ago and the effect would have been seen earlier. Large regional differences of antimicrobial consumption are observed suggesting that the termination of AGPs is not the cause of localised increased antimicrobial consumption. In addition, the increase in water medication and the possible significance of PMWS are all factors supporting the assumption that the increase in antimicrobial consumption is not related to the termination of AGPs .

To reduce the ongoing increase in antimicrobial use in pigs the Danish Veterinary and Food Administration in collaboration with the Danish Institute for Food and Veterinary Research, Danish Bacon and Meat Council and Danish Veterinary Association has initiated a three-tiered strategy directed towards veterinary swine practitioners and pig producers:

1) Revise the treatment guidelines for veterinary practitioners
2) Audit and a one-year follow up of antimicrobial usage for the veterinary swine practitioners using most antimicrobial per pig
3) An investigation of production facilities, management and prevalence of diseases in pig herds with a high antimicrobial consumption, in order to find potential causes for the high consumption

Antimicrobial consumption in cattle

In 2004, the antimicrobial consumption in cattle was estimated at 11 tonnes or 10% of the total consumption in food animals. The vast majority (78% in kg active compound) of the antimicrobials were used in cows, mainly dairy cattle, i.e. for diseases related to milk production. Therefore, only the consumption in calves and heifers should be compared directly with the consumption in other meat producing animal species. The consumption in calves, steers and heifers was 33 mg antimicrobial/kg meat.

From 2003 to 2004, antimicrobial consumption in cattle did not change (Table 7). The consumption in ADD_{kg} in 2004 was approximately 88% in cows, 10% in calves, and 2% in heifers/steers.

From 2003 to 2004, the production of veal decreased by 10%, and the number of fattening calves slaughtered or exported decreased by 13%. During the same period the total antimicrobial consumption in

calves did not change significantly. The consumption of tetracyclines increased 5% from 2003 to 2004. Tetracyclines are now used in 24% of the treatments in calves.

The use of fluoroquinolones decreased significantly from 2001 to 2004, due to the legal restriction imposed from 2002. However, from 2003 to 2004, the fluoroquinolone consumption in calves almost doubled but remained at a very low level. Fluoroquinolones were not used in cows and heifers in 2004.

Consumption of prescribed antimicrobials in poultry

In 2004, 400 kg active compound or 0.4% of the total veterinary antimicrobial consumption was prescribed for poultry production.

The consumption of prescribed antimicrobials in broilers increased by 20% from 2003 to 2004. Despite this increase, consumption remained at a very low level (0.4 mg/kg meat produced).

The predominant antibacterial used in broilers was amoxicillin, accounting for 86% of all ADDs in 2004 (Table 8). The use of fluoroquinolones decreased by 10% and fluoroquinolones were prescribed in 7% of treatments in broilers in 2004.

From 2003 to 2004, the antibacterial use in rearing hens (for both eggs and broiler production) more than doubled. The use of fluoroquinolones increased three-fold (Table 8) despite legal restrictions on fluoroquinolone use. According to the practitioners, fluoroquinolones were prescribed primarily for intestinal infections and septicaemia caused by multiresistant *E. coli.*

Table 7. Consumption of antimicrobials in cattle given as Defined Animal Daily Doses (ADDs) 2002 to 2004, Denmark

DANMAP 2004

		Pharmacies and feed mills											
Age group		Cows, bulls			Calves <12 months			Heifers, steers >12 months			Age not given		
Animal standard weight		600 kg			100 kg			300 kg			600 kg		
		2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
ATC_{vet} group	Therapeutic group	ADD (1,000s)											
QJ01A	Tetracyclines	3	4	6	348	394	414	6	3	3	2	4	4
QJ01B	Amphenicols	<1	<1	<1	20	30	38	<1	<1	<1	<1	<1	<1
QJ01CE	Penicillin, β-lact. sen. b)	5	7	15	210	184	151	2	1	1	<1	3	2
QJ01CA/CR	Penicillins, other	3	3	2	121	188	186	1	1	2	<1	4	1
QJ01DA	Cephalosporins	<1	1	2	15	24	20	<1	<1	<1	<1	<1	<1
QJ01E	Sulfonamides/trimeth.	1	3	3	74	89	127	<1	<1	<1	<1	2	1
QJ01FA	Macrolides	3	4	5	30	39	29	<1	<1	<1	0	3	1
QJ01FF	Lincosamides	<1	1	<1	21	16	14	<1	<1	<1	<1	<1	<1
QJ01G/QA07AA	Aminoglycosides	<1	1	<1	51	66	62	<1	<1	0	<1	2	<1
QA07AA10	Colistin (local GI)	0	<1	0	3	5	4	0	0	0	0	<1	<1
QJ01MA	Fluoroquinolones	<1	<1	0	10	2	3	0	0	0	<1	<1	0
QJ01R	Combinations	<1	1	1	122	118	92	<1	<1	<1	<1	1	<1
QJ01X	Other antimicrobials	<1	<1	1	<1	3	1	<1	<1	<1	<1	3	2
QJ51	Intramammaries	22	30	49	<1	<1	1	<1	<1	<1	0	0	0
QG01AA	Gynecologic (local)	<1	<1	<1	0	0	0	0	0	0	0	0	0
Total		40	55	85	1,027	1,158	1,142	12	7	9	7	24	14

		Veterinary practice a)					
Age group		Cows, bulls		Calves <12 months		Heifers, steers >12 months	
Animal standard weight		600 kg		100 kg		300 kg	
		2003	2004	2003	2004	2003	2004
ATC_{vet} group	Therapeutic group	ADD (1,000s)					
QJ01A	Tetracyclines	124	160	137	145	10	14
QJ01B	Amphenicols	<1	<1	10	13	<1	<1
QJ01CE	Penicillin, β-lact. sen. b)	369	456	52	48	18	26
QJ01CA/CR	Penicillins, other	69	90	59	70	4	6
QJ01DA	Cephalosporins	29	56	13	17	2	4
QJ01E	Sulfonamides/trimeth.	51	68	36	51	2	3
QJ01FA	Macrolides	69	96	12	17	4	5
QJ01FF	Lincosamides	1	3	7	12	<1	<1
QJ01G/QA07AA	Aminoglycosides	2	3	25	41	<1	<1
QA07AA10	Colistin (local GI)	<1	0	1	2	0	0
QJ01MA	Fluoroquinolones	<1	<1	2	4	0	<1
QJ01R	Combinations	13	18	45	44	2	5
QJ01X	Other antimicrobials	<1	1	<1	0	0	0
QJ51	Intramammaries	885	1,091	0	0	11	14
QG01AA	Gynecologic (local)	54	119	0	0	2	4
Total		1667a)	2,162	400	465	55	82

a) Data from veterinary practice is shown separately because the use in cattle practice is underreported by an estimated 20% in 2003 and 0-5% in 2004

b) β-lactamase sensitive penicillins

The production of turkeys (slaughter and export) decreased by 50% from 2002 to 2003, but returned to the 2002 level in 2004. The antibacterial consumption in turkeys decreased in 2003 and 2004 to a very low level (5 ADD_{kg}/turkey) compared to 2002 (26 ADD_{kg}/turkey) (Table 9). The consumption of fluoroquinolones increased from 0.01 ADD_{kg} in 2002 to 1.3 ADD_{kg} per turkey produced. According to the veterinary practitioners the fluoroquinolones were mainly prescribed for intestinal infections caused by resistant *E. coli.* In 2005, the Danish Food and Veterinary Administration will in cooperation with veterinary practitioners specialised in poultry production look into the causes for the increase in fluoroquinolone consumption in turkeys and rearing hens.

As in previous years, the consumption of antimicrobials prescribed for game birds was high compared to the size of the production with an estimated consumption of 1.1 ADD_{kg}/bird (est. 1 mill. pheasants, 0.5 mill. ducks and 0.1 mill. other birds).

Antimicrobial consumption in fish, mink and companion animals

The production of farmed fish decreased by 13% from 2002 to 2004 (Table 1). The antimicrobial consumption in farmed fish was almost halved from 4 tonnes in 2002 to 2.4 tonnes in 2004, which corresponds to a decrease from 105 to 70 mg antimicrobial/kg meat produced. The high consumption in 2002 was likely due to an unusually warm summer, whereas the decline in 2004 was most likely due to an unusually cold summer with low water temperatures. In particular, the consumption of simple quinolones in aquaculture was reduced almost four-fold from 1,157 kg in 2002 to 310 kg in 2004, leaving sulfadiazin/trimethoprim as the predominant drug used in farmed fish.

The antimicrobial consumption in mink production increased by 40% as estimated from pharmacy and feed mill figures. However, the amount used in veterinary practice is unknown.

Table 8. Consumption of prescribed antimicrobials in domestic fowl a) given as Defined Animal Daily Doses (ADDs), Denmark

DANMAP 2004

Age group/production type		Broilers			Layers			Rearing hens			Production type not given b)		
Animal standard weight		1 kg			1 kg			1 kg			1 kg		
		2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
ATC_{vet} code	Therapeutic group	ADD (1,000s)											
QA07AA	Aminoglycosides	0	133	233	0	0	0	0	67	67	0	0	0
QJ01A	Tetracyclines	10	64	4	4	7	4	276	122	44	93	10	56
QJ01CA	Amoxicillin	4,400	3,471	4,198	521	463	216	2,883	2,521	6,575	900	379	1,515
QJ01E/QP51	Sulfonamides c)	94	23	86	65	6	3	485	574	414	120	65	125
QJ01FA	Macrolides	36	6	4	13	11	1	237	150	79	40	19	6
QJ01FF	Lincosamides	<1	0	22	<1	0	0	40	0	2	2	0	0
QJ01MA	Fluoroquinolones	552	390	350	490	140	80	430	191	620	160	20	51
QJ01X	Tiamulin	3	3	0	0	0	0	5	0	3	0	3	0
Total		5,094	4,089	4,898	1,093	627	304	4,356	3,624	7,803	1,315	496	1,753

Includes data from all sources (pharmacies, feedmills and veterinary practice)
a) *Gallinus domesticus*
b) May include any type of poultry
c) Includes sulfaclozin, a coccidiostat/antibcaterial on prescription

Table 9. Consumption of antimicrobials in other poultry a) given as Defined Animal Daily Doses (ADDs), Denmark

DANMAP 2004

Age group/production type		Turkeys			Ducks, geese			Game birds		
Animal standard weight		1 kg			1 kg			1 kg		
		2002	2003	2004	2002	2003	2004	2002	2003	2004
ATC_{vet} code	Therapeutic group	ADD (1,000s)								
QA07AA	Aminoglycosides	0	0	8	0	0	0	0	100	133
QJ01A	Tetracyclines	0	0	0	6	154	14	99	92	124
QJ01CA	Amoxicillin	26,833	10,900	3,608	50	250	100	896	838	953
QJ01E/QP51	Sulfonamides b)	0	58	36	0	0	0	319	306	425
QJ01FA	Macrolides	0	6	13	25	0	11	246	260	89
QJ01FF	Lincosamides	0	0	0	0	0	0	50	0	0
QJ01MA	Fluoroquinolones	10	390	1,335	0	0	150	0	<1	30
QJ01X	Tiamulin	0	0	0	0	0	3	10	10	18
Total		26,843	11,354	5,001	81	404	278	1,634	1,606	1,772

Includes data from all source (pharmacies, feedmills and veterinary practice)
a) Poultry other than domestic fowl (*Gallinus domesticus*)
b) Includes sulfaclozin, a coccidiostat/antibcaterial on prescription

Report 1

Large consumption in companion animals of antimicrobials regarded as critically important in human medicine

The VetStat programme provides a comprehensive and detailed registration of use of medicines for animals in Denmark, including prescription medicines used in companion animals. So far, use of antimicrobials in companion animals has received little attention, as monitoring programmes have focused on food animals only.
Data generated by the VetStat programme in 2003 document, that for some antimicrobials the consumption in companion animals is substantial compared to the consumption in food animals [Heuer O. E., Jensen V. F. and Hammerum A. M. Emerg. Infect. Dis. 2005; 11:344-5]. These antimicrobials include fluoroquinolones and cephalosporins, which are ranked by the US Federal Drug Administration (FDA) as critically important for use in human medicine, and for which emergence of resistant bacteria is especially undesirable [FDA Guidance (152) Federal Register 2003; 68:61221]. Considering the shared environment of humans and companion animals, and the importance of these antimicrobials in human medicine, emergence of antimicrobial resistance in companion animals is a matter of concern.

Reports on transfer of resistant bacteria or mobile resistance determinants from companion animals to humans are scarce, however occurrence of the same resistance genes in companion animals and in humans, as well as possible transfer of bacteria between companion animals and humans have been reported [Butaye *et al.*, Antimicrob. Agents Chemother. 2001. 45:1374-8; Lanz *et al.*, Vet Microbiol. 2003. 91:73-84; Simjee *et al.*, J. Clin. Microbiol. 2002. 40:4659-65].

Companion animal owners and their families are presumably in close daily contact with their animals, providing possibility for transfer of bacteria between companion animals and humans. Twenty percent of families in Denmark own dogs (550,000 dogs) and 16 percent of families own cats (650,000 cats) [Statistics Denmark (www.dst.dk)]. Due to legal restrictions on the usage of fluoroquinolones in food animals, the total consumption of fluoroquinolones in animals (companion and food animals) in Denmark was reduced from 183 kg in 2001 to 53 kg in 2003. However, fluoroquinolones remain widely used for companion animals, even though emergence of fluoroquinolone resistance in bacteria is especially undesirable and regarded a human health hazard. In 2003, almost half (24 kg) of the total consumption of fluoroquinolones in animals (53 kg) was used in companion animals. The total consumption of cephalosporins in animals (companion and food animals) in Denmark in 2003 was 461 kg, of which more than half (254 kg) was used in companion animals. (Data based on reporting on use in veterinary practice and sales from pharmacies on prescription).

Thus, a comparatively small number of companion animals (550,000 dogs and 650,000 cats) consume approximately the same amount of fluoroquinolones and cephalosporins as consumed annually in food animals in Denmark (24 million slaughter pigs, 130 million broiler chicken and 1.2 million cattle and dairy cows) [Statistics Denmark (www.dst.dk)]. We have no reason to believe that antimicrobials are more generously prescribed for companion animals in Denmark than in other industrialised countries. Rather, these consumption data reflect the apparent contrast between policies of antimicrobial use in food animals and companion animals.

The animal reservoir of resistant bacteria extends to companion animals for which a high consumption of critically important antimicrobials, including fluoroquinolones and cephalosporins, have been documented in the VetStat programme. The use of these antimicrobials is avoided or restricted in food animals in order to minimize spread of resistance, while in companion animals prescription continues unimpeded. This may create undesirable antimicrobial resistance that may subsequently spread to humans from the previously neglected reservoir in companion animals.

Ole E. Heuer, Vibeke F. Jensen and Anette M. Hammerum; Emerg. Infect. Dis. 2005. 11:344-5.

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The total consumption of cephalosporins increased from 302 kg in 2001 to 513 kg in 2004. The main reason is an increased consumption in companion animals for which 282 kg was used in 2004. A relatively large part of the antimicrobials prescribed for companion animals are drugs that are considered critically important in human medicine (see Report 1).

Antimicrobial growth promoters

After 1999, antimicrobials for growth promotion in Denmark include only those agents approved by the EU as feed additives, currently avilamycin, flavomycin, and the ionophores salinomycin and monensin. Following the official ban on the growth promoter virginiamycin in January 1998, the Danish food animal industries decided to voluntarily discontinue all further use of antimicrobial growth promoters (AGPs). This became effective in broilers, slaughter pigs and cattle by early 1998. The use of AGPs was phased out in weaning pigs during 1999 (Figure 2).

In recent years, use of AGPs has included very small quantities of flavomycin, avilamycin and monensin, which are among the four AGPs remaining approved by the EU until 2006 (Table 10). AGPs sold in 2002-2003, were sold to an exporting feed mill and to a single pig farm. In 2004, only small amounts of monensin were used on this particular farm. The farm is presumably exporting weaned pigs to other European countries and thus avoiding the industries code of conduct.

Coccidiostats

Antimicrobials used as coccidiostats in poultry feed must have EU approval as feed additives.

Table 11 shows consumption of coccidiostats in poultry production. Almost all of the coccidiostats used belonged to the ionophore group of compounds. The consumption of coccidiostats has decreased since 1999 but increased by 19% from 2003 to 2004. The reason for this increase is not known but the consumption of coccidiostats is considerably below the level in 1996, before the ban of growth promoters. When taking the size of the poultry production into account, the consumption of ionophores in 2004 is still lower than the consumption before the ban of growth promoters.

Table 10. Consumption of antimicrobial growth promoters (kg active compound), Denmark a) DANMAP 2004

Antimicrobial group	Growth promoter	1990	1992	1994	1996	1998	1999	2000	2001	2002	2003	2004
Bacitracin	Bacitracin	3,983	5,657	13,689	8,399	3,945	63	n b)	0	0	0	0
Flavofosfolipols	Flavomycin	494	1,299	77	18	6	665	n	11 c)	15 c)	4 c)	0
Glycopeptides	Avoparcin	13,718	17,210	24,117	0	0	0	n	0	0	0	0
Ionophores	Monensin	2,381	3,700	4,755	4,741	935	0	n	0	0	0	5 c)
	Salinomycin	12	0	213	759	113	0	n	0	0	0	0
Macrolides	Spiramycin	0	0	95	15	0.3	0	n	0	0	0	0
	Tylosin	42,632	26,980	37,111	68,350	13,148	1,827	n	0	0	0	0
Oligosaccharides	Avilamycin	10	853	433	2,740	7	91	n	3 c)	0	0	0
Quinoxalines	Carbadox	850	7,221	10,012	1,985	1,803	293	n	0	0	0	0
	Olaquindox	11,391	21,193	22,483	13,486	28,445	9,344	n	0	0	0	0
Streptogramins	Virginiamycin	3,837	15,537	2,801	5,055	892	0	n	0	0	0	0
Total		79,308	99,650	115,786	105,548	49,294	12,283	n	14	15	4	5

a) Data from the Danish Plant Directorate until 2002 and VetStat from 2001
b) n = not monitored, assumed to be zero
c) Sold to exporting feed mill companies and three farms (pigs treated are presumably exported for slaughter)

Table 11. Consumption of coccidiostats in poultry (kg active compound), Denmark a) DANMAP 2004

Coccidiostats		1990	1992	1994	1996	1998	1999	2000	2001	2002	2003	2004
Pyranes and hydropyranes (ionophores)	Monensin	0	108	1,016	3,405	3,709	8,664	3,962	1,361	1,159	674	840
	Lasalocid	75	0	5	773	1,677	895	606	872	760	634	243
	Narasin	1,588	5,157	6,370	3,905	3,177	5,806	5,073	2,687	863	264	1,294
	Salinomycin	7,783	10,298	6,018	4,531	7,884	8,812	6,338	12,801	11,213	9,422	10,070
	Narasin/Nicarbazin	0	0	0	0	0	32	20	1	0	35	662
Carbanilides	Nicarbazin	0	0	0	115	36	4	0	0	0	0	0
Triazines	Diclazuril	0	0	18	34	3	1	0	2	5	4	1
Imidazole derivates	Dimetridazol	0	0	0	38	0	106	0	0	0	0	0
	Amprolium/Ethopabate	3,562	2,716	2,342	1,339	275	839	0	13	0	0	0
Others	DOT	0	0	300	0	0	13	0	0	0	0	0
	Halofuginon	0	0	19	8	0	2	0	0	0	0	0
	Robenidin	33	295	858	293	367	85	0	2	41	100	126
	Metichlorpindol/ Methylbenzoate	89	1,503	3,360	4,857	930	155	0	0	0	0	0
	Nifursol	0	395	0	146	234	79	0	0	0	0	0
Total		13,569	20,472	20,306	19,444	18,292	25,493	15,999	17,739	14,043	11,133	13,236

a) Data from the Danish Plant Directorate until 2002 and VetStat from 2001

Antimicrobial residues

The frequency of violations of antibacterial residue limits for finishers (pigs) did not exceed 0.02% from 1987 to 2002, which is extremely low compared to international levels. As a result, the frequency of sampling has been reduced by 85% since 2002.

In the 2004 monitoring programme, 4 positive samples were found among 1,375 targeted samples of sows while no positive samples were found among 2,452 targeted samples of pigs, 437 targeted samples of cattle, 9 sheep, 5 horses, 231 targeted samples of poultry, 51 aquaculture trouts, 210 milk samples, 139 eggs, 23 samples of farmed game nor in 27 samples of honey. The monitoring of other residues in eggs showed very low amounts (less than 3 µg/kg) of coccidiostats in 5 of 139 egg samples. Furthermore, one positive result for the banned antimicrobial substance chloramphenicol was found for the first time in Denmark among 96 targeted samples of chickens.

In 2004, samples taken based on suspicion revealed antimicrobial residues in one heifer and one cow, 5 sows and 7 milk samples. Furthermore, tetracycline was found during inspection at one pig farm and malachite green was found during inspection at one aquaculture trout farm.

Annual reports on monitoring residues in animals and foods are available on the Internet at the homepage of the Danish Veterinary and Food Administration (www.fvst.dk/Foedevare/Foedevarekontrol/Indberetninger_EU/forside.htm).

Further information on the monitoring of residues in Denmark and Europe can be obtained from Senior Scientific Adviser Flemming Kæreby (fk@fvst.dk).

Antimicrobial consumption in humans

Overall

In 2005, the Danish Medicines Agency retrospectively updated data on the consumption of antibacterials in primary health care and in hospitals for 1997-2004. Data on the number of hospital bed-days from the National Board of Health has also been updated and corrected. This update has led to only minor changes in the reported consumption. For hospitals, the consumption in the present report only shows minor increases of less than 4% in average as compared to previous reports. The distribution of antibacterials used in both health care sectors remains unchanged and conclusions from previous years are therefore unchanged.

In 2004, the overall consumption of antibacterials for systemic use (ATC group J01, 2005 definition) in humans in Denmark increased to 30.8 million DDDs or 15.6 DDD/1,000 inhabitant-days representing an increase of 4.1% compared to 2003. To follow overall changes in the consumption of antibacterials and to allow comparison with consumption in animals, total human consumption is presented in kilo-grams (Table 12). In 2004, 44.1 tonnes of antibacterials for systemic use were used in humans in Denmark, representing an increase of 2.7% as compared to 2003.

Primary health care sector

The consumption of antibacterials for systemic use in primary health care is presented in Table 13 as a number of DDDs per 1,000 inhabitant-days, and in Table 14 as a number of treated patients per 1,000 inhabitants. Since 2000, there has been a small but steady annual increase in antibacterial consumption in DDD/1,000 inhabitant-days ranging from 2 to 5%. In 2004, consumption increased by 3.9% as compared to 2003. In 2004, β-lactamase sensitive penicillins still represented 37.0% of the total antimicrobial consumption followed by penicillins with extended spectrum (18.7%) and macrolides (15.9%). These proportions were very similar to previous years.

Figure 8 shows the changes in consumption for selected classes of antibacterials for 1997-2004. Between 2003 and 2004, the total consumption of macrolides increased by 4.7%. This increase was solely due to an increased consumption of roxithromycin, whereas consumption of the other macrolides decreased. The introduction of generic roxithromycin on the Danish market resulted in a 76% reduction in the price per roxithromycin DDD between 2001 and 2004 and could explain the increase in consumption.

Table 12. 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

DANMAP 2004

ATC group a)	Therapeutic group	Year								
		1997	1998	1999	2000	2001	2002	2003	2004	(lowest cal.limit - highest cal.limit) b)
J01AA	Tetracyclines	1,519	1,486	1,383	1,486	1,475	1,501	1,542	1,636	
J01B	Amphenicols	1	1	0	0	1	0	0	0	
J01CA	Penicillins with extended spectrum	5,525	5,477	5,202	5,141	5,385	5,356	5,295	5,346	
J01CE	ß-lactamase sensitive penicillins	18,840	19,969	18,825	19,749	20,730	21,263	21,630	22,230	
J01CF	ß-lactamase resistant penicillins	1,919	2,120	2,425	2,655	3,230	3,738	4,075	4,377	
J01CR	Combinations of penicillins, including ß-lactamase inhibitors	49	56	52	93	146	249	336	480	
J01D	Cephalosporins and related substances	626	614	650	692	739	811	830	894	(436 - 1,352)
J01EA	Trimethoprim and derivatives	245	256	258	262	280	293	307	334	
J01EB	Short-acting sulfonamides	3,503	3,497	3,296	3,142	3,113	3,092	3,064	3,067	
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	350	330	286	291	289	288	273	185	
J01FA	Macrolides	3,093	3,332	3,075	2,962	3,020	3,069	2,889	2,834	(2,810 - 2,857)
J01FF	Lincosamides	25	34	30	29	37	40	45	53	(49 - 56)
J01G	Aminoglycosides	61	35	42	32	30	31	28	31	
J01MA	Fluoroquinolones	384	405	383	344	398	452	611	722	(660 - 784)
J01MB	Other quinolones	15	17	16	0	0	0	0	0	
J01XA	Glycopeptides	25	27	33	37	36	42	43	46	
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70	59	59	58	52	
J01XD	Imidazoles	129	129	142	155	168	179	191	195	
J01XE	Nitrofuran derivatives (nitrofurantoin)	141	144	145	151	155	163	166	171	
J01XX05	Methenamine	2,234	2,132	1,956	1,788	1,637	1,662	1,590	1,473	
J01XX08	Linezolid	0	0	0	0	0	3	4	5	
J01	Antibacterials for systemic use (total) c)	38,760	40,133	38,276	39,081	40,927	42,293	42,979	44,131	(43,836 - 44,677)

cal.limit = calculated limit

a) From the 2005 edition of the ATC classification system

b) When two different DDDs of an antimicrobial existed for different presentations, i.e. oral and parenteral, e.g. for cefuroxime, erythromycin, clindamycin and ciprofloxacin, an average DDD was used. Estimates using the lowest and the highest DDD are given in parentheses

c) Does not include polymyxins

The consumption of fluoroquinolones (primarily ciprofloxacin, J01MA02), which increased from 0.18 to 0.25 DDD/1,000 inhabitant-days between 2002 and 2003, further increased to 0.28 DDD/1,000 inhabitant-days in 2004. Expressed in DDD/1,000 inhabitant-days, fluoroquinolones were the 10th most used antibacterial class in primary health care (Table 13), whereas they were the 7th most used antibacterial class when expressed as a number of patients treated/1,000 inhabitants (Table 14). This difference could be explained by short course treatments, e.g. for urinary tract infections. A very low price per DDD, as well as low resistance as compared to other antibacterial drugs, could be reasons for the continuing increase in consumption of fluoroquinolones. However, this increased consumption has already resulted in increasing resistance to fluoroquinolones, e.g. in *Escherichia coli* isolates from community-acquired urinary tract infections. This increase in resistance related to increasing consumption requires close surveillance and prescribers should again be alerted on the ecological risks of a continued increase in fluoroquinolone consumption.

Table 13. Consumption of antibacterials for systemic use in human primary health care (DDD/1,000 inhabitant-days), Denmark

DANMAP 2004

ATC group a)	Therapeutic group	Year							
		1997	1998	1999	2000	2001	2002	2003	2004
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	0.99	1.04	1.07	1.17
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	2.47	2.51	2.52	2.63
J01CE	ß-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	4.91	5.00	5.07	5.20
J01CF	ß-lactamase resistant penicillins	0.34	0.40	0.48	0.52	0.65	0.77	0.85	0.92
J01CR	Combinations of penicillins, incl. ß-lactamase inhibitors	0.02	0.03	0.02	0.02	0.03	0.04	0.05	0.06
J01D	Cephalosporins and related substances	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.02
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	0.35	0.36	0.38	0.41
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	0.36	0.36	0.36	0.36
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.08	0.04	0.03	0.03	0.04	0.03	0.03	0.00
J01FA	Macrolides	2.03	2.28	2.17	2.02	2.10	2.15	2.13	2.23
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01GB	Aminoglycosides	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.22	0.23	0.20	0.15	0.17	0.18	0.25	0.28
J01XB	Polymyxins	0.03	0.02	0.03	0.03	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
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	0.39	0.41	0.42	0.43
J01XX05	Methenamine	0.46	0.43	0.40	0.36	0.33	0.34	0.32	0.30
J01XX08	Linezolid	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

a) From the 2005 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 14. Consumption of antibacterials for systemic use in human primary health care (No. treated patients/1,000 inhabitants), Denmark

DANMAP 2004

ATC group a)	Therapeutic group	Year					
		1999	2000	2001	2002	2003	2004
J01AA	Tetracyclines	12.1	12.0	11.8	11.5	11.4	11.6
J01CA	Penicillins with extended spectrum	66.7	65.6	69.4	69.0	68.6	70.3
J01CE	ß-lactamase sensitive penicillins	163.9	168.9	173.3	173.0	172.2	170.8
J01CF	ß-lactamase resistant penicillins	14.0	15.6	19.2	23.8	26.3	27.0
J01CR	Combinations of penicillins, incl. ß-lactamase inhibitors	0.6	0.6	0.7	1.0	1.1	1.3
J01D	Cephalosporins and related substances	0.4	0.4	0.4	0.4	0.4	0.5
J01EA	Trimethoprim and derivatives	4.1	4.1	4.2	4.5	4.6	5.0
J01EB	Short-acting sulfonamides	34.4	33.5	33.2	33.0	33.1	33.3
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.8	0.8	0.8	0.7	0.6	0.0
J01FA	Macrolides	73.5	65.7	67.7	66.8	64.1	65.8
J01FF	Lincosamides	0.2	0.2	0.2	0.3	0.3	0.4
J01GB	Aminoglycosides	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
J01XA	Glycopeptides	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
J01XC	Steroid antibacterials (fusidic acid)	0.6	0.5	0.5	0.4	0.3	0.3
J01XE	Nitrofuran derivatives (nitrofurantoin)	5.7	5.8	5.7	6.1	6.2	6.4
J01XX05	Methenamine	0.7	0.6	0.5	0.6	0.5	0.5
J01XX08	Linezolid	-	-	-	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

a) From the 2005 edition of the 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

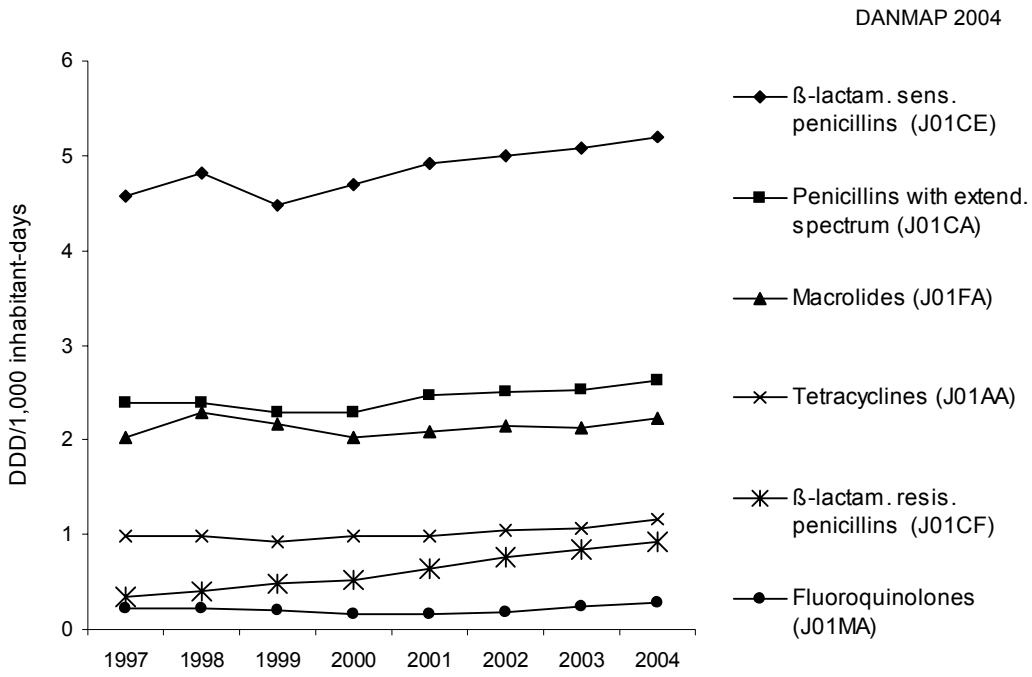

Figure 8. Consumption of selected antibacterials for systemic use in primary health care, Denmark

Table 15. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 occupied bed-days), Denmark. Data represented close to 99% of the total DDDs used in Danish hospitals in 2004. Psychiatric hospitals, private hospitals and seven rehabilitation centers were excluded

DANMAP 2004

ATC group a)	Therapeutic group	Year							
		1997	1998	1999	2000	2001	2002	2003 b)	2004 c)
J01AA	Tetracyclines	3.4	3.3	2.8	2.9	2.8	3.2	3.1	3.4
J01CA	Penicillins with extended spectrum	112.1	113.4	112.7	115.7	116.1	115.2	118.0	116.2
J01CE	ß-lactamase sensitive penicillins	80.2	89.2	95.3	100.3	106.5	114.3	120.0	121.2
J01CF	ß-lactamase resistant penicillins	44.4	45.8	48.3	53.5	60.2	62.8	66.1	68.8
J01CR	Combinations of penicillins, including ß-lactamase inhibitors	0.3	0.4	0.4	0.9	1.7	3.1	4.9	8.4
J01DB	First-generation cephalosporins	1.3	1.0	1.2	1.0	1.2	1.4	1.4	1.7
J01DC	Second-generation cephalosporins	39.9	41.9	44.0	47.4	52.1	58.5	63.3	69.6
J01DD	Third-generation cephalosporins	5.0	5.4	6.4	6.7	6.5	6.5	6.7	6.7
J01DF	Monobactams	0.6	0.1	0.1	0.2	0.1	0.0	0.0	0.1
J01DH	Carbapenems	3.6	2.4	3.2	3.9	4.2	6.0	6.8	8.4
J01EA	Trimethoprim and derivatives	4.2	4.4	3.8	3.7	4.3	4.2	4.4	4.1
J01EB	Short-acting sulfonamides	12.9	13.3	12.9	12.3	12.5	12.4	11.6	10.6
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	4.4	13.9	13.7	14.0	13.4	14.6	15.2	18.1
J01FA	Macrolides	34.5	35.3	33.5	32.8	32.6	32.3	30.6	28.9
J01FF	Lincosamides	1.3	1.8	1.5	1.6	1.7	1.9	1.9	2.3
J01GB	Aminoglycosides	33.8	23.6	27.6	21.3	18.5	17.7	17.3	19.9
J01MA	Fluoroquinolones	14.6	15.5	18.8	23.1	28.4	35.2	39.2	49.1
J01XA	Glycopeptides	2.1	2.3	2.8	3.3	3.2	3.7	4.1	4.6
J01XB	Polymyxins	0.4	0.2	0.3	0.4	0.3	0.3	0.3	0.6
J01XC	Steroid antibacterials (fusidic acid)	2.5	2.5	2.6	2.3	2.0	1.9	2.2	2.2
J01XD	Imidazoles	14.2	14.4	16.2	17.9	19.6	21.1	23.4	24.3
J01XE	Nitrofuran derivatives	3.7	3.5	3.0	2.9	2.9	2.8	2.7	2.8
J01XX05	Methenamine	1.8	1.8	1.6	1.4	1.3	1.2	0.8	1.0
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.7
J01	Antibacterials for systemic use (total)	421.3	435.3	452.9	469.5	492.1	521.0	544.5	573.6

a) From the 2005 edition of the ATC classification system
b) Calculation based on provisional data for number of bed-days
c) Calculated estimate of the number of bed-days in 2004 is based on past trends

Hospitals

The consumption of antibacterials for systemic use in hospitals is presented in Table 15 as a number of DDD per 1,000 occupied bed-days, and in Table 16 as a number of DDD per 1,000 discharged patients. From 1997 to 2003, total consumption in hospitals increased by 29.2% from 421 to 544 DDD/1,000 occupied bed-days. This increase in consumption was due to a 14% increase in the number of DDDs of antibacterials registered by hospital pharmacies (from 2.60 million DDDs in 1997 to 2.97 million DDDs in 2003), while there was a concurrent 10.2% decrease in the total number of hospital bed-days registered in Denmark in the same period. Between 2003 and 2004, hospital

consumption showed another 5.3% increase from 544 to 573 DDD/1,000 bed-days. However, the results for 2004 should be interpreted with caution since they are based on an estimate of the number of occupied bed-days calculated from previous trends and these have recently changed. Between 2002 and 2003, the total number of occupied bed-days suddenly decreased by 4.1%, which is much more than the annual 1% average decrease observed previously.

When expressed as a number of DDDs per 1,000 discharged patients the total consumption in hospitals increased from 2,413 in 1997 to 2,657 in 2004 (Table16) corresponding to a much smaller increase in consumption (10.1%) as when expressed in DDD/ 1,000 bed-days (36.2%). This can be explained by the fact that, while the number of occupied bed-days decreased, the number of discharged patients increased by 7.7% from 1.05 million in 1997 to 1.3 million in 2003. Figure 9 shows the changes in consumption expressed as a number of DDD per 1,000 discharged patients for all classes of antibacterials from 2000 to 2004, using an estimate for the number of discharges in 2004 based on previous trends.

In 2003, the consumption of β-lactamase sensitive and extended spectrum penicillins (J01CE and J01CA) represented 43.7% of the total hospital antibacterial consumption in Denmark. This percentage decreased to 41.4% in 2004. Inversely, the consumption of cephalosporins (J01DB, J01DC, J01DD), fluoroquinolones (J01MA) and carbapenems (J01DH), which represented 17.4% of the hospital antibacterial consumption in 2000, increased to 21.6% in 2003 and then to 23.7% in 2004. Figure 10 illustrates the steady shift towards increasing consumption of newer, broad-spectrum antibacterials in Danish hospitals. Additionally, Figure 11 shows detailed data on this increase by specialty, i.e. intensive care, onco-haematology, surgery, and medicine.

This change in the pattern of antibacterial consumption in hospitals seems to be associated with an increase in resistance to cefuroxime and ciprofloxacin in *Escherichia coli* isolates from blood and urine clinical samples, respectively. A continued increase in the consumption of broad spectrum antibacterials is likely to result in increased resistance in hospitals. This will require close surveillance in the coming years.

Table 16. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 discharged patients), Denmark. Data represents close to 99% of the total DDDs used in Danish hospitals in 2004. Psychiatric hospitals, private hospitals and seven rehabilitation centers were excluded

DANMAP 2004

ATC group a)	Therapeutic group	Year							
		1997	1998	1999	2000	2001	2002	2003	2004 b)
J01AA	Tetracyclines	19.3	18.2	15.2	15.4	14.8	16.3	14.8	15.8
J01CA	Penicillins with extended spectrum	641.8	634.4	608.7	610.5	604.9	578.0	563.2	538.0
J01CE	Beta-lactamase sensitive penicillins	459.3	498.9	514.6	529.1	555.0	573.5	572.8	561.5
J01CF	Beta-lactamase resistant penicillins	254.4	256.3	260.9	282.5	313.7	314.9	315.6	318.7
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	1.8	2.1	2.4	4.9	8.9	15.6	23.5	38.7
J01DB	First-generation cephalosporins	7.4	5.4	6.7	5.2	6.1	7.2	6.8	7.7
J01DC	Second-generation cephalosporins	228.7	234.2	237.7	250.2	271.5	293.6	302.0	322.2
J01DD	Third-generation cephalosporins	28.6	29.9	34.8	35.6	34.0	32.5	31.8	31.1
J01DF	Monobactams	3.3	0.7	0.8	0.9	0.5	0.2	0.2	0.2
J01DH	Carbapenems	20.6	13.5	17.2	20.6	21.9	29.9	32.5	39.0
J01EA	Trimethoprim and derivatives	24.0	24.6	20.7	19.5	22.6	21.0	20.9	19.1
J01EB	Short-acting sulfonamides	73.9	74.4	69.7	64.9	64.9	62.2	55.5	49.2
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	25.1	77.5	74.1	73.6	70.0	73.2	72.7	83.7
J01FA	Macrolides	197.6	197.3	180.8	173.1	170.1	161.9	145.9	134.0
J01FF	Lincosamides	7.6	10.0	8.1	8.5	9.0	9.5	9.0	10.4
J01GB	Aminoglycosides	193.6	131.9	149.0	112.5	96.4	88.6	82.4	92.4
J01MA	Fluoroquinolones	83.4	86.8	101.4	121.8	148.1	176.7	187.0	227.2
J01XA	Glycopeptides	12.3	13.1	15.3	17.2	16.6	18.8	19.7	21.3
J01XB	Polymyxins	2.5	1.4	1.8	2.1	1.5	1.7	1.4	2.7
J01XC	Steroid antibacterials (fusidic acid)	14.4	14.1	14.2	12.1	10.2	9.7	10.5	10.2
J01XD	Imidazoles	81.5	80.6	87.6	94.5	102.0	106.0	111.9	112.7
J01XE	Nitrofuran derivatives	21.3	19.5	16.3	15.5	15.0	14.2	13.0	12.8
J01XX05	Methenamine	10.2	10.3	8.6	7.5	6.7	6.1	3.8	4.6
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	2.2	2.1	3.4
J01	Antibacterials for systemic use (total)	2,412.6	2,435.2	2,446.2	2,477.6	2,564.6	2,613.3	2,599.2	2,656.7

a) From the 2005 edition of the ATC classification system
b) Calculated estimate of the number of discharged patients in 2004 is based on past trends

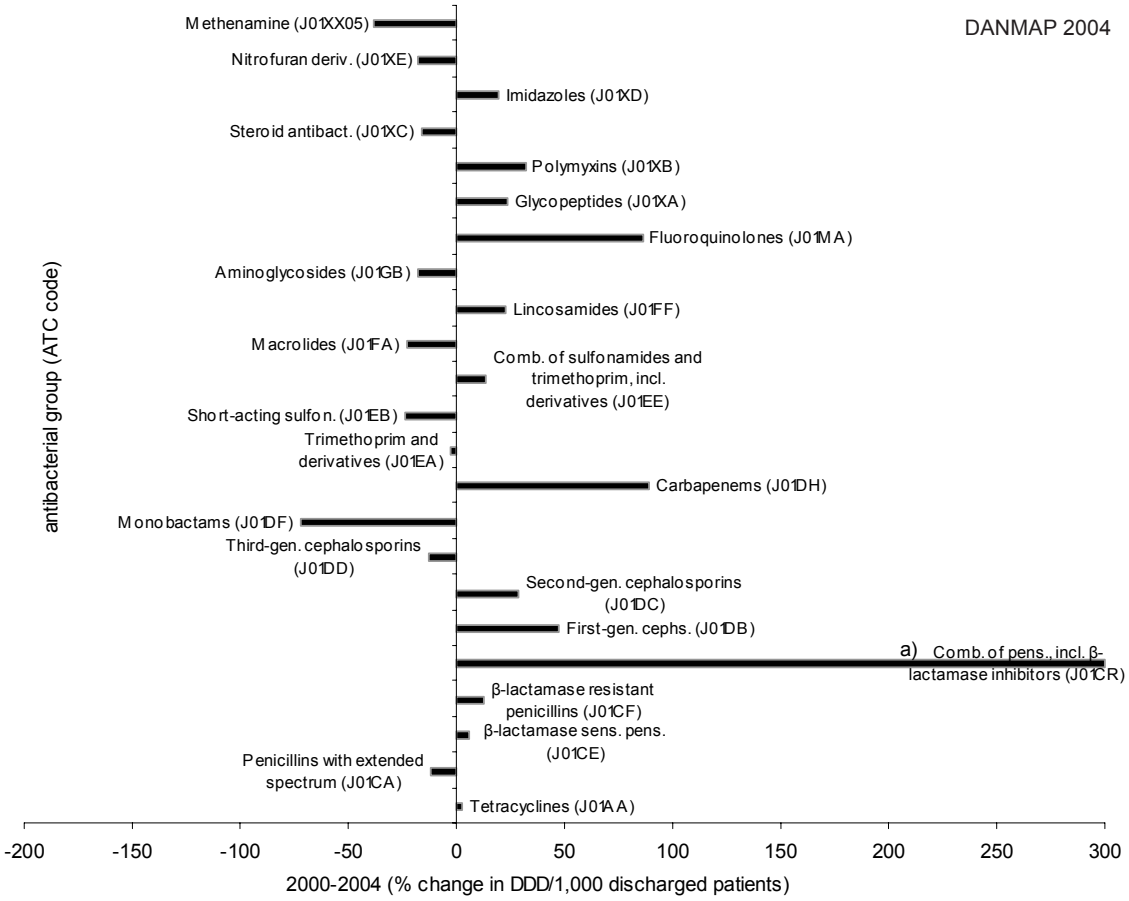

Figure 9. Changes in hospital consumption of antibacterials for systemic use between 2000 and 2004, Denmark
a) The consumption of J01CR increased by 681% from 4.9 to 38.7 DDD/1,000 discharged patients

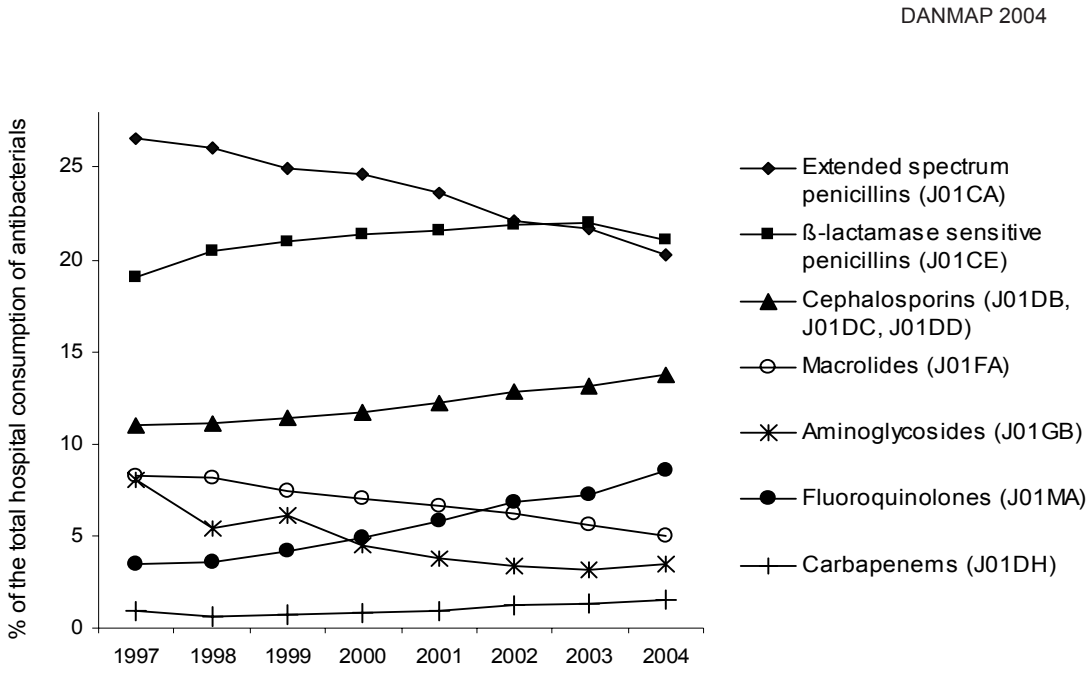

Figure 10. Changes in hospital consumption of selected antibacterials for systemic use, 1997-2004, Denmark

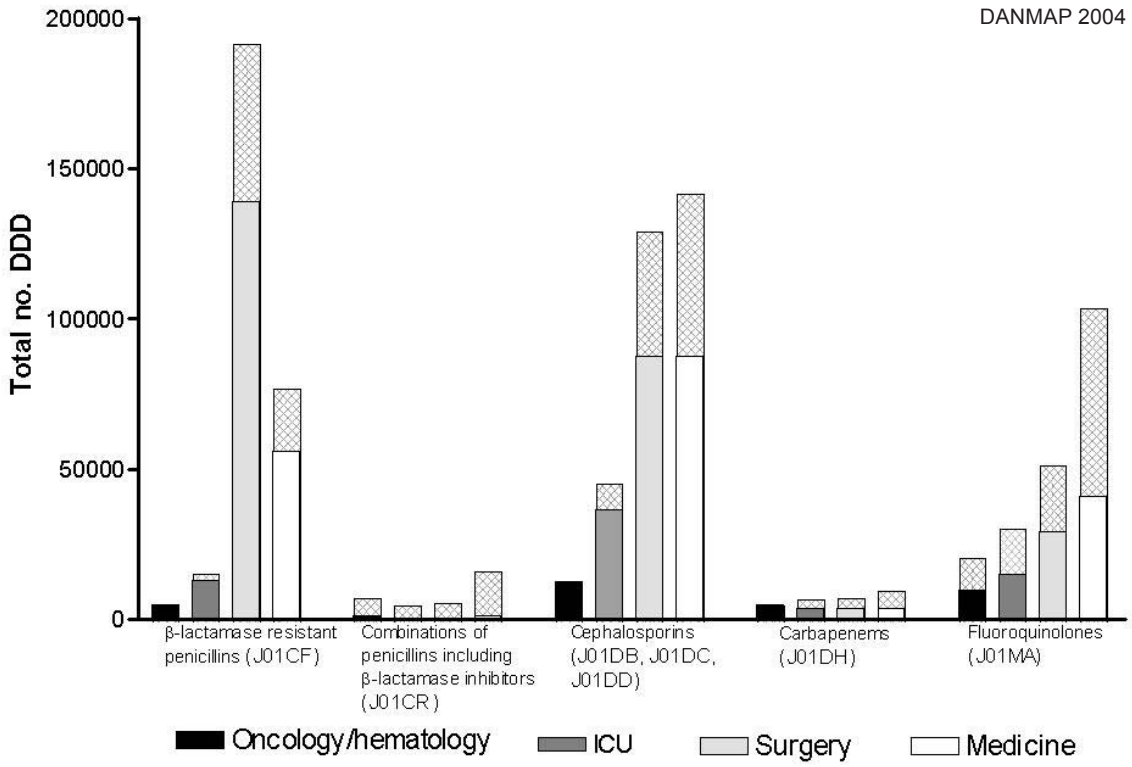

Figure 11. Total no. DDD for 5 selected classes of antibacterials in hospitals by specialty in 2000 and 2004, Denmark. Hatched bars represent 2004 data

Resistance in zoonotic bacteria

Salmonella

Table 17 shows the *Salmonella* serotype distribution of isolates from food animals, foods and humans in 2004. The phage type distributions of *Salmonella* Enteritidis and *Salmonella* Typhimurium are presented in Tables 18 and 19.

Salmonella from food animals

Salmonella isolates from pigs and poultry (broilers and layers) were mainly from subclinical 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 20 shows the MIC distributions and the occurrence of antimicrobial resistance in *S.* Typhimurium from poultry, cattle and pigs in 2004. Only 6 *S.* Enteritidis isolates from poultry were obtained.These isolates were susceptible to all antimicrobials in the test panel and therefore the MIC distributions and the occurrence of resistance are not shown.

From 2003 to 2004, no significant changes in the occurrence of resistance were observed among *S*. Typhimurium from pigs. However, from 1999 to 2004 a significant increase in resistance to tetracycline (P<0.0001) and ampicillin (P<0.0001) has been

Table 17. Distribution (%) of Salmonella *serotypes isolated from food animals, foods and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2004

Serotypes	Poultry a)	Broiler meat b)	Cattle a)	Beef	Pigs a)	Pork c)	Humans
	%	%	%	%	%	%	%
Agona		12			1		1
Derby	3			8	21	9	1
Dublin			55	33			2
Enteritidis	10	40			<1		36
Hadar		2		42			1
Infantis	20	7			5	9	2
Newport							2
Stanley					<1		2
Typhimurium	30	9	37	8	64	44	30
Virchow		1			<1		3
Others including non-typeable	37	29	8	9	8	38	20
Number of isolates	61	153	75	12	1,278	177	1,535

a) Only one isolate per serotype per farm
b) All but four isolates originated from imported meat
c) Only 25 isolates originated from Danish pork

Table 18. Distribution (%) of Salmonella *Enteritidis phage types from broiler meat and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2004

Phage type	Broiler meat a)	Humans b)
	%	%
1	4	15
4	51	25
6	2	7
6a		2
8	3	22
14b		3
21/21b	28	12
Others including non-typeable	12	13
Number of isolates	61	513

a) All but two isolates originated from imported broiler meat
b) Not all isolates selected for susceptibility testing were phage typed

Table 19. Distribution (%) of Salmonella *Typhimurium phage types from food animals, foods and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2004

Phage type	Poultry	Broiler meat a)	Cattle	Pigs	Pork b)	Humans c)
	%	%	%	%	%	%
1				<1		<1
3				<1		
12	24		21	25	6	18
17	6		14	7	1	<1
41	24		4	<1		2
66				3		1
104/104b	6	64	21	9	12	11
110	6			<1		<1
120			4	15	14	15
135	6			1		1
170	12		4	11	1	4
193			4	4	12	4
U302				1	12	4
Others including non-typeable	16	36	28	23	42	39
Number of isolates	18	14	28	814	77	456

a) All isolates originated from imported broiler meat
b) Only 13 isolates originated from Danish pork
c) Not all isolates selected for susceptibility testing were phage typed

Table 20. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from poultry (broilers and layers) (n=18), cattle (n=28), and pigs (n=814), Denmark*

DANMAP 2004

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	17	[3.6-41.4]							77.8	5.6				16.7					
	Cattle	21	[8.3-41.0]							78.6			3.6	3.6	14.3					
	Pigs	40	[36.5-43.4]							55.9	3.8	0.4	0.1	4.7	35.1					
Chloramphenicol	Poultry	6	[0.1-27.3]							11.1	55.6	16.7	11.1			5.6				
	Cattle	14	[4.0-32.7]							7.1	60.7	17.9			7.1	7.1				
	Pigs	9	[7.4-11.5]							0.6	45.8	41.3	2.9	0.2	0.9	8.2				
Florfenicol	Poultry	0	[0.0-18.5]							33.3	55.6	11.1								
	Cattle	7	[0.9-23.5]							14.3	71.4	7.1		3.6	3.6					
	Pigs	5	[3.8-7.0]							1.5	76.4	14.0	2.8	4.1	0.5	0.7				
Ampicillin	Poultry	17	[3.6-41.4]						77.8		5.6				16.7					
	Cattle	32	[15.9-52.4]						57.1	10.7					32.1					
	Pigs	22	[19.4-25.3]						60.3	15.0	2.3	0.1			22.2					
Amoxicillin/ clavulanic acid a)	Poultry	0	[0.0-18.5]							83.3		16.7								
	Cattle	0	[0.0-12.3]							67.9	3.6	21.4	7.1							
	Pigs	0	[0.0-0.5]							77.6	5.3	11.2	5.9							
Cephalothin	Poultry	0	[0.0-18.5]							72.2	5.6	16.7	5.6							
	Cattle	0	[0.0-12.3]							42.9	32.1	25.0								
	Pigs	1	[0.6-2.2]							40.3	40.0	14.6	3.8	1.1	0.1					
Ceftiofur	Poultry	0	[0.0-18.5]					83.3	11.1	5.6										
	Cattle	0	[0.0-12.3]					60.7	35.7	3.6										
	Pigs	0	[0.0-0.5]					34.9	57.5	7.6										
Sulfonamide	Poultry	11	[1.4-34.7]												88.9					11.1
	Cattle	32	[15.9-52.4]												57.1	10.7				32.1
	Pigs	38	[34.4-41.1]												53.6	8.7				37.7
Trimethoprim	Poultry	0	[0.0-18.5]								100									
	Cattle	4	[0.1-18.3]								96.4				3.6					
	Pigs	6	[4.7-8.2]								93.7				6.3					
Apramycin	Poultry	0	[0.0-18.5]								88.9	11.1								
	Cattle	0	[0.0-12.3]								92.9	7.1								
	Pigs	1	[0.7-2.4]								96.6	2.0	0.1			1.4				
Gentamicin	Poultry	0	[0.0-18.5]						94.4	5.6										
	Cattle	0	[0.0-12.3]						96.4	3.6										
	Pigs	1	[0.8-2.6]						97.4	1.1		0.2	0.4	0.7	0.1					
Neomycin	Poultry	6	[0.1-27.3]							94.4					5.6					
	Cattle	0	[0.0-12.3]							92.9	7.1									
	Pigs	8	[6.0-9.8]							89.3	2.6	0.4		0.9	6.9					
Spectinomycin	Poultry	0	[0.0-18.5]										5.6	38.9	55.6		7.7			
	Cattle	32	[15.9-52.4]											60.7	7.1		32.1			
	Pigs	15	[12.4-17.4]										1.4	78.1	5.8	2.0	12.8			
Streptomycin	Poultry	17	[3.6-41.4]									22.2	61.1		5.6	11.1				
	Cattle	39	[21.5-59.4]								3.6	32.1	25.0		10.7	28.6				
	Pigs	37	[34.0-40.8]								1.6	46.3	14.7	2.9	3.8	30.6				
Ciprofloxacin	Poultry	0	[0.0-18.5]	88.9	11.1															
	Cattle	0	[0.0-12.3]	96.4	3.6															
	Pigs	<1	[0.3-1.8]	90.9	8.2	0.7		0.1												
Nalidixic acid	Poultry	0	[0.0-24.7]										100							
	Cattle	0	[0.1-18.3]										100							
	Pigs	<1	[0.03-0.9]										99.0	0.7		0.1	0.1			
Colistin	Poultry	0	[0.0-18.5]								100									
	Cattle	0	[0.0-12.3]								100									
	Pigs	0	[0.0-0.5]								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

observed (Figure 12). This increase coincides with increased tetracycline and broad spectrum penicillin consumption in pigs in the same period. A low number of *S*. Typhimurium isolates were available from poultry and cattle in 2003 and 2004, which makes it difficult to detect differences in the occurrence of resistance from year to year.

Salmonella from foods

In 2004, *Salmonella* isolates from foods were obtained from Danish and imported broiler meat, beef and pork sold at wholesale and retail outlets. A total of 77 *S*. Typhimurium isolates were obtained from pork (13 isolates from Danish pork and 64 isolates from imported pork). In addition, 14 *S*. Typhimurium isolates were obtained from imported broiler meat. The results of the susceptibility testing are shown in Table 21. Fifty-nine *S*. Enteritidis isolates were obtained from imported broiler meat and only two isolates from Danish broiler meat. The results of the susceptibility testing of the 59 isolates from imported broiler meat are shown in Table 22. For further discussion of the occurrence of resistance in food isolates please see "*Salmonella* from animals, foods and humans".

Salmonella in humans

In 2004, 1,538 cases of human salmonellosis occurring in Denmark were reported to the Statens Serum Institut. This represents a decrease in incidence from 32 cases per 100,000 inhabitants in 2003 to 28 cases per 100,000 inhabitants in 2004.This is a continuation of the steady decline apparent since 1997 (EPI-NEWS 2005, no. 9: http://www.ssi.dk/sw26374.asp) and is probably due to the successful implementation of measures to control *Salmonella* in eggs, poultry and pigs. The proportion of *Salmonella* infections reported as acquired abroad was 13%. This is probably an underestimated percentage as information on travel is often missing and some cases reported as domestically acquired may in fact have been acquired abroad. Therefore, comparisons of data between those infections acquired abroad and those acquired domestically should be interpreted with caution.

The distribution of *Salmonella* serotypes is presented in Table 17. Susceptibility testing was performed for 59% of *S.* Enteritidis isolates and for >99% of the *S*. Typhimurium isolates obtained. Tables 23 and 24 present the MIC distributions and occurrence of antimicrobial resistance among *S.* Enteritidis and *S.* Typhimurium from humans by origin of infection in 2004.

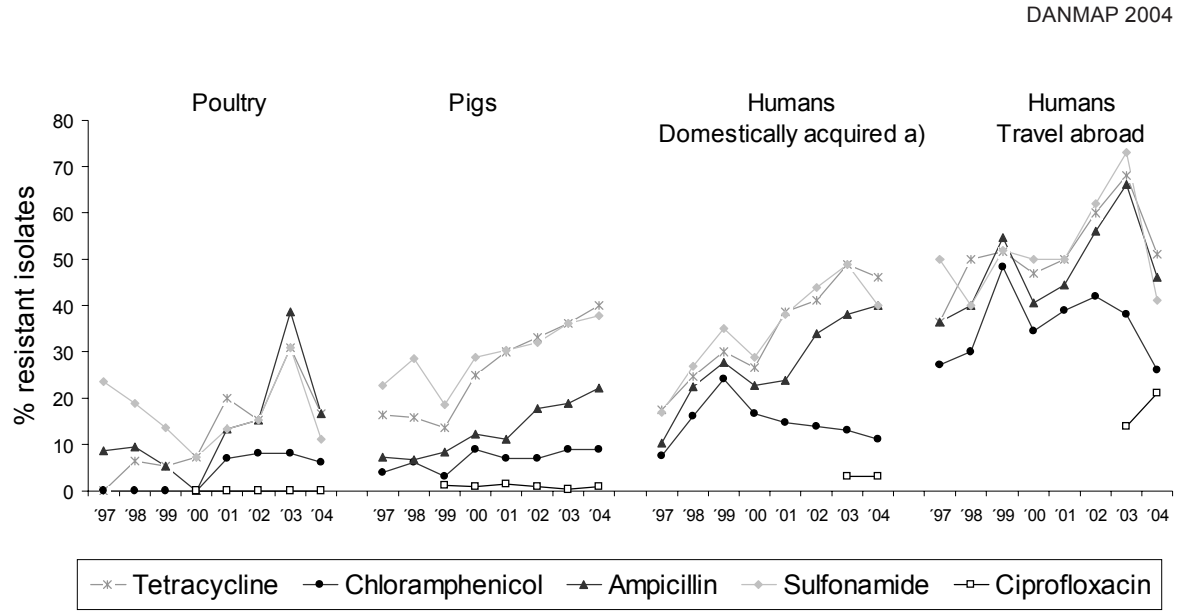

Figure 12. Trends in resistance to selected antimicrobials among Salmonella *Typhimurium isolated from poultry and pigs and from human cases, Denmark*

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

Table 21. Distribution of MICs and occurrence of resistance in Salmonella *Typhimurium from broiler meat (imported n=14) and pork (Danish n=13; imported n=64), Denmark*

DANMAP 2004

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	Broiler meat	Imported	79	[49.2-95.3]							21.4				28.6	50.0					
	Pork	Danish	92	[64.0-99.8]							7.7					92.3					
		Imported	80	[67.8-88.7]							15.6	4.7		1.6	18.8	59.4					
Chloramphenicol	Broiler meat	Imported	43	[17.7-71.1]							7.1	7.1	42.9				42.9				
	Pork	Danish	15	[1.9-45.4]							46.2		38.5				15.4				
		Imported	38	[25.7-50.5]							1.6	29.7	31.3			6.3	31.3				
Florfenicol	Broiler meat	Imported	43	[17.7-71.1]							7.1	7.1	42.9		7.1	35.7					
	Pork	Danish	0	[0.0-24.7]								76.9	7.7	15.4							
		Imported	25	[15.0-37.4]								57.8	6.3	10.9	17.2	3.1	4.7				
Ampicillin	Broiler meat	Imported	79	[49.2-95.3]						14.3	7.1					78.6					
	Pork	Danish	54	[25.1-80.8]						30.8	15.4					53.8					
		Imported	63	[49.5-74.3]						31.3	4.7	1.6			1.6	60.9					
Amoxicillin/ clavulanic acid a)	Broiler meat	Imported	0	[0.0-23.2]							21.4		42.9	35.7							
	Pork	Danish	0	[0.0-24.2]							46.2	30.8	15.4	7.7							
		Imported	2	[0.04-8.4]							37.5	6.3	21.9	32.8		1.6					
Cephalothin	Broiler meat	Imported	0	[0.0-23.2]							7.1	21.4	71.4								
	Pork	Danish	8	[0.2-36.0]							23.1	38.5	23.1	7.7	7.7						
		Imported	2	[0.04-8.4]							18.8	46.9	21.9	10.9			1.6				
Ceftiofur	Broiler meat	Imported	0	[0.0-23.2]					7.1	85.7	7.1										
	Pork	Danish	0	[0.0-24.7]					7.7	92.3											
		Imported	0	[0.0-5.6]					28.1	62.5	9.4										
Sulfonamide	Broiler meat	Imported	79	[49.2-95.3]												21.4					78.6
	Pork	Danish	69	[38.6-90.9]												23.1	7.7				69.2
		Imported	69	[55.9-79.8]												28.1	3.1				68.8
Trimethoprim	Broiler meat	Imported	36	[12.8-64.9]								64.3				35.7					
	Pork	Danish	31	[9.1-61.4]								69.2				30.8					
		Imported	23	[13.8-35.7]								76.6				23.4					
Apramycin	Broiler meat	Imported	0	[0.0-23.2]								100									
	Pork	Danish	8	[0.2-36.0]								92.3					7.7				
		Imported	2	[0.04-8.4]								95.3	3.1				1.6				
Gentamicin	Broiler meat	Imported	0	[0.0-23.2]						100											
	Pork	Danish	8	[0.2-36.0]						92.3				7.7							
		Imported	3	[0.4-10.8]						95.3	1.6			1.6		1.6					
Neomycin	Broiler meat	Imported	36	[12.8-64.9]							64.2				7.1	28.6					
	Pork	Danish	0	[0.0-24.7]							100										
		Imported	6	[1.7-15.2]							93.8				6.3						
Spectinomycin	Broiler meat	Imported	79	[49.2-95.3]											21.4		28.6	50.0			
	Pork	Danish	15	[1.9-45.4]											84.6			15.4			
		Imported	47	[34.3-59.8]											50.0	3.1	3.1	43.8			
Streptomycin	Broiler meat	Imported	86	[57.2-98.2]									7.1	7.1	7.1	35.7	42.9				
	Pork	Danish	46	[19.2-74.9]								7.7	23.1	23.1	7.7		38.5				
		Imported	59	[46.4-71.5]								1.6	26.6	12.5	6.3	12.5	40.6				
Ciprofloxacin	Broiler meat	Imported	50	[23.0-77.0]	14.3	35.7		35.7	14.3												
	Pork	Danish	0	[0.0-24.7]	92.3	7.7															
		Imported	8	[2.6-17.3]	84.4	7.8		7.8													
Nalidixic acid	Broiler meat	Imported	50	[23.0-77.0]									50.0				7.1	42.9			
	Pork	Danish	0	[0.0-24.7]										100							
		Imported	8	[2.6-17.3]										92.2				7.8			
Colistin	Broiler meat	Imported	0	[0.0-23.2]								100									
	Pork	Danish	0	[0.0-24.7]								92.3	7.7								
		Imported	0	[0.0-5.6]								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

Resistance was generally low in domestically acquired *S.* Enteritidis isolates as well as in those acquired abroad, except for ciprofloxacin and nalidixic acid resistance (Tables 23 and 25). In isolates from infections acquired in Denmark and abroad, resistance to ciprofloxacin and nalidixic acid was at the same level as in 2003. However, in 2004 resistance to ciprofloxacin (*P*=0.009) and nalidixic acid (*P*=0.02) was significantly higher in *S.* Enteritidis isolates from infections acquired abroad than from infections acquired in Denmark.

In *S.* Typhimurium isolates, resistance to chloramphenicol (*P*=0.02), florfenicol (*P*=0.008), spectinomycin (*P*=0.002), ciprofloxacin (*P*=0.0001) and nalidixic acid (*P*=0.0001) was significantly higher in cases where the infection was acquired abroad, compared to cases where the infection was acquired in Denmark (Tables 24 and 26). Among *S.* Typhimurium isolates from cases with infection acquired in Denmark, a significant decrease in resistance to sulfonamide (*P*=0.006) and streptomycin (*P*=0.008) was observed in 2004. However, a significant increase (*P*=0.008) in resistance to trimethoprim occurred. In *S.* Typhimurium

Table 22. Distribution of MICs and occurrence of resistance in Salmonella *Enteritidis from broiler meat (imported n=59) a), Denmark*

DANMAP 2004

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	2	[0.04-9.1]							94.9	3.4				1.7					
Chloramphenicol	0	[0.0-6.1]							1.7	79.7	18.6								
Florfenicol	0	[0.0-6.1]							1.7	94.9	3.4								
Ampicillin	8	[2.8-18.7]						40.0	50.9	1.7			6.8	1.7					
Amoxicillin/ clavulanic acid b)	0	[0.0-6.1]							100										
Cephalothin	0	[0.0-6.1]							25.4	71.2	3.4								
Ceftiofur	0	[0.0-6.1]					8.5	88.1	3.4										
Sulfonamide	0	[0.0-6.1]												69.5	30.5				
Trimethoprim	0	[0.0-6.1]								100									
Apramycin	2	[0.04-9.1]								94.9	3.4				1.7				
Gentamicin	2	[0.04-9.1]						98.3			1.7								
Neomycin	0	[0.0-6.1]							100										
Spectinomycin	2	[0.04-9.1]										71.2	27.1			1.7			
Streptomycin	0	[0.0-6.1]								89.8	8.5	1.7							
Ciprofloxacin	58	[44.1-70.4]	42.4		1.7	22.0	33.9												
Nalidixic acid	58	[44.1-70.4]									42.4				1.7	55.9			
Colistin	0	[0.0-6.1]								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) Only two isolates from Danish broiler meat. Results of susceptibility testing is not shown
b) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

Table 23. S. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from human cases acquired domestically (n=262) or associated with travel abroad (n=64), Denmark*

DANMAP 2004

Compound	Origin	% 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	Domestic	2	[0.6-4.4]							97.7	0.4				1.9					
	Travel abroad	5	[1.0-13.1]							95.3					4.7					
Chloramphenicol	Domestic	<1	[0.1-2.7]							0.8	80.2	17.9	0.4		0.4	0.4				
	Travel abroad	0	[0.0-5.6]							1.6	79.7	18.8								
Florfenicol	Domestic	0	[0.0-1.4]							1.5	96.9	1.1	0.4							
	Travel abroad	0	[0.0-5.6]							1.6	98.4									
Ampicillin	Domestic	2	[0.6-4.4]						10.7	85.9	0.8	0.4	0.4		1.9					
	Travel abroad	3	[0.4-10.8]						6.3	90.6					3.1					
Amoxicillin/ clavulanic acid a)	Domestic	<1	[0.0-2.1]							97.3	0.8	1.5			0.4					
	Travel abroad	0	[0.0-5.6]							96.9		3.1								
Cephalothin	Domestic	0	[0.0-1.4]							56.9	39.7	3.4								
	Travel abroad	0	[0.0-5.6]							40.6	54.7	4.7								
Ceftiofur	Domestic	0	[0.0-1.4]					80.2	19.5	0.4										
	Travel abroad	0	[0.0-5.6]					79.7	18.8	1.6										
Sulfonamide	Domestic	<1	[0.1-2.7]												98.1	1.1				0.8
	Travel abroad	2	[0.0-8.4]												98.4					1.6
Trimethoprim	Domestic	<1	[0.0-2.1]								99.2	0.4			0.4					
	Travel abroad	2	[0.0-8.4]								98.4				1.6					
Apramycin	Domestic	0	[0.0-1.4]								100									
	Travel abroad	0	[0.0-5.6]								100									
Gentamicin	Domestic	0	[0.0-1.4]						99.6	0.4										
	Travel abroad	0	[0.0-5.6]						100											
Neomycin	Domestic	<1	[0.0-2.1]							99.6				0.4						
	Travel abroad	2	[0.0-8.4]							98.4					1.6					
Spectinomycin	Domestic	1	[0.2-3.3]										80.9	16.8	1.1	0.8	0.4			
	Travel abroad	3	[0.4-10.8]										84.4	10.9	1.6	3.1				
Streptomycin	Domestic	<1	[0.0-2.1]								96.9	2.7		0.4						
	Travel abroad	2	[0.0-8.4]								95.3	3.1				1.6				
Ciprofloxacin	Domestic	16	[11.5-20.6]	84.0	0.4		9.9	5.7												
	Travel abroad	31	[20.2-44.1]	68.8			14.1	14.1	3.1											
Nalidixic acid	Domestic	16	[11.8-21.0]									83.6	0.4			0.4	15.6			
	Travel abroad	30	[18.9-42.4]									68.8	1.6				29.7			
Colistin	Domestic	0	[0.0-1.4]								100									
	Travel abroad	0	[0.0-5.6]								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 24. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from human cases acquired domestically (n=425) or associated with travel abroad (n=39), Denmark*

DANMAP 2004

Compound	Origin	% 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	Domestic	46	[41.5-51.2]							52.9	0.7		2.1	6.1	38.1					
	Travel abroad	51	[34.8-67.6]							48.7			7.7	7.7	35.9					
Chloramphenicol	Domestic	11	[7.8-13.9]							3.3	65.9	20.2		0.2	1.9	8.5				
	Travel abroad	26	[13.0-42.1]								53.8	20.5		2.6	5.1	17.9				
Florfenicol	Domestic	4	[2.5-6.6]							5.6	82.1	4.5	3.5	3.8	0.2	0.2				
	Travel abroad	15	[5.9-30.5]								76.9	5.1	2.6	12.8	2.6					
Ampicillin	Domestic	40	[35.1-44.6]						41.9	17.6	0.7				39.8					
	Travel abroad	46	[30.1-62.8]						25.6	28.2					46.2					
Amoxicillin/	Domestic	1	[0.4-2.7]							59.3	4.9	21.4	13.2	1.2						
clavulanic acid a)	Travel abroad	3	[0.1-13.5]							53.8		23.1	20.5	2.6						
Cephalothin	Domestic	<1	[0.1-1.7]							49.4	34.8	13.6	1.6	0.2		0.2				
	Travel abroad	0	[0.0-9.0]							38.5	35.9	15.4	10.3							
Ceftiofur	Domestic	0	[0.0-0.9]					84.2	14.8	0.9										
	Travel abroad	0	[0.0-9.0]					71.8	25.6	2.6										
Sulfonamide	Domestic	40	[35.1-44.6]												60.0	0.2				39.8
	Travel abroad	41	[25.6-57.9]												59.0					41.0
Trimethoprim	Domestic	8	[5.2-10.5]								92.5				7.5					
	Travel abroad	8	[1.6-20.9]								92.3				7.7					
Apramycin	Domestic	0	[0.0-0.9]								99.3	0.7								
	Travel abroad	0	[0.0-9.0]								100									
Gentamicin	Domestic	<1	[0.2-2.1]						98.6	0.7			0.2	0.5						
	Travel abroad	5	[0.6-17.3]						92.3	2.6					5.1					
Neomycin	Domestic	2	[0.7-3.4]							98.4				0.7	0.9					
	Travel abroad	5	[0.6-17.3]							92.3	2.6				5.1					
Spectinomycin	Domestic	11	[8.5-14.7]										4.7	81.9	2.1	0.5	10.8			
	Travel abroad	31	[17.0-47.6]											69.2			30.8			
Streptomycin	Domestic	40	[35.3-44.8]								8.9	46.4	4.7		7.8	32.2				
	Travel abroad	44	[27.8-60.4]								2.6	51.3	2.6		17.9	25.6				
Ciprofloxacin	Domestic	3	[1.3-4.6]	96.5	0.9	0.7	0.7	0.2				0.9								
	Travel abroad	21	[9.3-36.5]	79.5		12.8	5.1	2.6												
Nalidixic acid	Domestic	2	[1.1-4.3]									97.4	0.2				2.4			
	Travel abroad	18	[7.5-33.5]									79.5	2.6				17.9			
Colistin	Domestic	0	[0.0-0.9]								100									
	Travel abroad	0	[0.0-9.0]								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 25. Comparison of resistance (%) among Salmonella *Enteritidis from imported broiler meat and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2004

Compound	Broiler meat	Humans	
	Imported %	Domestic a) %	Travel abroad %
Tetracycline	2	2	5
Chloramphenicol	0	<1	0
Florfenicol	0	0	0
Ampicillin	8	2	3
Amoxicillin/clavulanic acid	0	<1	0
Cephalothin	0	0	0
Ceftiofur	0	0	0
Sulfonamide	0	<1	2
Trimethoprim	0	<1	2
Apramycin	2	0	0
Gentamicin	2	0	0
Neomycin	0	<1	2
Spectinomycin	2	1	3
Streptomycin	0	<1	2
Ciprofloxacin	58	16	31
Nalidixic acid	58	16	30
Colistin	0	0	0
Number of isolates	59	262	64

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

isolates from infections acquired abroad, a significant decrease in resistance to sulfonamide (*P*=0.003) and streptomycin (*P*=0.03) was observed in 2004.

Table 27 presents the occurrence of resistance among *S.* Typhimurium isolates other than phage types DT104 and related phage types (DT104b, DTU302) from humans by origin of infection. The proportion of DT104 and related phage types (DT104b, DTU302) among the *S*. Typhimurium isolates was 15% in 2004.

Salmonella from animals, foods and humans

The occurrence of resistance among *S*. Enteritidis isolates from imported broiler meat and human cases acquired domestically or abroad are compared in Table 25. While in Table 26 the occurrence of resistance in *S*. Typhimurium isolates from Danish food animals, imported pork and human cases acquired domestically or abroad, is compared.

Measures to control Salmonella in the Danish pig production have been implemented and in 2004 the *Salmonella* prevalence in Danish pork sampled at slaughter was 1.3%. However, the *S.* Typhimurium prevalence was only 0.5% (Annual Report on Zoonoses in Denmark, 2004). The low S. Typhimurium prevalence is the reason why only 13 *S*. Typhimurium isolates were obtained from Danish pork (Table 21). This small sample size makes it difficult to detect differences in the occurrence of resistance between Danish and imported pork. From 2003 to 2004, the occurrence of tetracycline resistance significantly increased (*P*=0.02) among *S.* Typhimurium from Danish pork. In the same period no significant changes in resistance were observed among *S.* Typhimurium from imported pork. When comparing the occurrence of resistance in *S*. Typhimurium from Danish pigs and Danish pork tetracycline (*P*=0.0004), ampicillin (*P*=0.01) and sulfonamide (*P*=0.04) resistance were significantly more prevalent in isolates from Danish pork compared to isolates from Danish pigs (Table 26). However, when comparing the occurrence of resistance in S. Typhimurium from Danish pigs and imported pork the occurrences of tetracycline (*P*<0.0001), chloramphenicol (*P*<0.0001), florfenicol (*P*<0.0001), ampicillin (*P*<0.0001), sulfonamide (*P*<0.0001), trimethoprim (*P*<0.0001), spectinomycin (*P*<0.0001), streptomycin (*P*=0.0008), ciprofloxacin (*P*=0.0009), and nalidixic acid (*P*<0.0001) resistance were significantly higher in isolates from imported pork compared with isolates from Danish pigs. This indicates that the occurrence of resistance in *S*. Typhimurium from imported pork is more prevalent compared with isolates from Danish pork.

The proportion of multi-resistant *S*. Typhimurium among the isolates can have a strong influence on the frequency of resistance. Therefore, the *S*.

Table 26. Comparison of resistance (%) among Salmonella *Typhimurium from food animals, imported pork and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2004

Compound	Poultry	Cattle	Pigs	Pork	Humans	
	Danish %	Danish %	Danish %	Imported %	Domestic a) %	Travel abroad %
Tetracycline	17	21	40	80	46	51
Chloramphenicol	6	14	9	38	11	26
Florfenicol	0	7	5	25	4	15
Ampicillin	17	32	22	63	40	46
Amoxicillin/clavulanic acid	0	0	0	2	1	3
Cephalothin	0	0	1	2	<1	0
Ceftiofur	0	0	0	0	0	0
Sulfonamide	11	32	38	69	40	41
Trimethoprim	0	4	6	23	8	8
Apramycin	0	0	1	2	0	0
Gentamicin	0	0	1	3	<1	5
Neomycin	6	0	8	6	2	5
Spectinomycin	8	32	15	47	11	31
Streptomycin	17	39	37	59	40	44
Ciprofloxacin	0	0	<1	8	3	21
Nalidixic acid	0	0	<1	8	2	18
Colistin	0	0	0	0	0	0
Number of isolates	18	28	814	64	425	39

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

Typhimurium phage types DT104/104b and DTU302 were excluded from Table 27. However, in this case excluding these phage types did not change the proportions of resistant and sensitive isolates presented in Table 26.

In 2004, no *S*. Typhimurium isolates were available from Danish broiler meat. When comparing the occurrence of resistance in *S*. Typhimurium from Danish poultry (broilers and layers) and imported broiler meat tetracycline (*P*=0.002), chloramphenicol (*P*=0.003), florfenicol (*P*=0.003), ampicillin (*P*=0.002), sulfonamide (*P*=0.0003), trimethoprim (*P*=0.01), spectinomycin (*P*<0.0001), streptomycin (*P*=0.0004), ciprofloxacin (*P*=0.001), and nalidixic acid (*P*=0.001) resistance were significantly higher in isolates from imported broiler meat compared with isolates from Danish poultry. This may indicate that the occurrence of resistance in *S*. Typhimurium from imported broiler meat products was higher compared to isolates from Danish products.

In 2004, *S*. Enteritidis was rare in layers and broilers in Denmark and hardly isolated from other animal species, therefore only a few isolates were obtained from Danish animals. Table 25 compares the occurrence of resistance in *S.* Enteritidis from imported broiler meat and human cases acquired domestically or abroad. With the exception of resistance to ciprofloxacin and nalidixic acid, few *S*. Enteritidis isolates were resistant to the antimicrobials in the test panel. The occurrence of ciprofloxacin (*P*<0.0001) and nalidixic acid (*P*<0.0001) resistance was significantly higher in isolates from imported broiler meat compared to isolates from human cases acquired domestically. This finding is consistent with the national estimates of sources of human salmonellosis (Annual Report on Zoonoses in Denmark 2004) where imported chicken accounted for 6.9-12.0% of human Salmonella cases in 2004, while domestically produced eggs accounted for 5.0-8.2%. The high prevalence of nalidixic acid resistance among isolates from imported broiler meat was not significantly different from the nalidixic acid resistance prevalence found in *S*. Enteritidis from imported broiler meat in 2003 (susceptibility to ciprofloxacin among *S*. Enteritidis from imported broiler meat was not available in 2003).

Table 27. Comparison of resistance (%) among Salmonella *Typhimurium other than DT104, DT104b and DTU302 from food animals, imported pork and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2004

Compound	Poultry	Cattle	Pigs	Pork	Humans	
	Danish	Danish	Danish	Imported	Domestic a)	Travel abroad
	%	%	%	%	%	%
Tetracycline	12	14	37	76	44	45
Chloramphenicol	0	5	5	31	5	16
Florfenicol	0	0	1	14	<1	6
Ampicillin	18	27	19	53	33	42
Amoxicillin/clavulanic acid	0	0	0	2	<1	3
Cephalothin	0	0	1	2	<1	0
Ceftiofur	0	0	0	0	0	0
Sulfonamide	12	18	35	61	33	35
Trimethoprim	0	5	7	29	8	10
Apramycin	0	0	1	2	0	0
Gentamicin	0	0	1	4	<1	6
Neomycin	0	0	7	6	2	6
Spectinomycin	0	18	11	41	6	23
Streptomycin	18	27	35	49	38	39
Ciprofloxacin	0	0	<1	8	2	16
Nalidixic acid	0	0	<1	8	2	13
Colistin	0	0	0	0	0	0
Number of isolates	17	22	735	49	357	31

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

Campylobacter

Campylobacter from food animals

Table 28 presents the MIC distributions and occurrence of antimicrobial resistance among *C. jejuni* from broilers and cattle in 2004 and Table 29 presents data for *C. coli* from pigs in 2004. Trends in resistance to selected antimicrobials among *C. jejuni* and *C. coli* from 1996 to 2004 are presented in Figures 13 and 14, respectively. Among *C. jejuni* isolates from broilers and cattle few resistant isolates were observed and no significant changes in the occurrence of antimicrobial resistance were observed from 2003 to 2004.

Among *C. coli* from pigs ciprofloxacin/nalidixic acid resistance increased significantly (P=0.04) from 3% in 2003 to 16% in 2004 (Figure 14). This increase coincides with a decrease in fluoroquinolone consumption since 2001 due to legislation changes. The reason for the increase in ciprofloxacin/nalidixic acid resistance remains unknown. In *C. coli* isolates from pigs resistance to erythromycin was unchanged from 2003 to 2004 despite a substantial increase in macrolide consumption in weaned pigs in the same period. The occurrence of tetracycline resistance in *C. coli* isolates from pigs has remained below 5% from 1996 to 2004. Therefore, the increase in tetracycline consumption in pigs observed from 1999 to 2004 has not had any immediate effect on the occurrence of tetracycline resistance in *C. coli* isolates.

Campylobacter from foods

In total 204 *C. jejuni* isolates and 61 *C. coli* isolates obtained from broiler meat samples collected at retail outlets were subjected to susceptibility testing. The results are presented in Tables 30 and 31. Nine out of 61 *C. coli* isolates originated from Danish broiler meat products, while 103 out of 204 *C. jejuni* isolates originated from Danish products. This shows that among the broiler meat products included in DANMAP 2004 *C. coli* were significantly more prevalent (P<0.0001) in imported products where 34% (52 out of 153 isolates) were *C. coli,* compared to Danish products where 8% (9 out of 112 isolates) were *C. coli*.

In 2004, the occurrence of tetracycline (P<0.0001), nalidixic acid (P<0.0001) and ciprofloxacin (P<0.0001) resistance was significantly higher in *C. jejuni* isolates from imported broiler meat compared to *C. jejuni* isolates from broiler meat of Danish origin (Table 30). Among *C. coli* the occurrence of tetracycline (P=0.0004), nalidixic acid (P=0.03) and ciprofloxacin (P=0.03) resistance was also significantly higher in *C. coli* isolates from imported broiler meat compared to *C. coli* from broiler meat of Danish origin (Table 31).

Campylobacter in humans

In 2004, there were 3,724 laboratory confirmed cases of human campylobacteriosis occurring in Denmark making it the most common bacterial cause of diarrhoeal illness. This corresponds to an incidence rate of 69 per 100,000 inhabitants and reflects an increase in the number of cases by 5% over the

Table 28. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *isolates from broilers (n=77) and cattle (n=42), Denmark*

DANMAP 2004

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	>128
Tetracycline	Broilers	5	[1.4-12.8]					92.2		2.6			2.6		2.6		
	Cattle	0	[0.0-8.4]					100									
Chloramphenicol	Broilers	0	[0.0-4.7]						1.3	3.9	83.1	6.5	5.2				
	Cattle	0	[0.0-8.4]							38.1	42.9	19.0					
Erythromycin	Broilers	1	[0.0-7.0]					5.2	32.5	46.8	14.3				1.3		
	Cattle	0	[0.0-8.4]					11.9	31.0	52.4	4.8						
Gentamicin	Broilers	0	[0.0-4.7]				44.2	53.2	2.6								
	Cattle	0	[0.0-8.4]				71.4	26.2	2.4								
Neomycin	Broilers	0	[0.0-4.7]						87.0	13.0							
	Cattle	0	[0.0-8.4]						90.5	9.5							
Streptomycin	Broilers	3	[0.3-9.1]						46.8	46.8	3.9			2.6			
	Cattle	0	[0.0-8.4]						71.4	28.6							
Ciprofloxacin	Broilers	5	[1.4-12.8]		2.6	32.5	50.6	5.2	1.3	2.6			1.3	3.9			
	Cattle	2	[0.1-12.6]			28.6	61.9	7.1					2.4				
Nalidixic acid	Broilers	5	[1.4-12.8]								9.1	75.3	7.8	2.6			5.2
	Cattle	2	[0.1-12.6]								11.9	78.6	4.8	2.4			2.4

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 29. Distribution of MICs and occurrence of resistance among Campylobacter coli *isolates from pigs (n=100), Denmark*

DANMAP 2004

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	>128
Tetracycline	0	[0.0-5.4]					86.0	9.0	3.0	2.0						
Chloramphenicol	0	[0.0-3.6]							3.0	23.0	69.0	5.0				
Erythromycin	23	[15.5-32.5]				2.0	13.0	4.0	29.0	25.0	4.0			23.0		
Gentamicin	0	[0.0-3.6]				20.0	75.0	5.0								
Neomycin	0	[0.0-3.6]						92.0	8.0							
Streptomycin	47	[36.9-57.2]						5.0	31.0	17.0		1.0	5.0	19.0	22.0	
Ciprofloxacin	16	[9.4-24.7]		20.0	38.0	23.0	3.0			2.0	1.0	4.0	9.0			
Nalidixic acid	16	[9.4-24.7]								3.0	65.0	15.0	1.0	1.0	3.0	12.0

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

DANMAP 2004

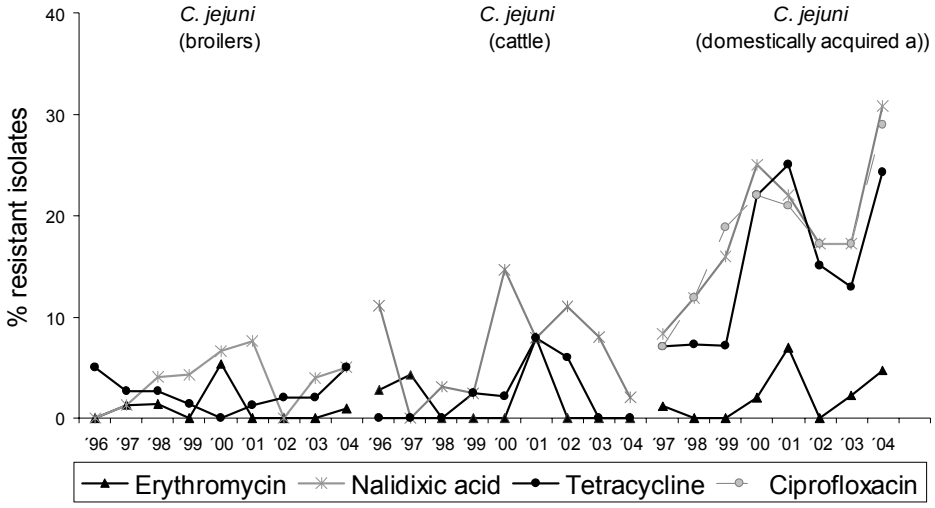

Figure 13. Trends in resistance to selected antimicrobials among Campylobacter jejuni *isolates from broilers, cattle and human domestic cases, Denmark*

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

DANMAP 2004

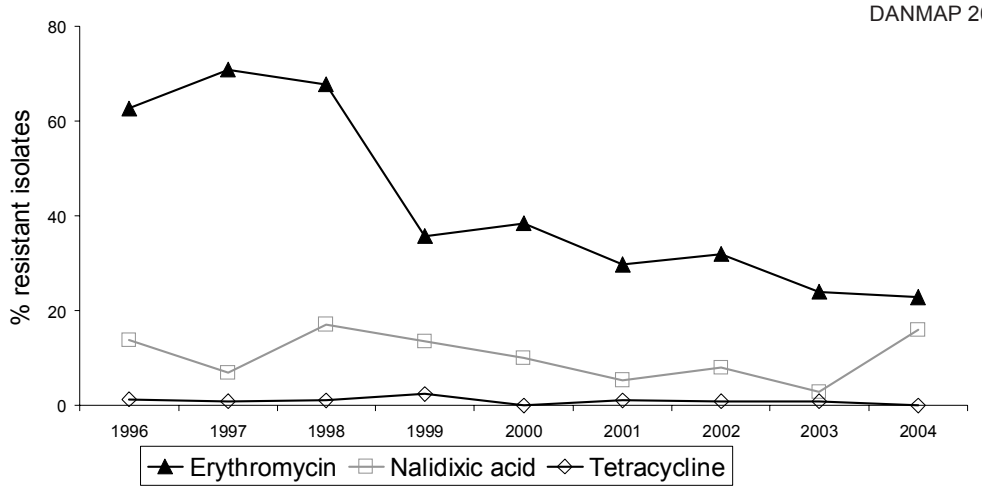

Figure 14. Trends in resistance to selected antimicrobials among Campylobacter coli *isolates from pigs, Denmark*

Table 30. Distribution of MICs and occurrence of resistance in Campylobacter jejuni *from broiler meat (Danish n=103; imported n=101), Denmark*

DANMAP 2004

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	>512
Tetracycline	Danish	<1	[0.02-5.3]					97.1	1.9				1.0						
	Imported	49	[38.5-58.7]					36.6	8.9	1.0	2.0	3.0		8.9	39.6				
Chloramphenicol	Danish	0	[0.0-3.5]						4.9	58.3	35.0	1.9							
	Imported	0	[0.0-3.6]						1.0	31.7	31.7	30.7	5.0						
Erythromycin	Danish	0	[0.0-3.5]				1.9	21.4	69.9	6.8									
	Imported	3	[0.6-8.4]				1.0	26.7	50.5	13.9	4.0	1.0			3.0				
Gentamicin	Danish	0	[0.0-3.5]				87.4	12.6											
	Imported	0	[0.0-3.6]				83.2	14.9	1.0	1.0									
Neomycin	Danish	0	[0.0-3.5]						100										
	Imported	<1	[0.03-5.4]						96.0		1.0	2.0	1.0						
Streptomycin	Danish	0	[0.0-3.5]						63.1	34.0	2.9								
	Imported	8	[3.5-15.0]						53.5	33.7	2.0	3.0		5.0	2.0	1.0			
Ciprofloxacin	Danish	3	[0.6-8.3]		9.7	79.6	6.8		1.0				2.9						
	Imported	48	[37.5-57.7]		11.9	20.8	11.9	5.9	2.0			17.8	17.8	11.9					
Nalidixic acid	Danish	3	[0.6-8.3]								66.0	30.1	1.0			1.0	1.9		
	Imported	48	[37.5-57.7]								25.7	16.8	9.9		1.0	5.9	40.6		

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 31. Distribution of MICs and occurrence of resistance among Campylobacter coli *from broiler meat (Danish n=9; imported n=52), Denmark*

DANMAP 2004

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	>512
Tetracycline	Danish	22	[2.8-60.0]					77.8						11.1	11.1				
	Imported	85	[71.9-93.1]					13.5				1.9	3.9	1.9	78.9				
Chloramphenicol	Danish	0	[0.0-33.6]							11.1	66.7	11.1	11.1						
	Imported	0	[0.0-6.9]							3.9	57.7	32.7	5.8						
Erythromycin	Danish	33	[7.5-70.1]				11.1	11.1	11.1	22.2	11.1				33.3				
	Imported	12	[4.4-23.4]					7.7	17.3	46.2	17.3				11.5				
Gentamicin	Danish	0	[0.0-33.6]				55.6	44.4											
	Imported	0	[0.0-6.9]				61.5	38.5											
Neomycin	Danish	0	[0.0-33.6]						88.9	11.1									
	Imported	0	[0.0-6.9]						100										
Streptomycin	Danish	56	[21.2-86.3]						22.2	11.1	11.1			44.4	11.1				
	Imported	37	[23.6-51.0]						30.8	26.9	5.8		9.6	15.4	9.6	1.9			
Ciprofloxacin	Danish	22	[2.8-60.0]		33.3	44.4							22.2						
	Imported	65	[50.9-78.0]		3.9	17.3	13.5				7.7	15.4	38.5	3.9					
Nalidixic acid	Danish	22	[2.8-60.0]								55.6	22.2				11.1	11.1		
	Imported	65	[50.9-78.0]								9.6	25.0			1.9	40.4	23.1		

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

previous year. A further decrease in the incidence of human campylobacteriosis seen in the last years has thus not been achieved (EPI-NEWS 2005, no. 9: http://www.ssi.dk/sw26374.asp). *Campylobacter* isolates from human infections were predominantly *Campylobacter jejuni* and the remaining were *Campylobacter coli*. About 9% of all reported cases of campylobacteriosis had a history of travel abroad. This is probably an underestimated percentage as information on travel is often missing and some cases reported as domestically acquired may in fact have been acquired abroad. Therefore, comparisons of data between those infections acquired abroad and those acquired domestically should be interpreted with caution.

Species determination was available for 122 (3%) of all *Campylobacter* isolates reported to the Unit of Gastrointestinal Infections at the Statens Serum Institut; 119 of these were *C. jejuni* and 3 were *C. coli*. Due to the low number of *C. coli* isolates, resistance data for this species are not presented. Table 32 shows the occurrence of resistance among *C. jejuni* isolates from humans by origin of infection. Trends in resistance to selected antimicrobials among *C. jejuni* in domestically acquired cases are shown in Figure 13.

Isolates of *C. jejuni* were generally susceptible to erythromycin (Table 32). Among *C. jejuni* isolates from infections without known travel information (considered as domestically acquired cases), a significant increase in resistance to nalidixic acid was observed in 2004, as compared to 2003 (*P*=0.04). 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 antimicrobials in *Campylobacter* infections acquired outside Denmark.

Campylobacter from animals, foods and humans

A comparison of the occurrence of resistance among *C. jejuni* isolates from Danish food animals, food of Danish and imported origin, and human cases acquired domestically or associated with travel abroad is presented in Table 32.

No significant differences in resistance levels were observed when comparing *C. jejuni* isolates from Danish broilers with *C. jejuni* isolates from Danish broiler meat (Table 32).

The occurrence of resistance to tetracycline (*P*=0.0005), ciprofloxacin (*P*<0.0001) and nalidixic acid (*P*<0.0001) was significantly higher in *C. jejuni* isolates from domestically acquired human cases compared with isolates from Danish broiler meat (Table 32). One explanation could be that information about travel is not systematically registered and some human cases reported as domestically acquired have actually been acquired abroad. It may also indicate that sources other than Danish broiler meat contribute to *C. jejuni* infections in humans. Resistance to tetracycline (*P*<0.0001), ciprofloxacin (*P*=0.002) and nalidixic acid (*P*=0.004) was significantly higher in *C. jejuni* isolates from imported broiler meat compared with isolates from domestically acquired human cases.

Table 32. Comparison of resistance among Campylobacter jejuni *from Danish food animals, broiler meat of Danish and imported origin and from human cases acquired domestically or associated with travel, Denmark*

DANMAP 2004

Compound	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestically acquired a)	Travel abroad
	%	%	%	%	%	%
Tetracycline	0	5	1	49	24	42
Erythromycin	0	1	0	3	5	8
Ciprofloxacin	2	5	3	48	29	58
Nalidixic acid	2	5	3	48	31	50
Number of isolates	42	77	103	101	107	12

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

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 2004, enterococci were not collected from samples from cattle.

The MIC distributions and the occurrence of antimicrobial resistance among enterococci from food animals are shown in Table 33 and 34. Trends in resistance among *E. faecium* isolates from broilers and pigs to antimicrobial growth promoters are presented in Figures 15-20.

From 2003 to 2004, erythromycin resistance significantly increased from 21% to 50% (P<0.0001) among *E. faecium* isolates from pigs (Figure 19). This coincides with a substantial increase in macrolide consumption in pigs in the same period (Table 6). In contrast, the occurrence of penicillin resistance among *E. faecium* from pigs significantly decreased from 39% to 15% from 2003 to 2004 (P<0.0001), although penicillin consumption increased during the same period in the pig production. The increased consumption of tetracycline in pigs did not have any immediate effect on the occurrence of tetracycline resistance in *E. faecium* isolates from pigs (Figure 21).

Resistance to penicillin among *E. faecium* isolates from broilers has remained at a relatively high level (50-60%) since 1999. From 2003 to 2004, a significant decrease in penicillin resistance from 54% to 35% (P<0.0001) was observed. The consumption of penicillins (amoxicillin) in broilers was reduced by 21% from 2002 to 2003 however, the consumption increased again by 21% from 2003 to 2004. This temporary decrease in penicillin consumption in broilers may to some extent explain the significant decrease in penicillin resistance observed from 2003 to 2004.

Table 33. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broilers (n=135) and pigs (n=148), Denmark* DANMAP 2004

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs													
				0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	4	[1.2-8.4]			95.6	0.7				2.2	1.5						
	Pigs	48	[39.7-56.3]			50.0		0.7	1.4	0.7	27.7	19.6						
Chloramphenicol	Broilers	0	[0.0-2.7]				0.7	24.4	68.1	6.7								
	Pigs	0	[0.0-2.5]				3.4	27.7	66.2	2.7								
Florfenicol	Broilers	0	[0.0-2.7]				35.6	64.4										
	Pigs	0	[0.0-2.5]				48.6	50.7	0.7									
Penicillin	Broilers	35	[26.8-43.5]				33.3	21.5	10.4	3.7	25.2	5.2	0.7					
	Pigs	15	[9.6-21.6]				40.5	9.5	35.1	14.9								
Erythromycin	Broilers	28	[20.8-36.5]		42.2	8.1	7.4	14.1	15.6	1.5	3.0	8.1						
	Pigs	50	[41.7-58.3]		10.8	1.4	10.8	27.0	23.6	2.0		24.3						
Gentamicin	Broilers	0	[0.0-2.7]				0.7	3.0	59.3	28.1	8.9							
	Pigs	0	[0.0-2.5]				0.7	4.7	48.0	39.9	5.4	1.4						
Kanamycin	Broilers	2	[0.5-6.4]										6.7	34.1	47.4	9.6	2.2	
	Pigs	22	[15.3-29.1]										16.9	30.4	22.3	8.8	1.4	20.3
Streptomycin	Broilers	2	[0.5-6.4]										97.8					2.2
	Pigs	26	[18.9-33.5]										68.2	2.0	2.0	2.0	7.4	18.2
Vancomycin	Broilers	2	[0.5-6.4]				95.6	2.2				2.2						
	Pigs	3	[1.1-7.7]				94.6	1.4	0.7			3.4						
Quinupristin/dalfopristin	Broilers	24	[16.8-31.8]		13.3	23.0	40.0	8.9	13.3	1.5								
	Pigs	13	[7.9-19.3]		19.6	6.8	60.8	12.2	0.7									
Avilamycin	Broilers	7	[3.1-12.3]				43.0	37.0	13.3	5.2		1.5						
	Pigs	0	[0.0-2.5]				90.5	8.8	0.7									
Salinomycin	Broilers	1	[0.02-4.1]				20.7	3.7	74.8	0.7								
	Pigs	0	[0.0-2.5]				100											
Linezolid	Broilers	0	[0.0-2.7]			11.1	74.1	14.8										
	Pigs	0	[0.0-2.5]			13.5	86.5											

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 concentration

Despite the fact that tetracycline is hardly used in the Danish broiler production, 41% of the *E. faecalis* isolates from broilers were tetracycline resistant. The reason for the high tetracycline resistance remains unknown. Finally, the occurrence of resistance in *E. faecalis* isolates from broilers and pigs remained unchanged from 2003 to 2004.

Enterococci from food

Isolation of enterococci from samples of broiler meat, beef and pork from retail outlets yielded 401 isolates of *E. faecium* and 434 isolates of *E. faecalis* in 2004.

The MIC distributions and occurrence of antimicrobial resistance among enterococci from foods are shown in Tables 35-37and 38-40. The *E. faecium* isolates were obtained from Danish pork (n=52) and imported pork (n=4), Danish beef (n=83), imported beef (n=19), Danish broiler meat (n=173), and from imported broiler meat (n=69).
The *E. faecalis* isolates were obtained from Danish pork (n=160) and imported pork (n=12), Danish beef (n=120), imported beef (n=28), Danish broiler meat (n=74), and from imported broiler meat (n=40).

(See "Comparison of resistance in enterococci from animals, foods and healthy human volunteers").

Enterococci from healthy human volunteers

In 2004, stool samples were collected from 125 randomly selected healthy human volunteers. In total 54 *E. faecium* isolates and 63 *E. faecalis* isolates were obtained.

The MIC distributions and occurrence of antimicrobial resistance among enterococci from humans are shown in Tables 41 and 42.

Resistance towards quinopristin/dalfopristin (Q/D) (35%) was most common among *E. faecium* isolates. All Q/D isolates had MIC=4, which is near to the breakpoint. In *E. faecium* isolates, erythromycin resistance significantly increased from 4% in 2003 to 28% in 2004 (*P*=0.001).
Resistance to tetracycline (44%) was most common among *E. faecalis* isolates in 2004 and significantly increased from 2003 to 2004 (*P*=0.005).
None of the enterococcal isolates were resistant to gentamicin, vancomycin or teicoplanin.

Table 34. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broilers (n=82) and pigs (n=153), Denmark*

DANMAP 2004

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs												
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	41	[30.7-52.9]		56.1			2.4	3.7	17.1	20.7						
	Pigs	86	[79.8-91.3]		13.1	0.7			2.0	20.9	63.4						
Chloramphenicol	Broilers	1	[0.03-6.6]			4.9	15.9	78.0			1.2						
	Pigs	10	[6.1-16.4]				19.0	69.9	0.7		6.5	3.9					
Florfenicol	Broilers	0	[0.0-4.4]			90.2	9.8										
	Pigs	0	[0.0-2.4]			56.9	42.5	0.7									
Penicillin	Broilers	0	[0.0-4.4]			39.0	59.8	1.2									
	Pigs	0	[0.0-2.4]			62.7	35.9	1.3									
Erythromycin	Broilers	18	[10.6-28.4]	35.4	14.6	22.0	9.8	1.2	6.1	2.4	8.5						
	Pigs	40	[32.1-48.1]	13.7	37.7	7.2	2.0				39.9						
Gentamicin	Broilers	0	[0.0-4.4]				3.7	53.7	34.1	8.5							
	Pigs	9	[5.1-14.9]				0.7	2.0	45.1	42.5	0.7				0.7	1.3	7.2
Kanamycin	Broilers	0	[0.0-4.4]									97.6	2.4				
	Pigs	26	[19.4-33.9]									71.9	2.0			0.7	25.5
Streptomycin	Broilers	0	[0.0-4.4]									87.8	11.0	1.2			
	Pigs	32	[24.7-40.0]									27.5	39.9		0.7	0.7	31.4
Vancomycin	Broilers	0	[0.0-4.4]			100											
	Pigs	0	[0.0-2.4]			100											
Avilamycin	Broilers	0	[0.0-4.4]			98.8	1.2										
	Pigs	1	[0.2-4.6]			98.0	0.7		0.7	0.7							
Flavomycin	Broilers	1	[0.03-6.6]			98.8					1.2						
	Pigs	<1	[0.02-3.6]			99.3					0.7						
Salinomycin	Broilers	0	[0.0-4.4]			79.3	17.1	3.7									
	Pigs	0	[0.0-2.4]			100.0											
Linezolid	Broilers	0	[0.0-4.4]		32.9	67.1											
	Pigs	0	[0.0-2.4]		24.8	74.5	0.7										

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 concentration

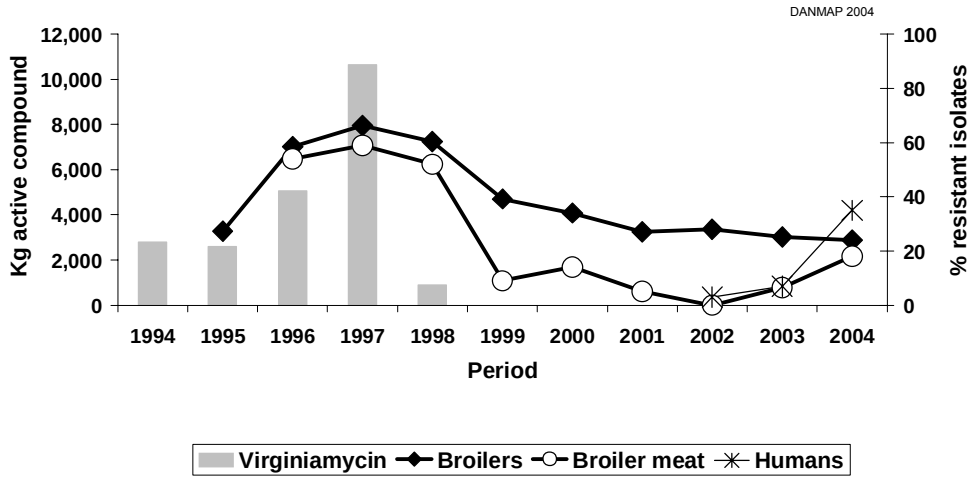

Figure 15. Trends in streptogramin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

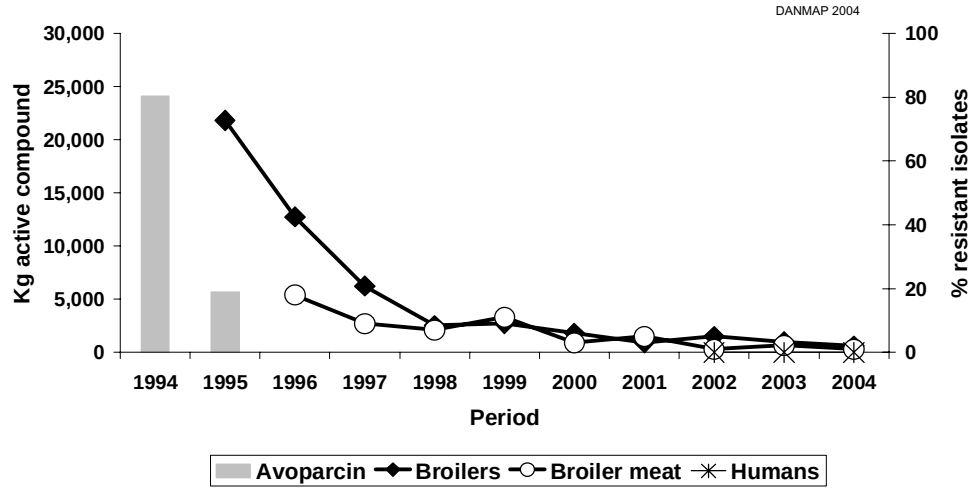

Figure 16. Trends in glycopeptide resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avoparcin in animals, Denmark*

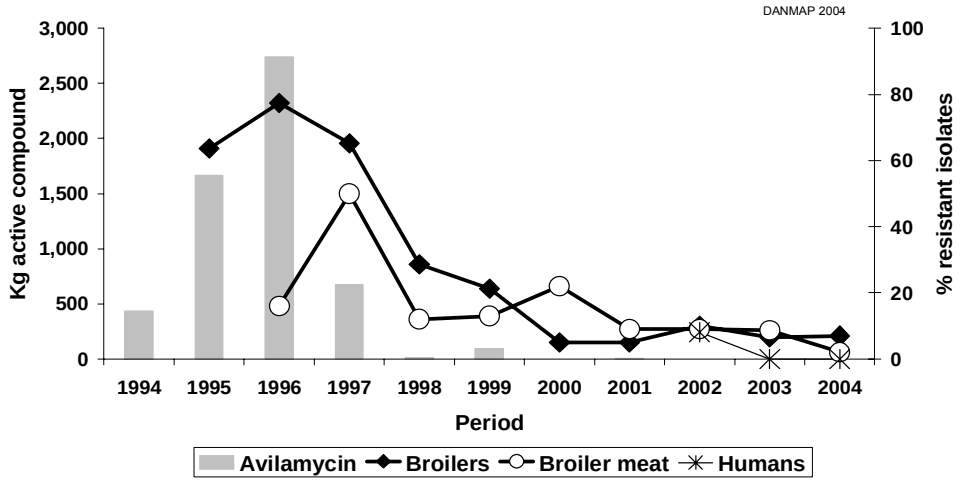

Figure 17. Trends in avilamycin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avilamycin in animals, Denmark*

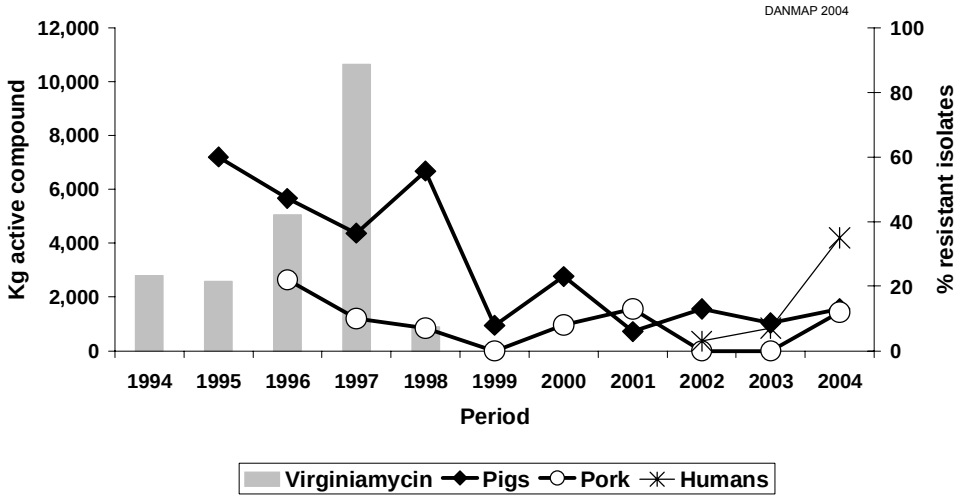

Figure 18. Trends in streptogramin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

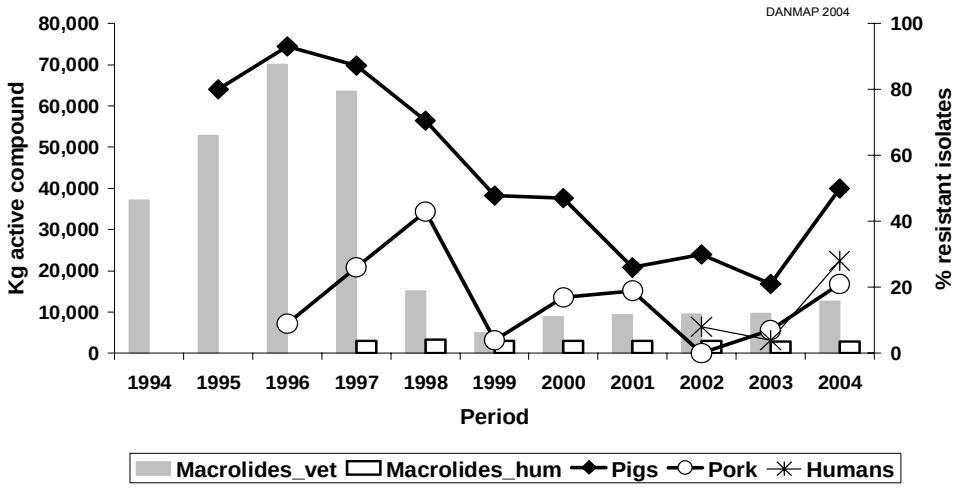

Figure 19. Trends in erythromycin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the total consumption of macrolides, both as growth promoters in animals and as prescribed antimicrobials in animals and humans, Denmark*

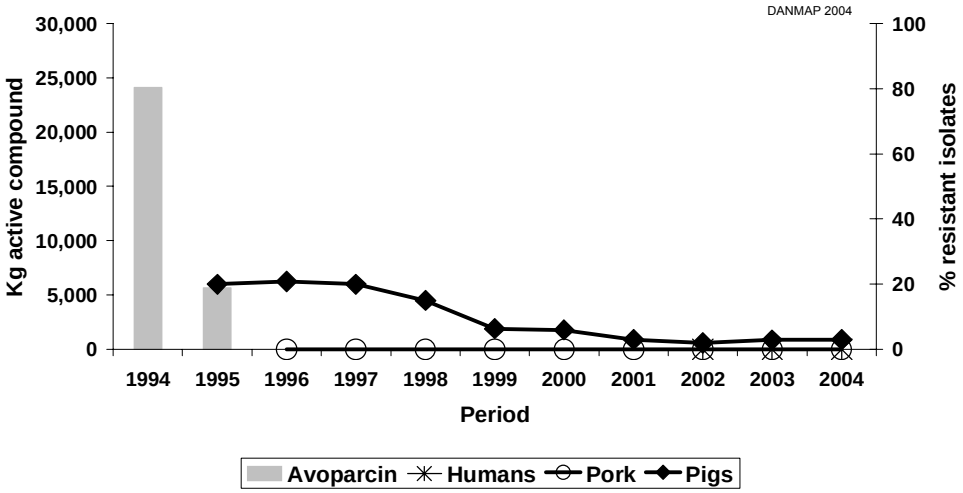

Figure 20. Trends in glycopeptide resistance among Enterococcus faecium *from pigs and pork and healthy humans in the community and the consumption of the growth promoter avoparcin, Denmark*

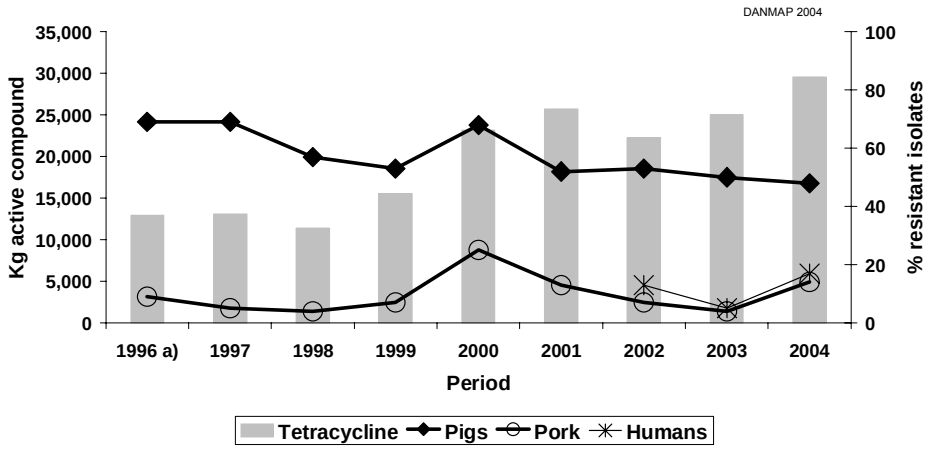

Figure 21. Trends in tetracycline resistance among Enterococcus faecium *from pigs, pork and healthy humans and the comsumption of tetracyclines in pig production, Denmark*

Table 35. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broiler meat (Danish n=173; imported n=69 a)), Denmark*

DANMAP 2004

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs													
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Danish	6	[2.8-10.4]		91.3	2.3		0.6	0.6	1.2	4.1						
	Imported	75	[63.5-85.0]		23.2	1.5			1.5	8.7	65.2						
Chloramphenicol	Danish	<1	[0.01-3.2]			11.0	33.5	54.9		0.6							
	Imported	3	[0.4-10.1]				18.8	68.1	10.1	2.9							
Florfenicol	Danish	0	[0.0-2.1]			73.4	26.6										
	Imported	0	[0.0-5.2]			44.9	55.1										
Penicillin	Danish	10	[5.8-15.3]			68.8	14.5	6.9	8.1	1.7							
	Imported	7	[2.4-16.1]			68.1	17.4	7.3	2.9		1.5	2.9					
Erythromycin	Danish	24	[18.1-31.4]	35.3	12.7	14.5	13.3	4.6	5.8	2.3	11.6						
	Imported	39	[27.6-51.6]	27.5	7.3	10.1	15.9	7.3	2.9	1.5	27.5						
Gentamicin	Danish	0	[0.0-2.1]			0.6	11.0	49.7	33.0	5.8							
	Imported	0	[0.0-5.2]				1.5	55.1	33.3	7.3	2.9						
Kanamycin	Danish	<1	[0.01-3.2]									32.4	44.5	19.1	3.5		0.6
	Imported	6	[1.6-14.2]									18.8	40.6	34.8		1.5	4.4
Streptomycin	Danish	2	[0.6-5.8]									94.8	2.3		0.6	0.6	1.7
	Imported	10	[4.2-19.8]									82.6	1.5	2.9	2.9	5.8	4.4
Vancomycin	Danish	1	[0.1-4.1]			96.5	2.3				1.2						
	Imported	0	[0.0-5.2]			98.6	1.5										
Quinupristin/	Danish	17	[11.5-23.2]	23.1	21.4	38.7	8.1	6.9	1.7								
dalfopristin	Imported	20	[11.6-31.7]	29.0	13.0	37.7	15.9	2.9		1.5							
Avilamycin	Danish	<1	[0.01-3.2]			90.8	6.4	2.3	0.6								
	Imported	7	[2.4-16.1]			78.3	10.1	4.4		1.5	5.8						
Salinomycin	Danish	2	[0.6-5.8]			29.5	12.7	55.5	2.3								
	Imported	0	[0.0-5.2]			63.8	11.6	24.6									
Linezolid	Danish	0	[0.0-2.1]		21.4	76.3	2.3										
	Imported	0	[0.0-5.2]		8.7	87.0	4.4										

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 concentration
a) One isolate is from a hen

Comparison of resistance in enterococci from animals, foods and healthy human volunteers

A comparison of resistance among enterococci from Danish food animals, foods of Danish and imported origin and humans are presented in Tables 43 and 44.

In general, the occurrence of resistance was significantly higher in *E. faecium* and *E. faecalis* isolates from imported broiler meat compared to isolates from Danish broiler meat. Comparison of the occurrence of resistance in Danish and imported pork was hampered by the low number of isolates from imported pork.

Table 36. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from beef (Danish n=83; imported n=19), Denmark*

DANMAP 2004

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs												
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Danish	7	[2.7-15.1]		91.6	1.2				4.8	2.4						
	Imported	26	[9.2-51.2]		68.4		5.3			15.8	10.5						
Chloramphenicol	Danish	0	[0.0-4.4]			1.2	33.7	65.1									
	Imported	0	[0.0-17.7]			15.8	36.8	47.4									
Florfenicol	Danish	0	[0.0-4.4]			66.3	33.7										
	Imported	0	[0.0-17.7]			79.0	21.1										
Penicillin	Danish	0	[0.0-4.4]			89.2	8.4	2.4									
	Imported	11	[1.3-33.1]			73.7	15.8		5.3	5.3							
Erythromycin	Danish	5	[1.3-11.9]	49.4	3.6	18.1	24.1		2.4		2.4						
	Imported	26	[9.2-51.2]	47.4		10.5	15.8		26.3								
Gentamicin	Danish	0	[0.0-4.4]			2.4	16.9	51.8	26.5	2.4							
	Imported	0	[0.0-17.7]					57.9	36.8	5.3							
Kanamycin	Danish	2	[0.3-8.4]									37.4	37.4	21.7	1.2		2.4
	Imported	5	[0.1-26.0]									10.5	57.9	26.3			5.3
Streptomycin	Danish	2	[0.3-8.4]									97.6					2.4
	Imported	0	[0.0-17.7]									84.2	10.5		5.3		
Vancomycin	Danish	0	[0.0-4.4]			98.8		1.2									
	Imported	0	[0.0-17.7]			100											
Quinupristin/	Danish	20	[12.4-30.8]	36.1	4.8	38.6	18.1	2.4									
dalfopristin	Imported	26	[9.2-51.2]	15.8	5.3	52.6	10.5	15.8									
Avilamycin	Danish	0	[0.0-4.4]			94.0	6.0										
	Imported	0	[0.0-17.7]			94.7	5.3										
Salinomycin	Danish	0	[0.0-4.4]			100											
	Imported	0	[0.0-17.7]			94.7		5.3									
Linezolid	Danish	0	[0.0-4.4]		10.8	88.0	1.2										
	Imported	0	[0.0-17.7]		21.1	79.0											

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 concentration

Table 37. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from pork (Danish n=52; imported n=4 data not shown), Denmark*

DANMAP 2004

Compound	% Resistant	[95% Confidence interval]	Distribution (%) of MICs												
			0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	13	[5.6-25.8]		86.5					7.7	5.8						
Chloramphenicol	0	[0.0-6.9]			1.9	57.7	40.4									
Florfenicol	0	[0.0-6.9]			80.8	19.2										
Penicillin	4	[0.5-13.2]			76.9	13.5	5.8	1.9	1.9							
Erythromycin	21	[11.1-34.7]	19.2	3.9	30.8	25.0	13.5	1.9		5.8						
Gentamicin	0	[0.0-6.9]				30.8	38.5	21.2	7.7	1.9						
Kanamycin	6	[1.2-16.0]									61.5	19.2	13.5		1.9	3.9
Streptomycin	4	[0.5-13.2]									92.3	1.9		1.9	1.9	1.9
Vancomycin	0	[0.0-6.9]			100											
Quinupristin/dalfopristin	13	[5.6-25.8]	28.9	7.7	50.0	11.5	1.9									
Avilamycin	0	[0.0-6.9]			100											
Salinomycin	0	[0.0-6.9]			100											
Linezolid	0	[0.0-6.9]		32.7	65.4	1.9										

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 concentration

Like in previous years *E. faecium* and *E. faecalis* isolates from pigs were significantly more resistant compared to isolates from Danish pork, while the occurrence of resistance in *E. faecium* and *E. faecalis* isolates from broilers and Danish broiler meat was similar for most antimicrobials. The reason for these differences between pigs/pork and broilers/broiler meat remains unknown. More details are presented below.

In *E. faecium* isolates from pigs, resistance to tetracycline (*P*<0.0001), erythromycin (*P*=0.0005), kanamycin (*P*=0.02) and streptomycin (*P*=0.002) was significantly higher compared to isolates from pork (Table 43). The resistance levels were similar for *E. faecium* isolates from Danish pork and healthy humans, except for quinopristin/dalfopristin. All human isolates had a MIC=4μg/ml, which is near to the breakpoint. From 2003 to 2004, quinopristin/dalfopristin resistance significantly increased among *E. faecium* from Danish broiler meat (*P*=0.04), beef (*P*=0.01) and pork (*P*=0.01). A majority of the resistant isolates from food from 2004 also had a MIC value of 4 μg/ml. Further investigation of the genetic background for this must be done in the future.

From 2003 to 2004, erythromycin resistance significantly increased among *E. faecium* isolates from pigs (*P*<0.0001) and healthy humans (*P*=0.001). This may be associated with increased macrolide consumption in pig production. In contrast, the increased consumption of tetracycline in pig production did not have any immediate effect on the occurrence of tetracycline resistance in *E. faecium* isolates from pigs and healthy human volunteers.

In general, the resistance levels in *E. faecium* isolates from broilers and Danish broiler meat were similar except for penicillin (*P*<0.0001) and avilamycin (*P*=0.006) resistance, for which resistance was significantly higher in isolates from broilers.

Comparing the occurrence of resistance in *E. faecium* from Danish and imported broiler meat showed that tetracycline (*P*<0.0001), erythromycin (*P*=0.03), kanamycin (*P*=0.02), streptomycin (*P*=0.01), and avilamycin (*P*=0.008) resistance was significantly higher in iso-lates from imported than in Danish broiler meat.

Table 38. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broiler meat (Danish n=74; imported n=40 a)), Denmark*

DANMAP 2004

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs													
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Danish	43	[31.8-55.3]		55.4	1.4				20.3	23.0						
	Imported	83	[67.2-92.7]		17.5					10.0	72.5						
Chloramphenicol	Danish	0	[0.0-4.9]			2.7	13.5	81.1	2.7								
	Imported	5	[0.6-16.9]				22.5	70.0	2.5		2.5	2.5					
Florfenicol	Danish	0	[0.0-4.9]			77.0	23.0										
	Imported	0	[0.0-8.8]			70.0	30.0										
Penicillin	Danish	1	[0.03-7.3]			87.8	10.8			1.4							
	Imported	0	[0.0-8.8]			92.5	7.5										
Erythromycin	Danish	34	[23.2-45.7]	23.0	24.3	12.2	6.8	2.7	2.7	2.7	25.7						
	Imported	33	[18.6-49.1]	32.5	27.5	7.5					32.5						
Gentamicin	Danish	0	[0.0-4.9]			1.4	2.7	23.0	58.1	14.9							
	Imported	5	[0.6-16.9]				2.5	7.5	60.0	25.0							5.0
Kanamycin	Danish	0	[0.0-4.9]									97.3	2.7				
	Imported	8	[1.6-20.4]									92.5					7.5
Streptomycin	Danish	8	[3.0-16.8]									64.9	25.7	1.4			8.1
	Imported	18	[7.3-32.8]									45.0	37.5			2.5	15.0
Vancomycin	Danish	0	[0.0-4.9]			97.3	2.7										
	Imported	0	[0.0-8.8]			100											
Avilamycin	Danish	0	[0.0-4.9]			98.7	1.4										
	Imported	0	[0.0-8.8]			97.5	2.5										
Flavomycin	Danish	3	[0.3-9.4]			96.0		1.4	1.4		1.4						
	Imported	0	[0.0-8.8]			100											
Salinomycin	Danish	0	[0.0-4.9]			58.1	31.1	10.8									
	Imported	0	[0.0-8.8]			92.5	7.5										
Linezolid	Danish	0	[0.0-4.9]		46.0	54.1											
	Imported	0	[0.0-8.8]		42.5	57.5											

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 concentration
a) One isolate is from a hen

In *E. faecium* from imported beef the occurrence of resistance was significantly higher for erythromycin (*P*=0.01), tetracycline (*P*=0.03) and penicillin (*P*=0.03) compared with isolates from Danish beef.

With the exception of *E. faecium* from imported broiler meat, where the level of tetracycline resistance was significantly higher than in humans (*P*<0.0001), resistance levels in *E. faecium* from humans were similar to levels seen in isolates from Danish and imported meat products. These observations are consistent with the assumption that resistance levels found in isolates from food should be reflected in the occurrence of resistance in the normal flora of healthy humans (Table 43).

Differences between levels of resistance were observed in *E. faecalis* isolates from pigs and Danish pork, where resistance to tetracycline (*P*<0.0001), chloramphenicol (*P*=0.003), erythromycin (*P*<0.0001), kanamycin (*P*<0.0001), and streptomycin (*P*<0.0001) was significantly lower in pork (Table 44).
The resistance levels were similar for *E. faecalis* isolates from Danish pork and healthy humans, except for tetracycline (*P*<0.0001) and erythromycin (*P*=0.006) (Table 44).

In *E. faecalis* isolates from broilers and Danish broiler meat the resistance levels were similar except for erythromycin resistance which was significantly higher (*P*=0.04) in isolates from broiler meat compared to broilers.

When comparing *E. faecalis* isolates from Danish and imported broiler meat the occurrence of tetracycline (*P*<0.0001), kanamycin (*P*=0.04) and streptomycin (*P*=0.009) resistance was significantly higher in isolates from imported meat (Table 44).

Table 39. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from beef (Danish n=120; imported n=28), Denmark*

DANMAP 2004

Compound	Origin	% Resistant	[95% Confidence interval]	Distribution (%) of MICs												
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Danish	18	[11.9-26.4]		80.0	0.8	0.8			3.3	15.0						
	Imported	14	[4.0-32.7]		85.7					3.6	10.7						
Chloramphenicol	Danish	2	[0.2-5.9]				46.7	50.8	0.8		1.7						
	Imported	0	[0.0-12.3]				50.0	50.0									
Florfenicol	Danish	0	[0.0-3.0]			84.2	15.8										
	Imported	0	[0.0-12.3]			85.7	14.3										
Penicillin	Danish	0	[0.0-3.0]			90.0	10.0										
	Imported	0	[0.0-12.3]			96.4	3.6										
Erythromycin	Danish	4	[1.4-9.5]	30.0	30.0	30.8	5.0				4.2						
	Imported	0	[0.0-12.3]	35.7	25.0	39.3											
Gentamicin	Danish	2	[0.2-5.9]			6.7	10.8	35.0	35.0	9.2	0.8	0.8				0.8	0.8
	Imported	0	[0.0-12.3]				3.6	50.0	35.7	10.7							
Kanamycin	Danish	6	[2.4-11.7]									94.2				1.7	4.2
	Imported	0	[0.0-12.3]									100					
Streptomycin	Danish	5	[1.9-10.6]									76.7	17.5		0.8	2.5	2.5
	Imported	0	[0.0-12.3]									82.1	17.9				
Vancomycin	Danish	0	[0.0-3.0]			100											
	Imported	0	[0.0-12.3]			100											
Avilamycin	Danish	<1	[0.02-4.6]			98.3	0.8		0.8								
	Imported	0	[0.0-12.3]			92.9	7.1										
Flavomycin	Danish	<1	[0.02-4.6]			98.3	0.8			0.8							
	Imported	4	[0.1-18.4]			96.4					3.6						
Salinomycin	Danish	0	[0.0-3.0]			100											
	Imported	0	[0.0-12.3]			96.4	3.6										
Linezolid	Danish	0	[0.0-3.0]		21.7	78.3											
	Imported	0	[0.0-12.3]		28.6	71.4											

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 concentration

Table 40. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from pork (Danish n=160; imported n=12), Denmark*

DANMAP 2004

Compound	Origin	% Resistant [95% Confidence interval]		Distribution (%) of MICs													
				0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Danish	12	[7.3-17.9]		87.5	0.6				3.8	8.1						
	Imported	42	[15.2-72.3]		58.3						41.7						
Chloramphenicol	Danish	2	[0.4-5.4]				39.4	58.8		0.6	1.3						
	Imported	0	[0.0-26.5]				41.7	58.3									
Florfenicol	Danish	0	[0.0-2.3]			78.8	21.3										
	Imported	0	[0.0-26.5]			83.3	16.7										
Penicillin	Danish	0	[0.0-2.3]			93.1	6.3	0.6									
	Imported	0	[0.0-26.5]			91.7	8.3										
Erythromycin	Danish	9	[4.9-14.3]	33.1	31.3	23.8	3.1	0.6		0.6	7.5						
	Imported	8	[0.2-38.5]	50.0	25.0	16.7					8.3						
Gentamicin	Danish	0	[0.0-2.3]			2.5	4.4	35.0	42.5	14.4	1.3						
	Imported	0	[0.0-26.5]					25.0	50.0	25.0							
Kanamycin	Danish	4	[1.4-8.0]									94.4	0.6	0.6	0.6		3.8
	Imported	8	[0.2-38.5]									83.3	8.3				8.3
Streptomycin	Danish	8	[4.4-13.5]									76.3	15.6			1.3	6.9
	Imported	8	[0.2-38.5]									41.7	50.0				8.3
Vancomycin	Danish	0	[0.0-2.3]			98.8	1.3										
	Imported	0	[0.0-26.5]			100											
Avilamycin	Danish	0	[0.0-2.3]			98.8	1.3										
	Imported	8	[0.2-38.5]			91.7					8.3						
Flavomycin	Danish	3	[0.7-6.3]			96.9		0.6		0.6	1.9						
	Imported	0	[0.0-26.5]			100											
Salinomycin	Danish	0	[0.0-2.3]			98.8	0.6	0.6									
	Imported	0	[0.0-26.5]			100											
Linezolid	Danish	0	[0.0-2.3]		23.8	76.3											
	Imported	0	[0.0-26.5]		41.7	58.3											

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 concentration

Table 41. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from healthy humans (n=54), Denmark*

DANMAP 2004

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs														
			0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	17	[7.9-29.3]			81.5			1.8			16.7						
Chloramphenicol	0	[0.0-6.6]					42.6	55.6	1.8								
Florfenicol	0	[0.0-6.6]				64.8	35.2										
Penicillin	9	[3.1-20.3]				31.5	38.9	20.4	3.7	3.7		1.8					
Erythromycin	28	[16.5-41.6]		13.0	13.0	29.6	16.6	18.5			9.3						
Gentamicin	0	[0.0-6.6]					25.9	40.7	13.0	7.4	13.0						
Kanamycin	7	[2.1-17.9]										42.6	29.6	14.8	5.6	1.8	5.6
Streptomycin	6	[1.2-15.4]										92.6			1.8		5.6
Vancomycin	0	[0.0-6.6]				100.0											
Quinupristin/dalfopristin	35	[22.7-49.4]		18.5	16.7	29.6	35.2										
Avilamycin	0	[0.0-6.6]				70.4	27.8	1.8									
Salinomycin	0	[0.0-6.6]				100.0											
Linezolid	0	[0.0-6.6]			16.7	83.3											

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 42. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from healthy humans (n=63), Denmark*

DANMAP 2004

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs													
			0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	44	[31.9-57.5]		52.4	3.2				3.2	41.2						
Chloramphenicol	11	[4.6-21.6]			12.7	23.8	50.8	1.6	4.7	6.4						
Florfenicol	0	[0.0-5.7]			82.5	17.5										
Penicillin	0	[0.0-5.7]			50.8	47.6	1.6									
Erythromycin	21	[11.5-32.7]	20.6	43.0	7.9	7.9				20.6						
Gentamicin	0	[0.0-5.7]			3.2	14.3	69.8	12.7								
Kanamycin	8	[2.6-17.6]									90.5	1.6				7.9
Streptomycin	8	[2.6-17.6]									90.5	1.6			3.2	4.7
Vancomycin	0	[0.0-5.7]			100											
Avilamycin	0	[0.0-5.7]			95.2	4.8										
Flavomycin	0	[0.0-5.7]			95.2	4.8										
Salinomycin	0	[0.0-5.7]			100											
Linezolid	0	[0.0-5.7]		50.8	47.6	1.6										

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

DANMAP 2004

Compound	Pigs	Pork	Beef		Broilers	Broiler meat		Healthy humans
	Danish %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	%
Tetracycline	48	13	7	26	4	6	75	17
Chloramphenicol	0	0	0	0	0	<1	3	0
Florfenicol	0	0	0	0	0	0	0	0
Penicillin	15	4	0	11	35	10	7	9
Erythromycin	50	21	5	26	28	24	39	28
Gentamicin	0	0	0	0	0	0	0	0
Kanamycin	22	6	2	5	2	<1	6	7
Streptomycin	26	4	2	0	2	2	10	6
Vancomycin	3	0	0	0	2	1	0	0
Quinupristin/dalfopristin	13	13	20	26	24	17	20	35
Avilamycin	0	0	0	0	7	<1	7	0
Salinomycin	0	0	0	0	<1	2	0	0
Linezolid	0	0	0	0	0	0	0	0
Number of isolates	148	52	83	19	135	173	69	54

Tabel 44. Comparison of resistance (%) among Enterococcus faecalis *from food animals, foods of Danish and imported origin and healthy humans, Denmark*

DANMAP 2004

Compound	Pigs	Pork		Beef		Broilers	Broiler meat		Healthy humans
	Danish %	Danish %	Import %	Danish %	Import %	Danish %	Danish %	Import %	%
Tetracycline	86	12	42	18	14	41	43	83	44
Chloramphenicol	10	2	0	2	0	1	0	5	11
Florfenicol	0	0	0	0	0	0	0	0	0
Penicillin	0	0	0	0	0	0	1	0	0
Erythromycin	40	9	8	4	0	18	34	33	21
Gentamicin	9	0	0	2	0	0	0	5	0
Kanamycin	26	4	8	6	0	0	0	8	8
Streptomycin	32	8	8	5	0	0	8	18	8
Vancomycin	0	0	0	0	0	0	0	0	0
Avilamycin	1	0	8	<1	0	0	0	0	0
Flavomycin	<1	3	0	<1	4	1	3	0	0
Salinomycin	0	0	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0	0
Number of isolates	153	160	12	120	28	82	74	40	63

Escherichia coli

Escherichia coli from food animals

Table 45 presents the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates from animals at slaughter. Overall, 447 isolates from broilers, cattle and pigs were collected and susceptibility tested in 2004. Figure 22 presents the trends in resistance to selected antimicrobials from 1996 to 2004.

From 2003 to 2004, significant increases in tetracycline (*P*=0.005), ampicillin (*P*=0.01), sulfonamide (*P*=0.0003), trimethoprim (*P*=0.04), and neomycin (*P*=0.0002) resistance were observed among indicator *E. coli* isolates from pigs. This coincides with increased tetracycline and broad-spectrum penicillin consumption in weaners and finishers, while sulfonamide/ trimethoprim consumption increased only in weaners and neomycin consumption remained unchanged in

Table 45. Distribution of MICs and occurrence of resistance among Escherichia coli *from broilers (n=142), cattle (n=97) and pigs (n=208), Denmark*

DANMAP 2004

Compound	Animal species	% 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	Broilers	11	[6.6-17.7]							88.0	0.7		0.7		10.6					
	Cattle	12	[6.6-20.6]							83.5	4.1				12.4					
	Pigs	44	[36.9-50.8]							52.4	3.4	0.5	0.5	3.4	39.9					
Chloramphenicol	Broilers	0	[0.0-2.6]							6.3	64.8	28.2	0.7							
	Cattle	0	[0.0-3.7]							1.0	39.2	59.8								
	Pigs	9	[5.6-13.9]							2.4	50.5	36.5	1.4	1.4	1.9	5.8				
Florfenicol	Broilers	0	[0.0-2.6]							9.2	74.6	16.2								
	Cattle	0	[0.0-3.7]							3.1	30.9	64.9	1.0							
	Pigs	0	[0.0-1.8]							6.3	48.1	43.8	1.9							
Ampicillin	Broilers	18	[11.7-24.9]						9.2	42.3	28.2	2.1	0.7		17.6					
	Cattle	8	[3.6-15.6]						4.1	35.1	49.5	3.1			8.2					
	Pigs	33	[26.8-40.0]						2.4	39.4	24.5	0.5		1.0	32.2					
Amoxicillin/ clavulanic acid a)	Broilers	<1	[0.02-3.9]							33.1	49.3	16.9		0.7						
	Cattle	0	[0.0-3.7]							25.8	63.9	10.3								
	Pigs	<1	[0.1-3.4]							32.7	36.5	25.5	4.3	1.0						
Cephalothin	Broilers	2	[0.4-6.1]							7.0	21.8	54.2	14.8	0.7	0.7	0.7				
	Cattle	1	[0.03-5.6]							1.0	7.2	71.1	19.6	1.0						
	Pigs	5	[2.3-8.7							1.9	17.3	49.0	26.9	3.4		1.4				
Ceftiofur	Broilers	0	[0.0-2.6]					98.6	0.7	0.7										
	Cattle	0	[0.0-3.7]					99.0	1.0											
	Pigs	0	[0.0-1.8]					99.0	0.5	0.5										
Sulfonamide	Broilers	18	[11.7-24.9]												81.7	0.7		3.5	4.9	9.2
	Cattle	14	[8.1-23.0]												85.6					14.4
	Pigs	47	[39.7-53.7]												53.4				3.8	42.8
Trimethoprim	Broilers	5	[2.0-9.9]								94.4	0.7			4.9					
	Cattle	3	[0.6-8.8]								96.9				3.1					
	Pigs	21	[15.4-26.8]								79.3				20.7					
Apramycin	Broilers	0	[0.0-2.6]								72.5	26.1	1.4							
	Cattle	0	[0.0-3.7]								80.4	17.5	2.1							
	Pigs	3	[1.4-6.8]								86.5	9.6	0.5			3.4				
Gentamicin	Broilers	0	[0.0-2.6]						99.3	0.7										
	Cattle	0	[0.0-3.7]						97.9	1.0	1.0									
	Pigs	3	[1.4-6.8]						92.3	3.4	1.0	1.0	2.4							
Neomycin	Broilers	1	[0.2-5.0]							98.6				0.7	0.7					
	Cattle	0	[0.0-3.7]							92.8	5.2	2.1								
	Pigs	16	[11.2-21.6]							77.4	6.7			1.4	14.4					
Spectinomycin	Broilers	4	[1.2-8.0]								0.7	14.1	72.5	8.5	0.7	2.1	1.4			
	Cattle	3	[0.6-8.8]									1.0	81.4	13.4	1.0	2.1	1.0			
	Pigs	34	[27.3-40.5]									1.4	49.0	8.7	7.2	11.5	22.1			
Streptomycin	Broilers	8	[4.4-14.3]								16.2	69.7	5.6	2.8	4.2	1.4				
	Cattle	18	[10.6-26.6]								19.6	57.7	5.2	3.1	4.1	10.3				
	Pigs	48	[40.7-54.6]								16.8	26.9	8.7	6.3	13.9	27.4				
Ciprofloxacin	Broilers	0	[0.0-2.6]	86.6	1.4	2.8	9.2													
	Cattle	0	[0.0-3.7]	100																
	Pigs	0	[0.0-1.8]	96.6			2.9	0.5												
Nalidixic acid	Broilers	13	[8.3-20.1]									85.2	1.4	1.4	0.7	9.9	1.4			
	Cattle	0	[0.0-3.7]									100								
	Pigs	3	[1.4-6.8]									96.6			0.5	2.9				
Colistin	Broilers	0	[0.0-2.6]								98.6	1.4								
	Cattle	0	[0.0-3.7]								100									
	Pigs	0	[0.0-1.8]								99.5	0.5								

Vertical lines indicate breakpoints for resistance. The dotted line indicates reduced susceptibility to ciprofloxacin.
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

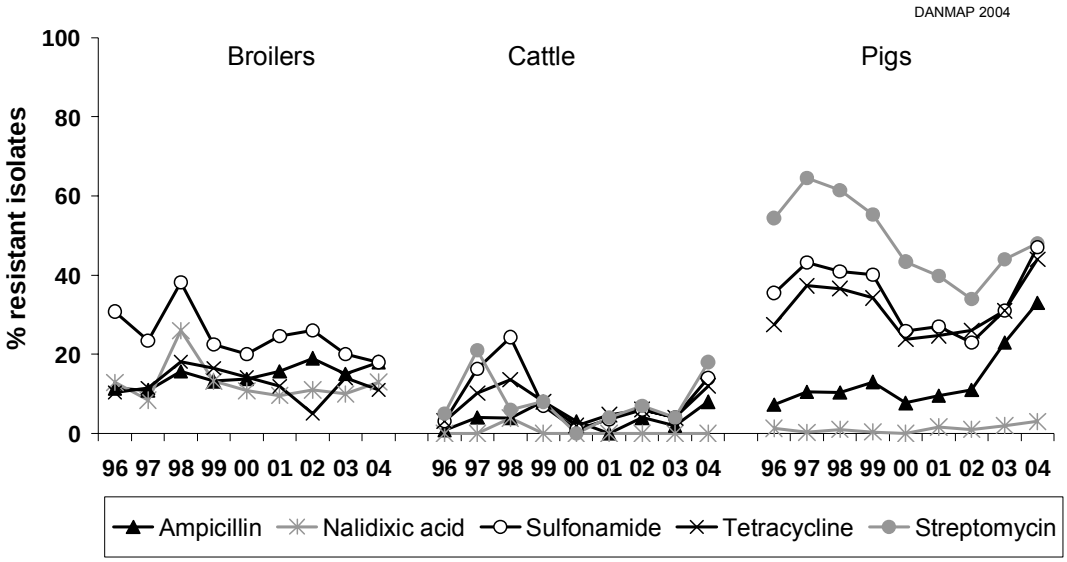

Figure 22. Trends in resistance to selected antimicrobials among Escherichia coli *from food animals, Denmark*

Table 46. Distribution of MICs and occurrence of resistance among Escherichia coli *from broiler meat (Danish n=216; imported n=93 a)), Denmark*

DANMAP 2004

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	9	[5.3-13.4]							90.3	0.9			1.4	7.4					
	Imported	59	[48.5-69.2]							39.8	1.1		1.1	1.1	57.0					
Chloramphenicol	Danish	<1	[0.01-2.6]							1.9	52.3	44.9	0.5			0.5				
	Imported	8	[3.1-14.9]							4.3	39.8	48.4		2.2		5.4				
Florfenicol	Danish	0	[0.0-1.7]							2.8	54.6	42.1	0.5							
	Imported	0	[0.0-3.9]							4.3	44.1	51.6								
Ampicillin	Danish	15	[10.4-20.3]						10.2	43.5	31.0	0.5			14.8					
	Imported	41	[30.8-51.5]							30.1	28.0	1.1			40.9					
Amoxicillin/ clavulanic acid b)	Danish	0	[0.0-1.7]							44.0	43.5	12.5								
	Imported	0	[0.0-3.9]							29.0	33.3	35.5	2.2							
Cephalothin	Danish	3	[1.0-5.9]							4.2	20.4	44.9	27.8	2.8						
	Imported	7	[2.4-13.5]								8.6	44.1	40.9	6.5						
Ceftiofur	Danish	0	[0.0-1.7]					99.5	0.5											
	Imported	0	[0.0-3.9]					98.9	1.1											
Sulfonamide	Danish	15	[10.8-20.8]												83.3	1.4				15.3
	Imported	45	[34.8-55.8]												52.7	2.2				45.2
Trimethoprim	Danish	3	[1.3-6.6]								96.3	0.5			3.2					
	Imported	30	[21.0-40.5]								69.9				30.1					
Apramycin	Danish	0	[0.0-1.7]								79.6	19.4	0.9							
	Imported	0	[0.0-3.9]								68.8	30.1	1.1							
Gentamicin	Danish	0	[0.0-1.7]						93.5	6.0	0.5									
	Imported	3	[0.3-7.6]						88.2	6.5	2.2	1.1			2.2					
Neomycin	Danish	0	[0.0-1.7]							88.0	11.6	0.5								
	Imported	3	[0.7-9.1]							79.6	17.2			2.2	1.1					
Spectinomycin	Danish	1	[0.3-4.0]									3.7	88.9	4.6	1.4	0.5	0.9			
	Imported	15	[8.5-24.0]									2.2	62.4	16.1	4.3	7.5	7.5			
Streptomycin	Danish	7	[3.6-10.6]								27.3	61.1	5.1	1.9	2.8	1.9				
	Imported	31	[22.0-41.6]								9.7	45.2	14.0	4.3	14.0	12.9				
Ciprofloxacin	Danish	0	[0.0-1.7]	94.0		0.9	3.2	1.9												
	Imported	4	[1.2-10.7]	74.2	1.1	5.4	11.8	3.2			1.1	3.2								
Nalidixic acid	Danish	6	[3.2-10.1]									94.0		0.5	3.2	0.5	1.9			
	Imported	24	[15.5-33.6]									75.3	1.1	2.2	7.5	8.6	5.4			
Colistin	Danish	0	[0.0-1.7]								100									
	Imported	0	[0.0-3.9]								100									

Vertical lines indicate breakpoints for resistance. The dotted line indicates reduced susceptibility to ciprofloxacin
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) Two isolates were from imported meat of hen
b) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

the same period. Neomycin, sulfonamide and trimethoprim resistance in indicator *E. coli* isolates from pigs often occurred in combination with ampicillin and tetracycline resistance. Consumption of ampicillin and tetracycline could have selected for neomycin, sulfonamide and trimethoprim resistance. In general, indicator *E. coli* isolates from pigs have become more resistant from 2002 to 2004 (Figure 22).

No significant changes in resistance were observed in indicator *E. coli* isolates from broilers from 2003 to 2004. Among indicator *E. coli* isolates from cattle, sulfonamide (*P*=0.02) and streptomycin (*P*=0.005), resistance significantly increased from 2003 to 2004.

Escherichia coli from food

Tables 46-48 present the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates collected from broiler meat, beef and pork sold at retail outlets. In 2004, 732 *E. coli* isolates were susceptibility tested. The isolates were obtained from Danish broiler meat (n=216), imported broiler meat (n=93), Danish beef (n= 96), imported beef (n=39), Danish pork (n=178), and imported pork (n=10). (See "Comparison of *Escherichia coli* from animals, foods and healthy human volunteers").

Escherichia coli from healthy human volunteers

In 2004, stool samples from 125 healthy human volunteers were collected and 111 *E. coli* isolates were subsequently isolated. Table 49 presents the MIC distributions and occurrence of antimicrobial resistances in these 111 isolates. As in 2002 and 2003, resistance to sulfonamide, ampicillin, tetracycline, and streptomycin were most common. None of the isolates were resistant to gentamicin. Nalidixic acid resistance was observed in one percent of the isolates. No significant change in resistance was observed between 2003 and 2004.

Table 47. Distribution of MICs and occurrence of resistance among Escherichia coli *from beef (Danish n=196; imported n=39 a)), Denmark*

DANMAP 2004

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	9	[5.5-14.1]							87.8	3.1			0.5	8.7					
	Imported	15	[5.9-30.5]							79.5	5.1				15.4					
Chloramphenicol	Danish	1	[0.1-3.6]							1.0	25.5	72.4				1.0				
	Imported	0	[0.0-9.0]							2.6	28.2	66.7	2.6							
Florfenicol	Danish	0	[0.0-1.9]							1.0	26.5	71.9	0.5							
	Imported	0	[0.0-9.0]								20.5	74.4	5.1							
Ampicillin	Danish	8	[4.3-12.3]						0.5	30.6	57.7	3.1	0.5		7.7					
	Imported	13	[4.3-27.4]							33.3	53.8				12.8					
Amoxicillin/ clavulanic acid a)	Danish	0	[0.0-1.9]							20.4	67.3	12.2								
	Imported	0	[0.0-9.0]							23.1	61.5	15.4								
Cephalothin	Danish	5	[2.1-8.5]							0.5	4.6	51.5	38.8	4.1	0.5					
	Imported	21	[9.3-36.5]								10.3	41.0	28.2	15.4	2.6	2.6				
Ceftiofur	Danish	0	[0.0-1.9]					98.5	1.5											
	Imported	3	[0.06-13.5]					97.4					2.6							
Sulfonamide	Danish	7	[4.0-11.7]												92.3	0.5				7.1
	Imported	13	[4.3-27.4]												87.2					12.8
Trimethoprim	Danish	4	[1.8-7.9]								95.9				4.1					
	Imported	5	[0.6-17.3]								94.9				5.1					
Apramycin	Danish	0	[0.0-1.9]								79.6	18.9	1.5							
	Imported	0	[0.0-9.0]								87.2	10.3	2.6							
Gentamicin	Danish	0	[0.0-1.9]						90.8	7.7	1.5									
	Imported	0	[0.0-9.0]						97.4	2.6										
Neomycin	Danish	2	[0.6-5.1]							86.8	9.7	1.5		0.5	1.5					
	Imported	3	[0.1-13.5]							92.3	5.1				2.6					
Spectinomycin	Danish	2	[0.6-5.1]									0.5	84.7	10.2	2.6	1.0	1.0			
	Imported	5	[0.6-17.3]									2.6	87.2	5.1		2.6	2.6			
Streptomycin	Danish	9	[5.5-14.1]								15.8	68.4	6.6	1.0	2.6	5.6				
	Imported	18	[7.5-33.5]								23.1	51.3	7.7	5.1	2.6	10.3				
Ciprofloxacin	Danish	0	[0.0-1.9]	100																
	Imported	3	[0.06-13.5]	97.4								2.6								
Nalidixic acid	Danish	0	[0.0-1.9]									100					0.4			
	Imported	3	[0.06-13.5]									97.4					2.6			
Colistin	Danish	0	[0.0-1.9]								100									
	Imported	0	[0.0-9.0]								100									

Vertical lines indicate breakpoints for resistance. The dotted line indicates reduced susceptibility to ciprofloxacin
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 48. Distribution of MICs and occurrence of resistance among Escherichia coli *from pork (Danish n=178; imported n=10), Denmark*

DANMAP 2004

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	26	[20.1-33.5]							70.2	3.4			1.7	24.7					
	Imported	10	[0.3-44.5]							90.0					10.0					
Chloramphenicol	Danish	2	[0.6-5.7]							5.6	38.8	53.4		1.1	1.1					
	Imported	0	[0.0-30.8]								70.0	20.0	10.0							
Florfenicol	Danish	1	[0.1-4.0]							11.2	38.8	48.3	0.6	1.1						
	Imported	0	[0.0-30.8]							10.0	50.0	40.0								
Ampicillin	Danish	15	[10.2-21.3]						2.8	42.7	36.5	2.8			15.2					
	Imported	10	[0.3-44.5]						10.0	40.0	40.0				10.0					
Amoxicillin/ clavulanic acid a)	Danish	<1	[0.01-3.1]							30.3	52.2	15.2	1.7	0.6						
	Imported	0	[0.0-30.8]							40.0	40.0	20.0								
Cephalothin	Danish	5	[2.0-8.7]							0.6	15.7	53.4	25.8	3.9	0.6					
	Imported	10	[0.3-44.5]								10.0	50.0	30.0	10.0						
Ceftiofur	Danish	0	[0.0-2.1]					100												
	Imported	0	[0.0-30.8]					100												
Sulfonamide	Danish	18	[12.6-24.4]												80.9	1.1			0.6	17.4
	Imported	40	[12.2-73.8]												60.0					40.0
Trimethoprim	Danish	10	[5.7-14.9]								90.4				9.6					
	Imported	30	[6.7-65.2]								70.0				30.0					
Apramycin	Danish	0	[0.0-2.1]								77.0	20.2	2.8							
	Imported	0	[0.0-30.8]								90.0	10.0								
Gentamicin	Danish	0	[0.0-2.1]						94.9	3.9	1.1									
	Imported	0	[0.0-30.8]						100											
Neomycin	Danish	3	[1.2-7.2]							87.1	7.9	1.7		1.1	2.2					
	Imported	0	[0.0-30.8]							90.0	10.0									
Spectinomycin	Danish	13	[8.8-19.4]									3.4	73.0	6.7	3.4	5.6	7.9			
	Imported	0	[0.0-30.8]										80.0	10.0	10.0					
Streptomycin	Danish	28	[21.6-35.3]								20.2	44.9	6.7	4.5	12.4	11.2				
	Imported	30	[6.7-65.2]								40.0	30.0		10.0		20.0				
Ciprofloxacin	Danish	0	[0.0-2.1]	96.1	2.2		1.7													
	Imported	0	[0.0-30.8]	100																
Nalidixic acid	Danish	2	[0.3-4.8]									98.3			0.6	0.6	0.6			
	Imported	0	[0.0-30.8]									100								
Colistin	Danish	0	[0.0-2.1]								99.4	0.6								
	Imported	0	[0.0-30.8]								100									

Vertical lines indicate breakpoints for resistance. The dotted line indicates reduced susceptibility to ciprofloxacin
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

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

There is neither data on treatment effect of fluoroquinolones against *E. coli* with reduced susceptibility to fluoroquinolones, nor any reports on treatment failures. In 2004, it was decided to define indicator *E. coli* isolates with reduced susceptibility to ciprofloxacin as isolates with MIC-values greater than or equal to 0.125 μg/ml and less than 4 μg/ml. *E. coli* isolates with MIC values greater than or equal to 4 μg/ml are considered resistant to ciprofloxacin.

The occurrence of resistance in food animals, Danish food, imported food, and healthy human volunteers are compared in Table 50.

No significant differences in resistance were observed between *E. coli* from broilers and Danish broiler meat, with the exception that nalidixic acid resistance was significantly lower in isolates from Danish broiler meat compared to isolates from broilers. When comparing isolates from Danish and imported broiler meat, the occurrence of tetracycline (P<0.0001), chloramphenicol (P=0.001), ampicillin (P<0.0001), sulfonamide (P<0.0001), trimethoprim (P<0.0001), spectinomycin (P<0.0001), streptomycin (P<0.0001), and nalidixic acid (P<0.0001) resistance was significantly higher among *E. coli* isolates from imported broiler meat than among isolates from Danish broiler meat (Table 46). In addition, the occurrence of reduced susceptibility to ciprofloxacin was significantly higher (P<0.0001) in *E. coli* isolates from imported broiler meat compared to isolates from Danish broiler meat (Table 46).

From 2003 to 2004, no significant change in resistance was observed among *E. coli* from Danish broiler meat. In the same period, sulfonamide resistance (P=0.01) and the occurrence of reduced susceptibility to ciprofloxacin (P=0.006) significantly increased in isolates from imported broiler meat.

In *E. coli* isolates from healthy human volunteers, resistance levels were similar to resistance levels in *E. coli* from Danish meat products and *E. coli* isolates from imported beef and pork for most of the antimicrobials tested. However, *E. coli* isolates from imported broiler meat were significantly more resistant to tetracycline (*P*<0.0001), ampicillin (*P*=0.01), sulfonamides (*P*=0.0006), trimethoprim (*P*=0.007), and nalidixic acid (*P*<0.0001) compared to isolates from human volunteers. In addition, the occurrence of reduced susceptibility to ciprofloxacin was significantly higher (*P*<0.0001) in *E. coli* from imported broiler meat compared to isolates from human volunteers.

Trends in resistance among *E. coli* isolates from pigs, pork and humans to tetracycline, sulfonamide and ampicillin are presented in Figures 23-25. Sulfonamide and tetracycline resistance in *E. coli* from pork and humans were, for most years, lower than the occurrence of resistance in isolates from pigs. The increased consumption of antimicrobials in pigs as well as the concurrent increase in the occurrence of resistance in indicator *E. coli* from pigs, were not reflected in the occurrence of resistance in *E. coli* from Danish pork. In 2004, *E. coli* isolates from pigs were significantly more resistant to tetracycline (*P*=0.0006), chloramphenicol (*P*=0.008), ampicillin (*P*<0.0001), sulfonamide (*P*<0.0001), trimethoprim (*P*=0.004), apramycin (*P*=0.04), gentamicin (*P*=0.04), neomycin (*P*<0.0001), spectinomycin (*P*<0.0001), and streptomycin (*P*=0.0001) than *E. coli* from Danish pork. There is a competition among *E. coli* isolates in meat, which could favor the susceptible isolates due to a higher growth rate when no selective antimicrobial pressure is present.

The sample sizes for imported beef and pork were small, which makes it difficult to detect differences in resistance between *E. coli* isolates from Danish and imported products. However, the occurrence of cephalothin resistance was significantly higher (*P*=0.002) in *E. coli* isolates from imported beef than in isolates from Danish beef. Among all *E. coli* isolates from food one isolate from imported beef was resistant to ceftiofur. This is the first *E. coli* isolate with extended spectrum β-lactamase resistance ever found in foodstuff sold in Denmark.

Table 49. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy humans (n=111), Denmark*

DANMAP 2004

Compound	% 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	17	[10.6-25.4]							82.9				2.7	14.4					
Chloramphenicol	4	[1.0-9.0]							4.5	37.8	54.1			1.8	1.8				
Florfenicol	0	[0.0-3.3]							5.4	58.6	36.0								
Ampicillin	23	[15.9-32.4]						2.7	26.1	43.3	4.5			23.4					
Amoxicillin/ clavulanic acid a)	<1	[0.02-4.9]							18.0	51.4	24.3	5.4	0.9						
Cephalothin	3	[0.6-7.7]							0.9	16.2	58.6	21.6		2.7					
Ceftiofur	0	[0.0-3.3]					99.1	0.9											
Sulfonamide	22	[14.4-30.4]												77.5	0.9			1.8	19.8
Trimethoprim	14	[7.8-21.3]								86.5				13.5					
Apramycin	0	[0.0-3.3]								84.7	15.3								
Gentamicin	0	[0.0-3.3]						99.1		0.9									
Neomycin	0	[0.0-3.3]							99.1	0.9									
Spectinomycin	5	[1.5-10.2]									6.3	78.4	6.3	4.5	3.6	0.9			
Streptomycin	21	[12.1-27.5]								44.2	30.6	3.6	1.8	5.4	14.4				
Ciprofloxacin	0	[0.0-3.3]	98.2	0.9	0.9														
Nalidixic acid	<1	[0.02-4.9]									98.2	0.9			0.9				
Colistin	0	[0.0-3.3]								99.1	0.9								

Vertical lines indicate breakpoints for resistance. The dotted line indicates reduced susceptibility to ciprofloxacin
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 50. Comparison of resistance (%) among Escherichia coli *from Danish animals, foods of Danish and imported origin and humans, Denmark*

DANMAP 2004

Compound	Broilers	Broiler meat		Cattle	Beef		Pigs	Pork		Humans
	Danish	Danish	Imported	Danish	Danish	Imported	Danish	Danish	Imported	
	%	%	%	%	%	%	%	%	%	%
Tetracycline	11	9	59	12	9	15	44	26	10	17
Chloramphenicol	0	<1	8	0	1	0	9	2	0	4
Florfenicol	0	0	0	0	0	0	0	1	0	0
Ampicillin	18	15	41	8	8	13	33	15	10	23
Amoxicillin/ clavulanic acid	<1	0	0	0	0	0	<1	<1	0	<1
Cephalothin	2	3	6	1	5	21	5	5	10	3
Ceftiofur	0	0	0	0	0	3	0	0	0	0
Sulfonamide	18	15	45	14	7	13	47	18	40	22
Trimethoprim	5	3	30	3	4	5	21	10	30	14
Apramycin	0	0	0	0	0	0	3	0	0	0
Gentamicin	0	0	3	0	0	0	3	0	0	0
Neomycin	1	0	3	0	2	3	16	3	0	0
Spectinomycin	4	1	15	3	2	5	34	13	0	5
Streptomycin	8	6	31	18	9	18	48	28	30	22
Ciprofloxacin	0	0	4	0	0	3	0	0	0	0
Nalidixic acid	13	6	24	0	0	3	3	2	0	<1
Colistin	0	0	0	0	0	0	0	0	0	0
Number of isolates	142	216	93	97	196	39	208	178	10	111

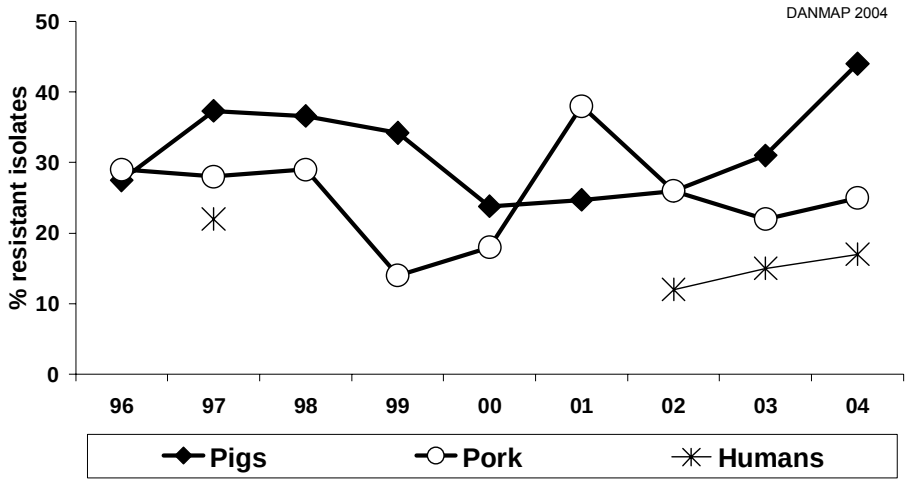

Figure 23. Trends in tetracycline resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

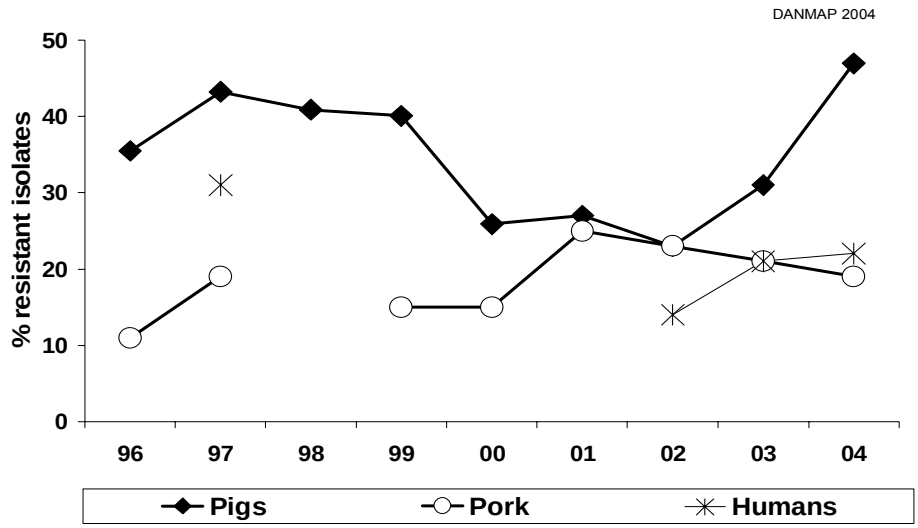

Figure 24. Trends in sulfonamide resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

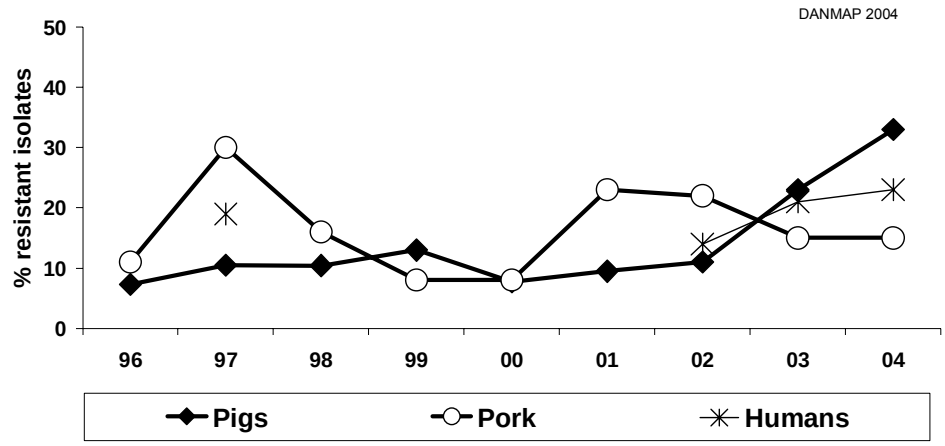

Figure 25. Trends in ampicillin resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

Resistance in bacteria from diagnostic submissions

Bacteria from food animals

The DANMAP programme monitors resistance in the following bacterial species isolated from diagnostic submissions from food animals: *Escherichia coli* from cattle and pigs and *Staphylococcus hyicus* from pigs. Most isolates from diagnostic submissions originate from animals already in antimicrobial therapy, or animals with a history of previous antimicrobial therapy. For this reason a higher frequency of resistance is expected in bacterial isolates from diagnostic submissions compared with indicator bacteria isolates originating from healthy animals sampled at slaughter.

Escherichia coli

The MIC distributions and the occurrence of resistance in *E. coli* isolates from cattle and pigs are presented in Table 51. Figure 26 presents trends in resistance to selected antimicrobials among *E. coli* from pigs and cattle. From 2003 to 2004, significant increases in sulfonamide (P=0.02) and neomycin (P=0.02) resistance were observed among *E. coli* isolates from diagnostic submissions from cattle. Among *E. coli* from diagnostic submissions from pigs no significant changes in resistance were observed from 2003 to 2004.

Staphylococci

The *Staphylococcus hyicus* isolates originated from skin infections in pigs, e.g. 'greasy pig disease'. The MIC distributions and the occurrence of resistance among *S. hyicus* from pigs are presented in Table 52. Trends in resistance to some selected antimicrobials from diagnostic submissions from pigs are presented in Figure 27.

Resistance to penicillin among *S. hyicus* isolates from pigs increased significantly from 54% in 2000 to 84% in 2003 and remained at a high level (78%) in 2004. Methicillin-resistance was not observed. For all other antimicrobials in the test panel, the frequency of resistance was unchanged from 2000 to 2004, except for an increase in resistance to tetracycline in 2001.

Table 51. Distribution of MICs and occurrence of resistance among Escherichia coli *from diagnostic submissions from cattle (n=32) and pigs (n=49), Denmark*

DANMAP 2004

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs															
				0,03	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Cattle	81	[63.6-92.8]						18.8				3.1	78.1					
	Pigs	86	[72.8-94.1]						14.3			2.0	6.1	77.6					
Chloramphenicol	Cattle	19	[7.2-36.4]							21.9	59.4				18.8				
	Pigs	37	[23.4-51.7]						4.1	46.9	10.2	2.0	8.2	2.0	26.5				
Florfenicol	Cattle	3	[0.1-16.2]							28.1	68.8				3.1				
	Pigs	0	[0.0-7.3]						14.3	65.3	20.4								
Ampicillin	Cattle	78	[60.0-90.7]						12.5	6.3		3.1		78.1					
	Pigs	49	[34.4-63.7]					10.2	30.6	8.2		2.0		49.0					
Amoxicillin/ clavulanic acid a)	Cattle	0	[0.0-10.9]						9.4	18.8	56.3	15.6							
	Pigs	2	[0.1-10.9]						44.9	18.4	28.6	6.1		2.0					
Cephalothin	Cattle	6	[0.8-20.8]						3.1	3.1	65.6	21.9	6.3						
	Pigs	10	[3.4-22.2]							18.4	51.0	20.4	4.1		6.1				
Ceftiofur	Cattle	0	[0.0-10.9]				96.9	3.1											
	Pigs	0	[0.0-7.3]				98.0	2.0											
Sulfonamide	Cattle	91	[75.0-98.0]											9.4				3.1	87.5
	Pigs	76	[61.1-86.7]											24.5				6.1	69.4
Trimethoprim	Cattle	69	[50.0-83.9]							31.3				68.8					
	Pigs	41	[27.0-55.8]							59.2				40.8					
Apramycin	Cattle	0	[0.0-10.9]							96.9	3.1								
	Pigs	16	[7.3-29.7]							83.7					16.3				
Gentamicin	Cattle	6	[0.8-20.8]					90.6	3.1					6.3					
	Pigs	20	[10.2-34.3]					77.6	2.0		8.2	8.2	2.0	2.0					
Neomycin	Cattle	41	[23.7-59.4]						59.4				9.4	31.3					
	Pigs	33	[20.0-47.5]						65.3	2.0			4.1	28.6					
Spectinomycin	Cattle	28	[13.8-46.8]									40.6	9.4	21.9	12.5	15.6			
	Pigs	59	[44.2-73.0]									30.6	2.0	8.2	12.2	46.9			
Streptomycin	Cattle	84	[67.2-94.7]							6.3	9.4		6.3	25.0	53.1				
	Pigs	67	[52.5-80.1]							12.2	10.2	10.2	24.5	14.3	28.6				
Ciprofloxacin	Cattle	6	[0.8-20.8]	75.0	6.3	9.4	3.1				6.3								
	Pigs	0	[0.0-7.3]	69.4	8.2	20.4		2.0											
Nalidixic acid	Cattle	25	[11.5-43.4]								75.0			3.1	12.5	9.4			
	Pigs	29	[16.6-43.3]								71.4		2.0	14.3	8.2	4.1			
Colistin	Cattle	0	[[0.0-10.9]							100									
	Pigs	0	[0.0-7.3]							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

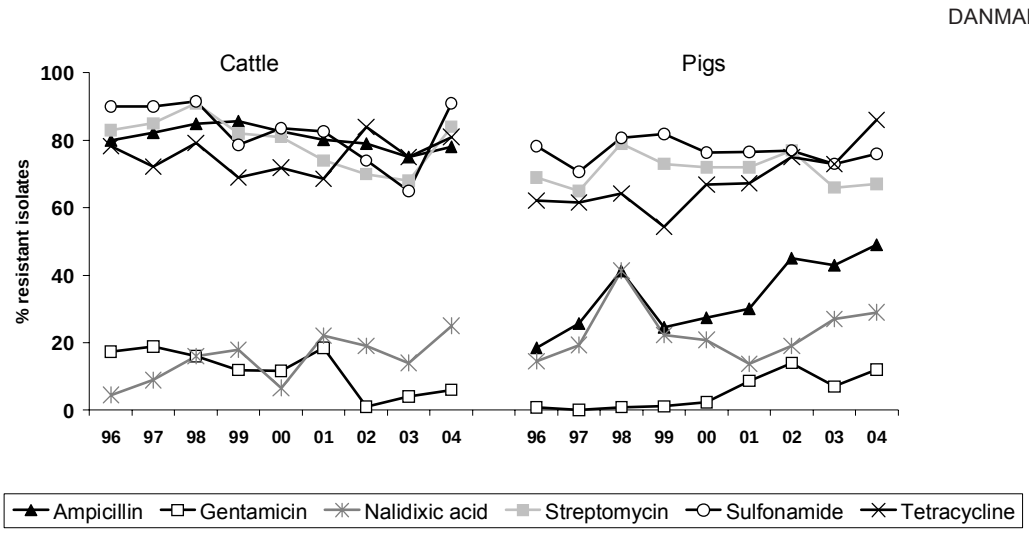

Figure 26. Trends in resistance to selected antimicrobials among Escherichia coli *from diagnostic submissions from animals, Denmark*

Table 52. Distribution of MICs and occurrence of resistance among Staphylococcus hyicus *from pigs (n=68), Denmark*

DANMAP 2004

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	24	[14.1-35.4]				63.2	13.2				2.9	16.2	4.4				
Chloramphenicol	1	[0.04-7.9]							80.9	17.6			1.5				
Florfenicol	0	[0.0-5.3]					1.5	91.2	7.4								
Penicillin	78	[66.2-87.1]	22.1			2.9	5.9	13.2	7.4	19.1	17.6	11.8					
Ceftiofur	0	[0.0-5.3]				42.6	55.9	1.5									
Sulfonamide	0	[0.0-5.3]								25.0	38.2	25.0	4.4	7.4			
Trimethoprim	15	[7.3-25.4]					57.4	16.2	10.3	1.5	1.5		13.2				
Erythromycin	13	[6.2-23.6]			2.9	80.9	2.9					13.2					
Gentamicin	0	[0.0-5.3]						100									
Spectinomycin	12	[5.2-21.9]										26.5	61.8	2.9		8.8	
Streptomycin	31	[20.2-43.3]						17.6	38.2	8.8	4.4	1.5	4.4	5.9	19.1		
Ciprofloxacin	0	[0.0-5.3]		79.4	17.6	2.9											
Vancomycin	0	[0.0-5.3]						100									
Quinupristin/dalfopristin	3	[0.4-10.2]				83.8	8.8	4.4	2.9								
Avilamycin	1	[0.04-7.9]						76.5	20.6	1.5	1.5						
Tiamulin	22	[12.9-33.8]				8.8	63.2	1.5		1.5	2.9	5.9	16.2				

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

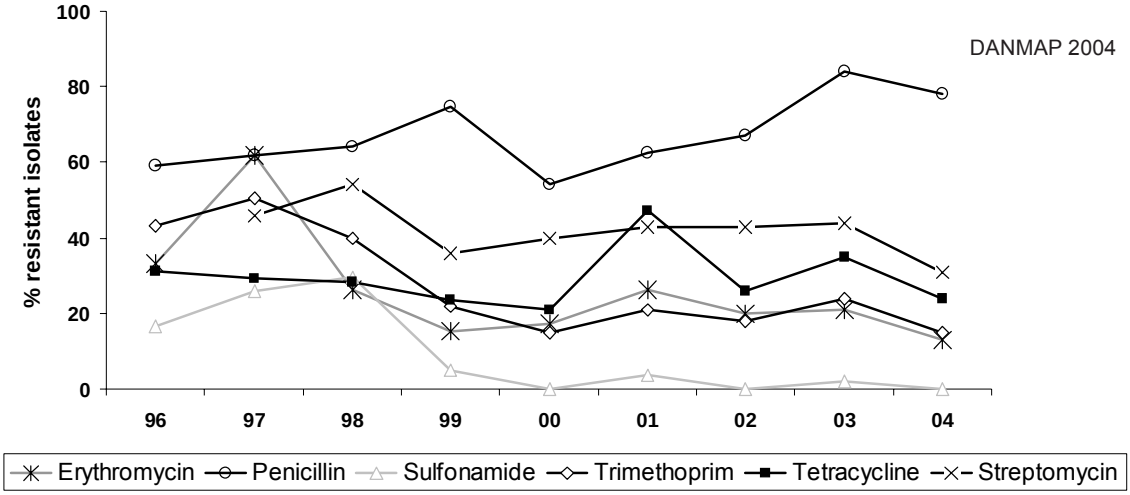

Figure 27. Trends in resistance to some selected antimicrobials among Staphylococcus hyicus *from diagnostic submissions from pigs, Denmark*

Bacteria from humans

Data on resistance levels in *Streptococcus pneumoniae* isolates, as well as *Salmonella* spp. and *Campylobacter* spp. isolates cover all 16 counties in Denmark. Data on resistance levels in *Staphylococcus aureus* isolates cover 15 counties in Denmark. 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, Ringkoebing, Aarhus, Viborg, and North Jutland, representing 95% of the Danish population. Demographic data is presented in Table 2.

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 1995-2004 are presented for each county in Figures 28-30, showing resistance in blood and urine isolates in *E. coli* to selected antimicrobials.

In *E. coli* blood isolates the generally high level of ampicillin resistance remained between 30 and 50%, with only one hospital, i.e. Rigshospitalet, reporting a level above 50% (Figure 28). Gentamicin resistance in *E. coli* blood isolates was reported from 11 counties between 2002 and 2004. It was 1.3% in 2002, 1.2% in 2003 and increased significantly to 1.7% in 2004 ($P<0.05$). Cefuroxime resistance in *E. coli* blood isolates was reported from 9 counties between 2002 and 2004. It significantly increased from 1.9% in 2002 to 2.7% in 2004 ($P<0.05$) (excluding Rigshospitalet). At the national referral hospital, Rigshospitalet, cefuroxime resistance in *E. coli* blood isolates increased from 2.3% in 2002 to 12% in 2004 ($P=0.005$) (Figure 28). High consumption of ceftriaxone was pointed out as a likely reason for the increased cefuroxime resistance found at this hospital in 2004. Recommendations for treatment, as well as patient isolation practices, were reinforced accordingly (Høiby N, personal communication). As registered by the Danish Medicines Agency, the consumption of second and third generation cephalosporins at Rigshospitalet has decreased by 12% and 24%, respectively, in 2004, as compared to 2003.

Data on resistance in *E. coli* urine isolates from primary health care in all participating counties are presented in Figure 29. Overall, ampicillin resistance in *E. coli* urine isolates from primary health care did not show any change in 2004 compared to 2003 and remained high at an average 39% (95% CI: 38.6-39.9). Sulfonamide resistance in *E. coli* urine isolates from primary health care was in average 37% (95% CI: 36.0-37.3), thus remaining unchanged compared to 2003 with the exception of Aarhus county. In this county, there was a significant decrease in sulfonamide resistance in *E. coli* urine isolates from 37.0% in 2004 to 34.1% in 2003 ($P=0.02$). This decrease in sulfonamide resistance was concomitant to an average 7% annual decrease in sulfonamide consumption in primary health care in Aarhus county since 2001. The high level of resistance to ampicillin and sulfonamides in *E. coli* from urine makes these drugs 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. As reported in DANMAP 2002, ampicillin and sulfonamide resistance in *E. coli* isolates from uncomplicated urinary tract infections in primary health care in Denmark is at around 20%.

Data on ciprofloxacin resistance in *E. coli* urine isolates from primary health care in 2004 were available from ten counties (see Figure 29**)**, representing 69% of the Danish population. A significant increase in resistance to ciprofloxacin was observed: from 1.9% in 2003 to 2.9% in 2004 ($P<0.0001$). Among counties reporting data in 2003, this increase was also highly significant ($P<0.0001$). The increase in ciprofloxacin resistance was concomitant to the increase in fluoroquinolone consumption in primary health care observed in Denmark since 2002 (see Table 13). Between 2003 and 2004 and for the ten counties which reported resistance data, consumption of fluoroquinolones in primary health care increased from 0.25 to 0.31 DDD per 1,000 inhabitant-days. 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. The main indications for prescribing ciprofloxacin are complicated and recurrent urinary tract infections, infections caused by bacteria resistant to other antibiotics, pyelonephritis, and certain gastrointestinal infections. In case of severe 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. The level of resistance to fluoroquinolones in *E. coli* is still low in Denmark, but the recent increase is significant and alarming.

Price is an important issue when prescribing drugs, however, the choice of antibiotic treatment should be based on recommendations rather than on price. 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 (EPI-NEWS 2004, no. 41: http://www.ssi.dk/sw18090.asp).

Data on resistance in urine isolates from hospitals in all participating counties are presented in Figure 30. There were no significant changes in ampicillin and sulfonamide resistance in *E. coli* urine isolates from hospitals. Of ten counties reporting data in 2004, three counties showed a significant increase in ciprofloxacin resistance in hospitals, as compared to 2003 (P=0.006 to P<0.0001). The overall comparison between eight counties providing data in 2003 and 2004 was an average increase from 2.3% to 3.1% (P<0.0001). This increase was concomitant to the steady increase in consumption of fluoroquinolones reported from both hospitals and primary health care in recent years.

Methicillin-resistant *Staphylococcus aureus*

In 2004, the number of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in Denmark continued to increase to reach 411 cases, which is approximately twice the number reported in 2003. Most infections were acquired in Denmark (90%) and had a community onset (62%). Community onset infections were dominated by skin and soft tissue infections. However, more alarmingly, the number of MRSA bacteraemia cases increased to 19 or 1.2% of all *S. aureus* bacteraemia cases in 2004, which is the highest reported number since the mid-1970s (See Report 2).

Coagulase-negative staphylococci

In 2004, the average level of penicillin resistance in coagulase-negative staphylococci blood isolates remained close to 80% (min. 70% - max. 91%), and has since 1996 been subject to small variations only. Resistance to erythromycin was unchanged compared to 2003 and averaged 36% (min. 25% - max. 61%). Resistance to methicillin was on average 46% (min. 21% - max. 80%). In nine counties reporting methicillin resistance data in 2003 and 2004, resistance increased from 43.5% to 50.5%. However, as stated in previous reports, it is 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 comparison of resistance levels between counties.

Streptococcus pneumoniae

The national reference center at Statens Serum Institut performs typing and susceptibility testing on *S. pneumoniae* isolates referred by the clinical microbiology laboratories in Denmark. In 2004, susceptibility testing was performed on 1,516 non-duplicate isolates from blood or cerebrospinal fluid samples. In 2004, the percentage of *S. pneumoniae* isolates not susceptible (resistant plus intermediate isolates) to penicillin remained at about 2%, which is much lower than reported in many other European countries (Figure 31).

Macrolide resistance in *S. pneumoniae* isolates from blood and spinal fluid has been around 5% since 2000. In 2004, this resistance decreased to 3.7% (P=0.06) despite a 10% increase in macrolide consumption between 2000 and 2004. However, there have been changes in the distribution of macrolides used between 2001 and 2004 with a 124% increase in roxithromycin consumption (from 0.29 to 0.65 DDD/1,000 inhabitant-days) and concomitant decreases in azithromycin and erythromycin consumption by 18% and 17%, respectively (also see human consumption in primary health care sector).

***Streptococcus pyogenes* (group A Streptococcus, GAS)**

In 2004, data were reported on 6,514 non-invasive GAS isolates from clinical samples in 12 counties. Resistance to macrolides in GAS isolates increased non-significantly from 1.8% in 2003 to 2.2% in 2004. County-to-county variations ranged from 0.0% to 5.0% in 2004. In 2004, data on 129 invasive GAS isolates were reported. Resistance to macrolides in invasive isolates was 1.6% in 2004 compared to 1.8% in 2003. As in previous years, resistance to penicillin in GAS was not reported in 2004.

Report 2

Staphylococcus aureus bacteraemia and methicillin-resistant *S. aureus*

In Denmark, nationwide surveillance of *Staphylococcus aureus* bacteraemia and of methicillin-resistant *S. aureus* (MRSA) is performed through referral of isolates from the local clinical microbiology laboratories to the Staphylococcus Laboratory, Statens Serum Institut. Clinical and epidemiological information regarding cases is retrospectively requested from hospitals and general practitioners.
In 2004, *S. aureus* bacteraemia isolates were submitted by 15 of the 16 local clinical microbiology laboratories, whereas MRSA isolates from bacteraemia and all other types of infection were submitted by all laboratories. Based on the clinical information, MRSA isolates were classified as "screening" or "infection" and according to origin ("imported", "hospital acquired", "community onset", or "unknown"). Community onset MRSA infections were further divided according to risk factors ("healthcare associated", "community risk", or "no apparent risk" (for further details see Faria *et al.* J. Clin. Microbiol. 2005;43:1936-42)). Only new cases, i.e. without recorded prior isolation of MRSA since 1997, are reported. The results of this surveillance are published annually and can be found at http://www.ssi.dk/sw3425.asp.

In 2004, a total of 1,575 *S. aureus* bacteraemia cases were reported corresponding to an incidence of 0.306/100,000 inhabitants. This is the highest number of *S. aureus* bacteraemia cases yet reported in Denmark.

In 2004, a total of 550 new MRSA cases were found and the numbers has thereby doubled each of the last two years. The number of new MRSA cases is now 10 times higher than in the mid-1990s (Figure 1).
Seventy-four percent of the cases were infections, whereas active screening accounted for 26% of cases. Ninety percent of infections were acquired in Denmark. The distribution of all cases according to origin is shown in figure 2. Sixty-two percent of the infections had a community onset and 41% of the infections were considered to be community acquired (CA-MRSA), i.e. community onset with community risk or no risk factor. CA-MRSA infections mainly were skin and soft tissue infections (88%). Among the 116 hospital acquired MRSA infections, 24% were bacteraemias or deep wound infections. Overall, the number of MRSA bacteraemia cases has increased from 7 (0.5% of *S. aureus* bacteraemia cases) in 2003 to 19 (1.2%) in 2004, which is the highest reported number since the mid-1970s.

In conclusion, both the number of *S. aureus* bacteraemia cases and the number of MRSA infections has increased in Denmark. The latter is of utmost concern as it confirms the increase in MRSA cases reported in 2003.This increase is predominantly seen in the community and parallels the increase in MRSA seen in other low prevalence countries with a "search and destroy" MRSA policy. However, the increase in MRSA in Denmark is also observed for hospital acquired infections, where it is responsible for severe infections including bacteraemia. In response to the increase in and the changing epidemiology of MRSA in Denmark, the national guidelines for control of MRSA are presently being revised and efforts are made to make reporting of MRSA infection and carriage mandatory.

For further information: Robert Skov (rsk@ssi.dk)

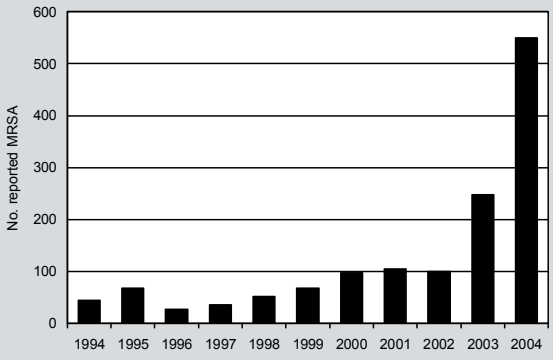

Figure 1. Reported methicillin-resistant *Staphylococcus aureus* (MRSA), Denmark, 1994-2004.

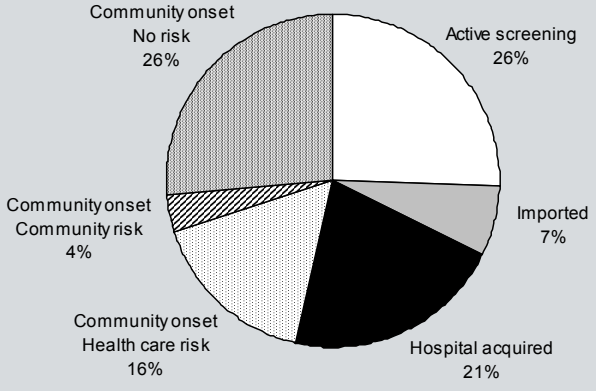

Figure 2. Distribution of reported methicillin-resistant *Staphylococcus aureus* (MRSA) according to origin, Denmark, 2004.

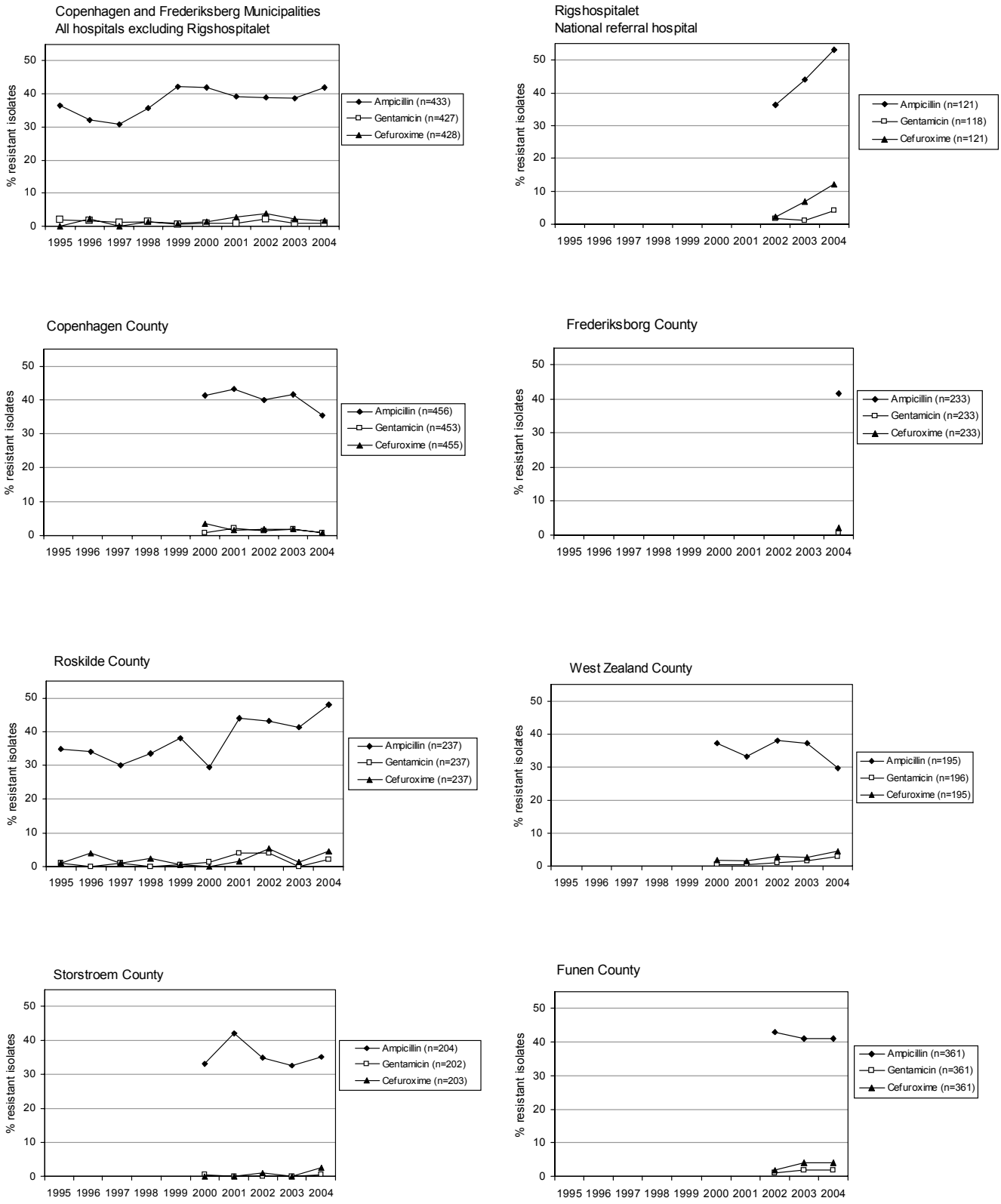

Figure 28. Resistance (%) to ampicillin, gentamicin and cefuroxime in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

DANMAP 2004

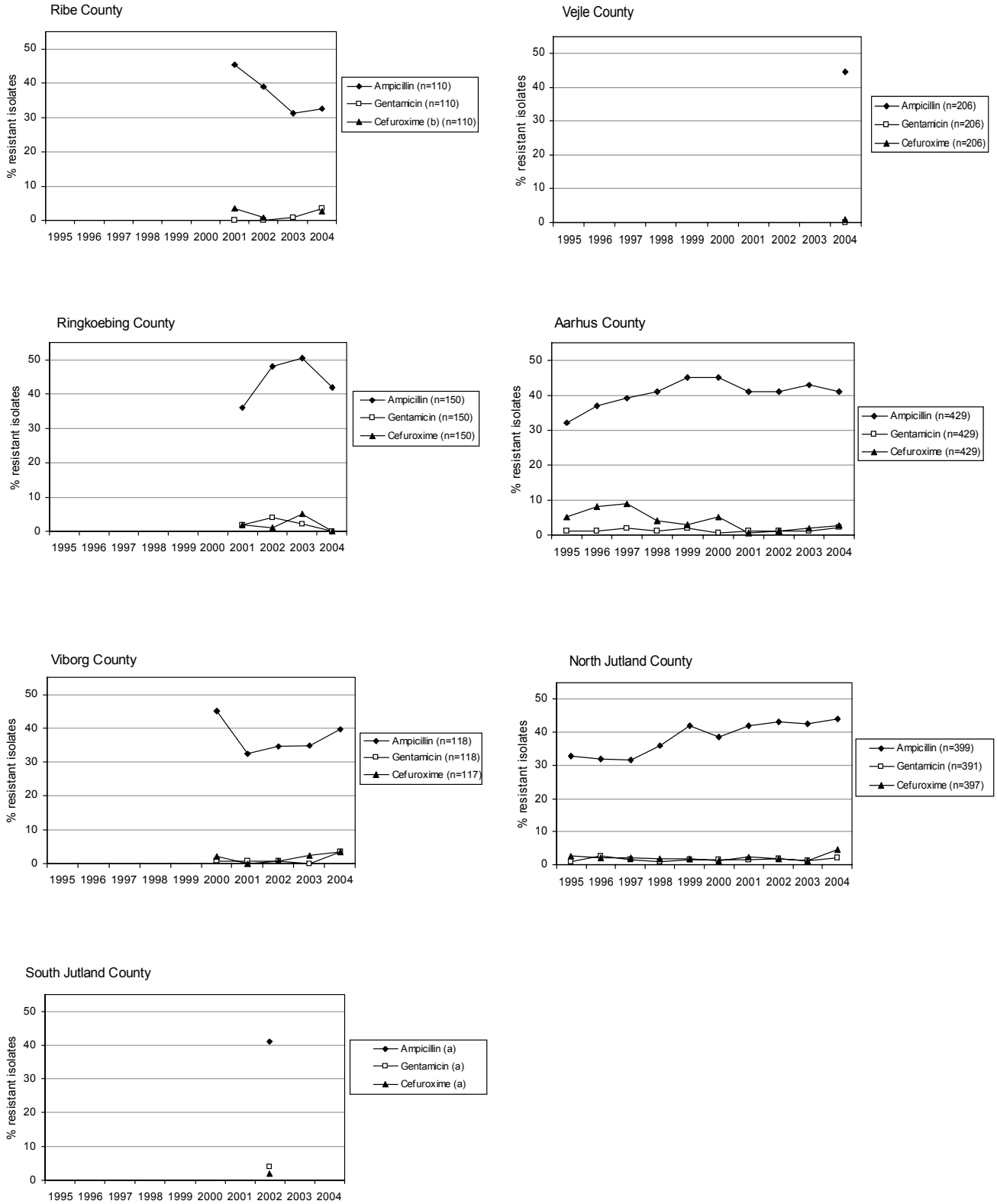

Figure 28. (Continued) Resistance (%) to ampicillin, gentamicin and cefuroxime in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

(a) South Jutland County, data for 2002 only
(b) Ribe County, cefuroxime, data for 2001, 2002 and 2004 only

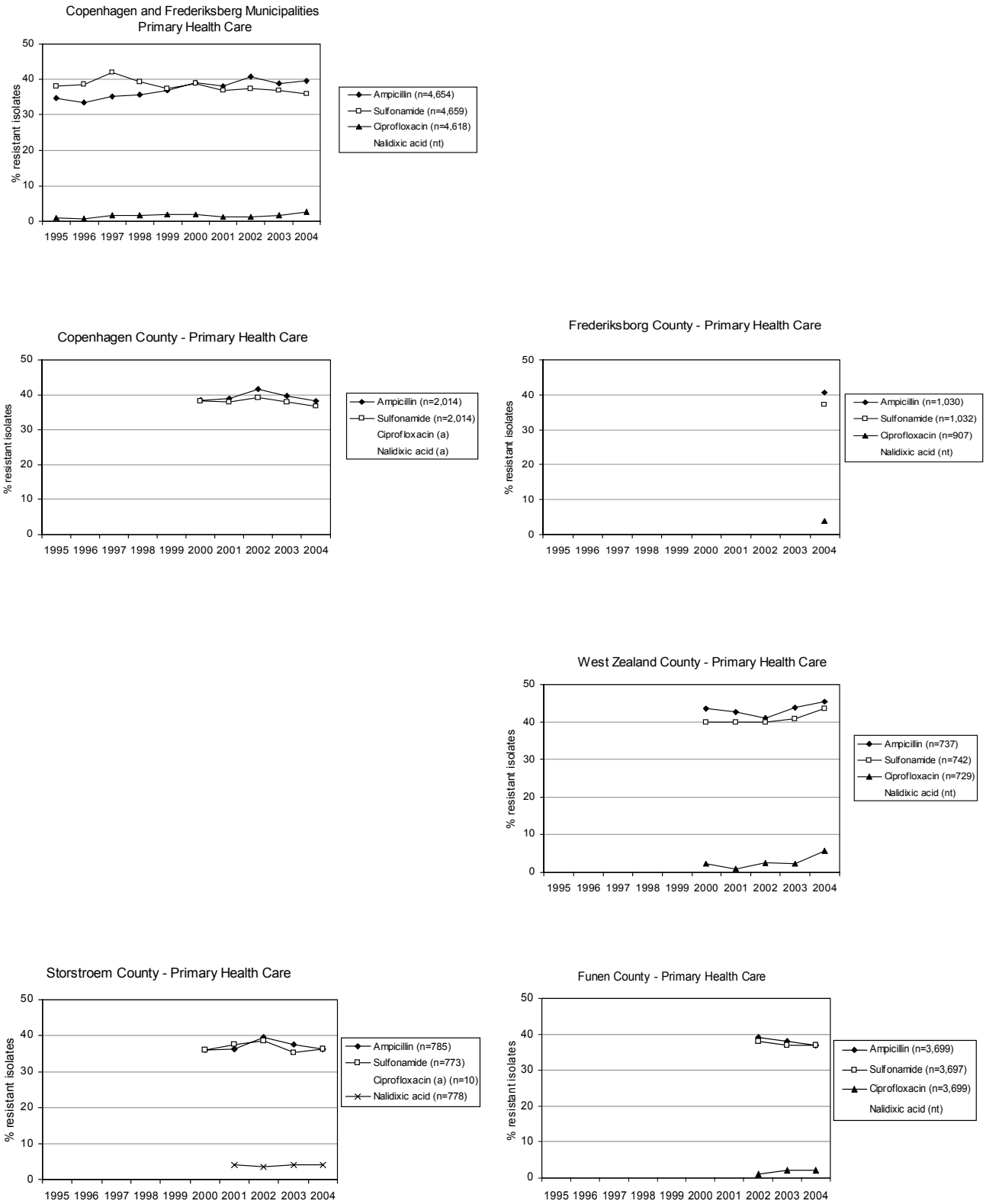

Figure 29. Resistance (%) to ampicillin, sulfonamide, ciprofloxacin, and nalidixic acid in Escherichia coli *urine isolates from humans in primary health care by county, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

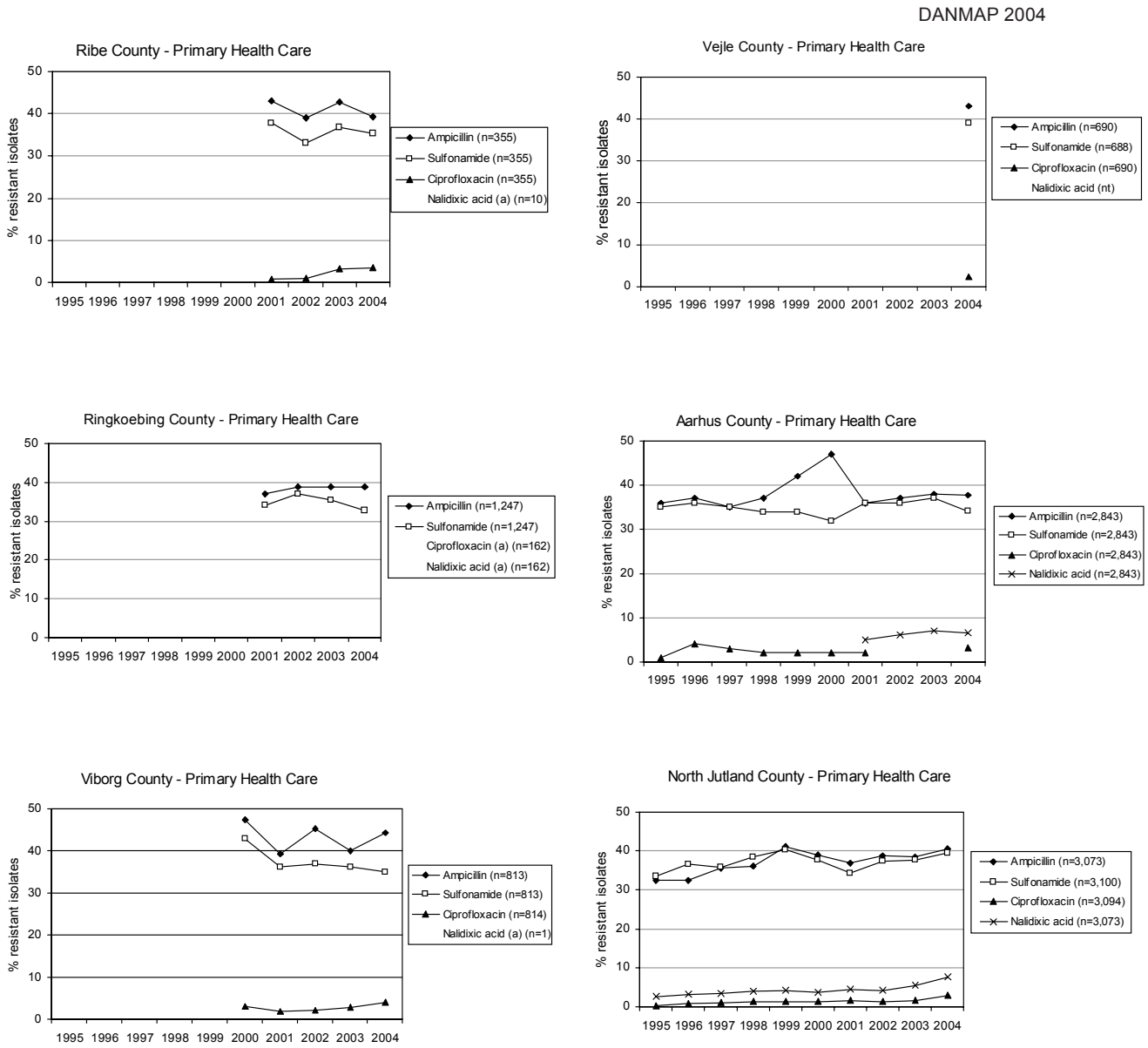

Figure 29. (Continued) Resistance (%) to ampicillin, sulfonamide, ciprofloxacin, and nalidixic acid in Escherichia coli *urine isolates from humans in primary health care by county, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

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

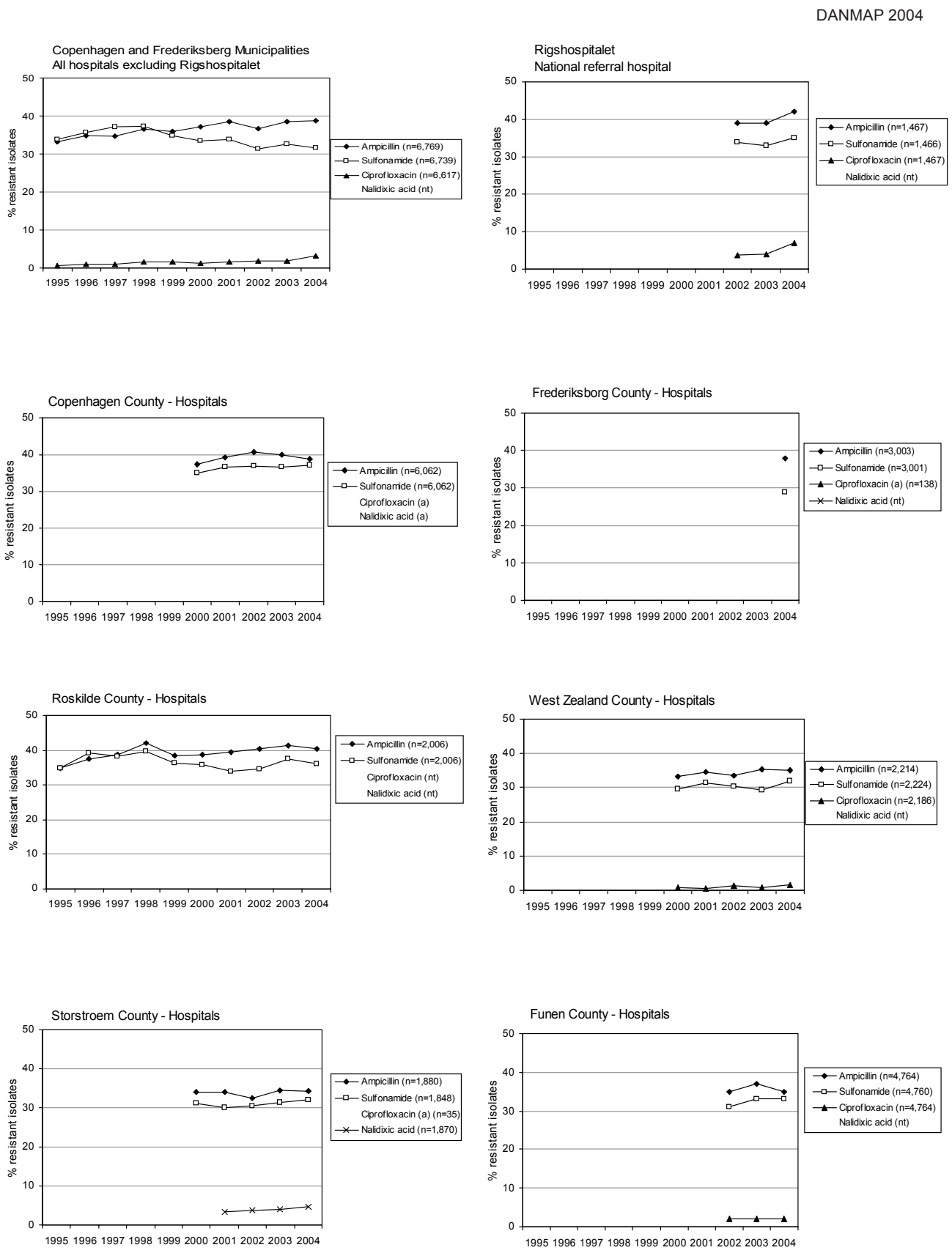

Figure 30. Resistance (%) to ampicillin, sulfonamide, ciprofloxacin, and nalidixic acid in Escherichia coli *urine isolates from humans in hospitals by county, Denmark. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

DANMAP 2004

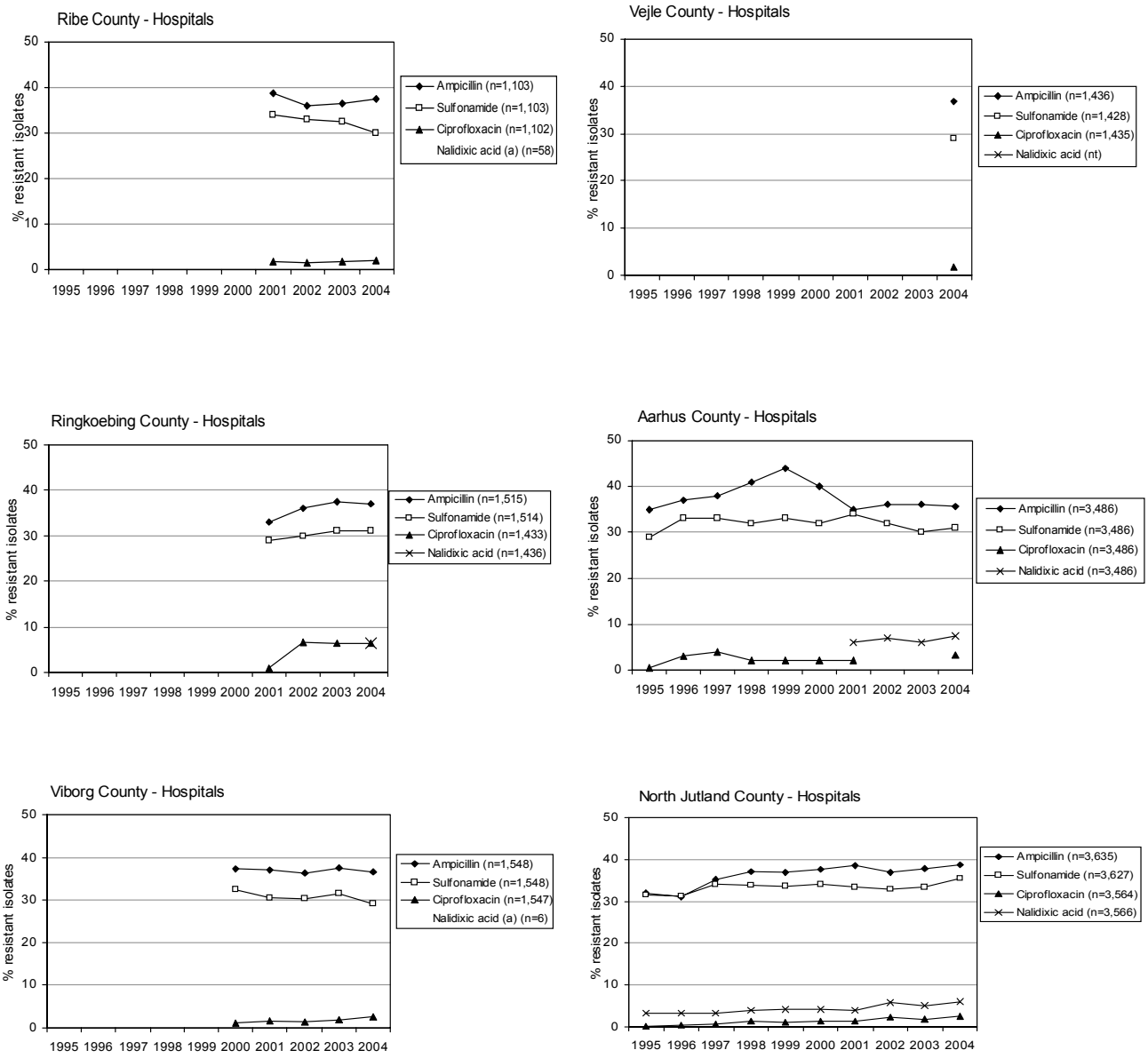

Figure 30. (Continued) Resistance (%) to ampicillin, sulfonamide, ciprofloxacin and nalidixic acid in Escherichia coli *urine isolates from humans in hospitals by county, Denmark. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2004*

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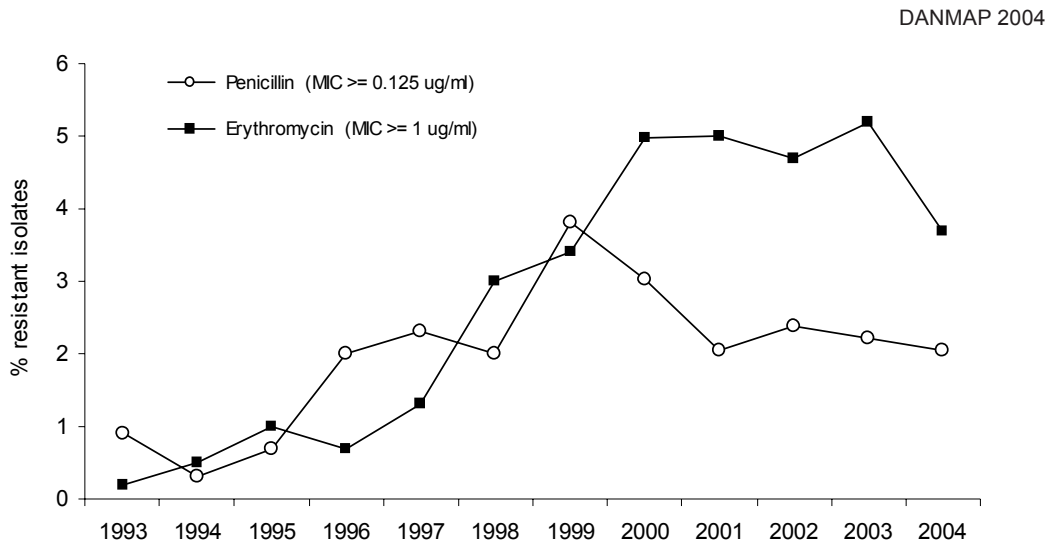

Figure 31. Resistance (%) to penicillin and macrolides in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

Appendix 1

Materials and Methods

Materials and methods

Data on consumption of antimicrobials

Consumption of antimicrobials 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 (99%). 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.

Antibacterials used in humans and/or animals are presented in Table A1.

Consumption of antimicrobials 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) and consumption of antimicrobials in hospitals is expressed as a number of DDD per 1,000 occupied beds and per day (DDD/1,000 bed-days). Since antimicrobial consumption expressed as DDD/1,000 bed-days does not necessarily reflect changes in hospital activity and production, consumption is also presented as DDD/1,000 discharged patients.
Data on the number of bed-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*), as well as from diagnostic submissions (*Staphylococcus hyicus* from pigs, *E. coli* from diarrhoea in cattle and pigs, *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 to the Danish Institute for Food and Veterinary Research (DFVF) for examination. The number of samples for each plant has been determined in proportion to the number of animals slaughtered per year. Each sample represents one herd or flock. They are collected once a month (weekly for broilers). The broiler, cattle and pig slaughter plants included in the surveillance programme account for 95%, 90% and 95%, respectively, of the total production of these animal species in Denmark. Accordingly, the bacterial isolates may be

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.

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ATC/ATCvet codes	Therapeutic group	Names of antibacterials in group
J01AA/*QJ01AA/QJ51AA*	Tetracyclines	Doxycycline, *chlortetracycline* , lymecycline, oxytetracycline, tetracycline
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*
J01DC	Second-generation cephalosporins	Cefuroxime
J01DD/*QJ01DD/QJ51DA*	Third-generation cephalosporins	Cefotaxime, ceftazidime, ceftriaxone, *ceftiofur, cefopezazone*
J01DE/*QJ51DA*	Fourth-generation cephalosporins	Cefepime, *cefquinome*
J01DF	Monobactams	Aztreonam
J01DH	Carbapenems	Meropenem, imipenem/cilastatin, ertapenem
J01EA	Trimethoprim and derivatives	Trimethoprim
J01EB/*QJ01EQ*	Short-acting sulfonamides	Sulfamethizole, *sulfadimidine*
J01EE/*QJ01EW*	Comb. of sulfonamides and trimethoprim, incl. derivatives	Sulfamethoxazole/trimethoprim, *sulfadiazine/trimethoprim* , *sulfadoxine/trimethoprim* , *sulfatroxazole/trimethoprim*
J01FA/*QJ01FA*	Macrolides	Erythromycin, *spiramycin* , roxithromycin, clarithromycin, azithromycin, *tylosin* , *tilmicosin, acetylisovaleryltylosin,*
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*	Other antibacterials	*Spectinomycin* , methenamine, linezolid, *tiamulin, valnemulin*
QP51AH	Pyranes and hydropyranes (ionophores)	*(Monensin, salinomycin)*
Not in ATCvet	Oligosaccharides	*(Avilamycin)*
Not in ATCvet	Flavofosfolipols	*(Flavomycin)*

a) Antibacterials for intrammamary use in animals are included. Antibacterials 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

regarded as representing a stratified random sample of the respective populations, so that the occurrence of resistance provides an estimate of the true occurrence in the populations.

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

Bacterial isolates from diagnostic submissions are selected by a pseudo-random process among isolates from submissions to the DFVF from the laboratory of the Federation of Danish Pig Producers and Slaughterhouses, 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) during the course of routine inspection carried out by the authorities, or on request specifically for the DANMAP surveillance programme. The collection of food samples for analyses of indicator bacteria (enterococci and *E. coli*) was planned and coordinated by the DFVF. The collected material consisted of Danish and imported foods. The food samples were collected according to the guidelines for microbiological examination of foods from the Danish Veterinary and Food Administration (Vejledning om mikrobiologisk kontrol af fødevarer, ISBN: 87-90978-46-3).

Isolates from humans

***Salmonella* spp. and *Campylobacter* spp.**
Antimicrobial susceptibility was tested 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. With a calculated response rate of 20% in total, 988 individuals were invited to participate in the study in 2004. 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 Unit of Gastrointestinal Infections at 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.

Streptococcus pneumoniae. All blood and spinal fluid isolates nationwide are sent to the Streptococcus Unit (national reference laboratory) at SSI for confirmation of susceptibility testing and typing.

Escherichia coli,* coagulase-negative staphylococci, *Streptococcus pyogenes. Data were 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, Storstroem, Funen, Ribe, Vejle, Ringkoebing, Aarhus, Viborg and North Jutland.

Isolation of bacteria

Examination of samples from animals

***Salmonella* spp.** Examination of samples from cattle and pigs was done by non-selective pre-enrichment of 22 g material in 200 ml of buffered peptone water (BPW) and incubated overnight at 37°C. A plate with Modified Semi-solid Rappaport-Vassiliadis medium was inoculated with 0.1 ml of BPW deposited on the agar as 3 drops. Overnight incubation at 41.5°C was followed by serotyping of suspect colonies by slide agglutination.

Samples from poultry were examined by non-selective pre-enrichment in BPW of paired sock samples, or homogenized organs, at a ratio of 1:9 and incubated at 37°C overnight, followed by selective enrichment by inoculation of 9.9 ml Rappaport-Vassiliadis broth with 0.1 ml pre-enrichment broth and incubation at 41.5°C overnight. The selective broth was inoculated onto Rambach agar. Presumptive *Salmonella* isolates were verified and typed by slide agglutination.

***Campylobacter* spp.** The samples were examined by direct inoculation of selective agar as well as by selective enrichment. As selective agar we used mCCD agar, which was incubated in micro-aerophilic atmosphere for 1-3 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 this enrichment culture was inoculated onto mCCD agar and incubated 1 - 3 days at 42°C. Campylobacter-like colonies were identified by their catalase activity, by their ability to hydrolyse hippurate and indoxyl acetate. For isolates from cattle and pigs, also oxidase activity was tested.

Escherichia coli. The material was inoculated directly onto Drigalski agar and incubated at 37°C overnight. Yellow colonies that were catalase positive and oxidase negative were identified according to the following standard criteria: indole, citrate, methyl red and Voges-Proskauer reaction.

Enterococci. Enterococci from pigs and cattle were isolated and identified by the following procedure. 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 then sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified according to the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose.

Enterococci from broilers were isolated and identified as follows. 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. A subset of all isolates verified as *E. faecium* or *E. faecalis* were subjected to antimicrobial susceptibility testing.

Pathogens. The diagnostic submissions were examined according to the standard procedures employed by the participating laboratories. All bacterial isolates from food animals have been stored at –80°C for further study as required.

Examination of samples from food

The isolation of indicator organisms from food samples was performed by the Regional Veterinary and Food Control Authorities. Subsequently, the isolates were transferred to standard transport media and shipped to the DFVF. Verifications of species identity and MIC-determinations were performed at the DFVF. One isolate per species per food sample was tested for antimicrobial susceptibility.

Escherichia coli. Analysis for *E. coli* was carried out by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulfphate-broth, which was incubated at 44°C for 18-24 hours, and subsequently streaked onto violet red bile agar and incubated for 24h at 44°C. Presumptive *E. coli* were sub-cultured onto blood agar, transferred to standard transport medium and shipped to the DFVF. The isolates were identified as *E. coli* by indole, citrate, methyl red and Voges-Proskauer reaction.

Enterococci. Analysis for enterococci was carried out by adding 5 g of the sample to 45 ml of azide dextrose broth, which was incubated at 44°C for 18-24 hours, 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*, respectively, were sub-cultured on blood agar, transferred to transport medium and shipped to the DFVF. At the DFVF, isolates were identified by motility, arginine dihydrolase test and by the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. Only *E. faecium* and *E. faecalis* were included in the surveillance.

Salmonella. isolates were isolated according to NMKL No. 71, 5th ed., 1999. Sero- and phage-typing was performed at DFVF.

***Campylobacter* spp.** Thermotolerant *Campylobacter* spp. was isolated by a semi-quantitative method. Twenty-five g food sample was mixed 1:4 with Mueller-Hinton broth supplemented with sodium pyrovate 0.25 mg/l, sodium metabisulphite 0.25 mg/l, ferrous sulphate 0.25 mg/l, cefaperazone 30 mg/l, and trimethoprim lactate 50 mg/l and the sample was stomachated. Dilutions 1:10 were prepared, and 1 ml from each dilution was enriched under micro-aerophilic conditions for 24 hours at 42°C in 9 ml of Mueller-Hinton bouillon with supplement (as described above).

After pre-enrichment 10 µl was striked on mCCDA and further incubated under microaerophilic conditions for 24-48 hours at 42°C. mCCDA plates were examined for the presence of *Campylobacter*-like colonies. Suspect colonies were verified by phase-contrast microscopy and positive oxidase reaction. Species identification was performed by hydrolysis of hippurate- and indoxyl acetate. Only isolates of *C. jejuni* and *C. coli* were included in the surveillance.

Examination of samples 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 with the hippurate test and the indoxyl acetate tests.

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 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).

Susceptibility testing

Isolates from animals and foods

Agar dilution MIC was used to test the susceptibility of *Campylobacter* isolates. All other susceptibility testing was done with Sensititre (Trek Diagnostic Systems Ltd.), a commercially available MIC technique using dehydrated antimicrobials in microtitre wells. The wells were inoculated and incubated according to the The Clinical and Laboratory Standards Institute (CLSI) guidelines (formerly NCCLS). The MIC was defined as the lowest concentration of antimicrobial with no visible growth. The breakpoints used are shown in Table A2. All staphylococci isolates were screened for *mec*A-mediated resistance (methicillin-resistance) by PCR.

The following strains were used for quality control: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212. In Sensititre, weekly quality control was performed by inoculation of a set of wells with the control strains. The MIC values for the strains were evaluated in accordance to the CLSI guidelines. For agar dilution (*Campylobacter*) all control strains and *Campylobacter jejuni* ATCC 35360 were inoculated on each agar plate.

Isolates from humans

***Salmonella* spp.** Susceptibility testing for *Salmonella* spp. isolates was performed with Sensititre (Trek Diagnostic Systems Ltd.). The breakpoints used are shown in Table A2. The wells were inoculated according to CLSI guidelines and incubated aerobically at 36°C for 18-22 hours. *Escherichia coli* ATCC 25922 was used for quality control.

***Campylobacter* spp.** Susceptibility testing for *Campylobacter* spp. isolates was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on 5% blood yeast extract-supplemented agar (SSI Diagnostika) and the breakpoints defined in Table A3.

***Staphylococcus aureus*.** Susceptibility testing was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar

Table A2 Breakpoints and range of dilutions used for testing bacteria from animals, foods and humans. Isolates with MIC higher than or equal to the figures shown were considered resistant DANMAP 2004

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

a) For animals and foods only
b) Concentration of amoxicillin given, testet with clavulanic acid in concentration ratio 2:1
c) >= 0.125 µg/ml was the ciprofloxacin breakpoint applied for *Salmonella* isolates
d) The trade name is Synercid

(Resistensplade, SSI). Methicillin susceptibility was screened for by a cefoxitin 60 mg tablet using <31 mm as a break-point. Methicillin resistance was confirmed by EVIGENE™ (SSI) (Skov RL, et al. J. Antimicrob. Chemother. 1999; 43: 467-475).

***Streptococcus pneumoniae*.** The Streptococcus Unit at SSI screens for penicillin-resistant *S. pneumoniae* using a 1 microgram oxacillin tablet (Neo-Sensitabs®, A/S Rosco) on 10% horse blood agar (SSI Diagnostika). Penicillin MICs are determined using the E-test (AB Biodisk, Solna, Sweden) on Danish Blood Agar (Resistensplade, SSI Diagnostika). The breakpoints used are those defined by the CLSI.

***Escherichia coli,* coagulase-negative staphylococci and *Streptococcus pyogenes*.** In 2004, the clinical microbiology laboratories serving Ribe, Roskilde, Storstroem 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. The clinical microbiology laboratory serving Vejle county used the above described method for testing isolates of *E. coli* and *Streptococcus pyogenes*. When testing isolates of coagulase-negative staphylococci this county used the Neo-Sensitabs® tablets on Müeller-Hinton II agar (SSI Diagnostika).The clinical microbiology laboratory serving North Jutland county also used the Neo-Sensitabs® tablets 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. For urine isolates from general practice tests were carried out using Vitek2 (BioMérieux). The laboratory serving Funen county used the tablet diffusion method (A/S

Table A3. Breakpoints used for Campylobacter *spp. from humans. Isolates were considered resistant if they had an inhibition zone less than shown in the table.*

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Antimicrobial agent	*Campylobacter*
Ciprofloxacin	27 mm
Erythromycin	27 mm
Nalidixic acid	27 mm
Tetracycline	32 mm

Rosco) on Danish Blood Agar for blood isolates and the same tablets on Mueller-Hinton II agar (SSI Diagnostika) for testing urine isolates.

In 2004, the clinical microbiology laboratories serving the Copenhagen and Frederiksberg Municipalities, Copenhagen county, Ringkoebing 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).

Indicator bacteria. Susceptibility testing of *E. faecium*, *E. faecalis* and *E. coli* were done with Sensititre (Trek Diagnostic Systems Ltd.), a commercially available MIC technique using dehydrated antimicrobials in micro-titre wells. The wells were inoculated according to CLSI guidelines and incubated aerobically at 35°C for 18-20 hours for the *E. coli* isolates and 20-22 hours for enterococcal isolates. The breakpoints used are shown in Table A2. The following strains were used for quality control: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212.

Quinolone resistance and breakpoint

The current CLSI (formerly NCCLS) breakpoint for resistance to the fluoroquinolone ciprofloxacin is >=4 μg/ml. There is now compelling evidence that the treatment efficacy of fluoroquinolones is reduced in humans infected with strains of *Salmonella enterica* with what is regarded as decreased susceptibility to fluoroquinolones (MIC values>=0.125 μg/ml). Thus, to reduce the risk for humans, it has been recommended that for *Salmonella* a breakpoint of >=0.125 μg/ml for fluoroquinolones should be used [Aarestrup *et al.* 2003. Antimicrob. Agents Chemother. 47: 827-9]. In this DANMAP report a breakpoint of >=0.125 μg/ml for fluoroquinolones is used for *Salmonella* isolates. There is neither data on treatment effect of fluoroquinolones against *E. coli* with reduced susceptibility to fluoroquinolones, nor any reports on treatment failures. In 2004, it was decided to define indicator *E. coli* isolates with reduced susceptibility to ciprofloxacin as isolates with MIC-values greater than or equal to 0.125 μg/ml and less than 4 μg/ml. *E. coli* isolates with MIC values greater than or equal to 4 μg/ml are considered resistant to ciprofloxacin.

Gentamicin and apramycin breakpoint

After evaluating the MIC distributions, breakpoints for gentamicin and apramycin (for *E. coli* and *Salmonella* spp.) were changed from >=16 μg/ml to >= 8 μg/ml and from >=16 μg/ml to >=32 μg/ml, respectively.

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 and the National Center for Antimicrobials and Infection Control at the SSI as well as the Section for Antimicrobial Resistance and Section of Poultry at DFVF received 4 *E. coli* strains, 4 *Salmonella* spp. and 4 *Enterococcus* spp. All four laboratories tested the strains in micro-broth dilution test according to recommendations by TREK diagnostics. A total of 688 susceptibility tests were performed and the overall results were 0.44% failures. The detailed results are shown in Table A4.

Data handling

Data on animal isolates

The results from the primary examination of slaughterhouse samples 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 and place of sampling and the species of animal. Information on the herd or flock of origin was also recorded. All handling and evaluation of results was carried out using SAS® Software, version 8.2 of the SAS System for Microsoft® Windows.

Data on food isolates

Results from the analysis of food samples were reported via the Food Microbiology Database. For each bacterial isolate information is available on the type of food sample, bacterial species, date of examination of the sample, the RFCA that collected and processed the sample, and an identification number, which makes it

Table A4. Results of performance testing (Correct result/number of tests performed) among laboratories participating in DANMAP 2004, Denmark

DANMAP 2004

Antimicrobial agent	*E. coli*		*Salmonella* spp.		*Enterococcus* spp.	
	S + I a)	R	S + I	R	S + I	R
Penicillin	-	-	-	-	3/3	9/9
Ampicillin	8/8	8/8	4/4	12/12	-	-
Amoxicillin/Clavulanat	16/16	-	16/16	-	-	-
Cefalothin	15/16		16/16	-	-	-
Ceftiofur	16/16	-	16/16	-	-	-
Erythromycin	-	-	-	-	6/6	6/6
Tetracycline	8/8	8/8	4/4	12/12	3/3	9/9
Chloramfenicol	16/16	-	4/4	12/12	12/12	-
Vancomycin	-	-	-	-	12/12	-
Linezolid	-	-	-	-	12/12	-
Quinopristin/dalfopristin	-	-	-	-	6/6	6/6
Nalidixic acid	12/12	4/4	8/8	8/8	-	-
Ciprofloxacin	12/12	4/4	8/8	8/8	-	-
Neomycin	16/16	-	12/12	4/4	-	-
Streptomycin	12/12	4/4	6/8	8/8	9/9	3/3
Apramycin	12/12	4/4	12/12	4/4	-	-
Gentamicin	12/12	4/4	12/12	4/4	9/9	3/3
Spectinomycin	16/16	-	4/4	12/12	-	-
Colistin	16/16	-	16/16	-	-	-
Sulfonamethoxazole	8/8	8/8	4/4	12/12	-	-
Trimethoprim	8/8	8/8	8/8	8/8	-	-
Florfenicol	16/16	-	12/12	4/4	-	-
Avilamycin	-	-	-	-	12/12	-
Flavomycin	-	-	-	-	6/6	6/6
Salinomycin	-	-	-	-	12/12	-
Total	219/220	52/52	162/164	108/108	102/102	42/42
	99.5%	100%	98.8%	100%	100%	100%

a) S + I: Suseptible and Intermediate, R: Resistant

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 at SSI. This register 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® Excel 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*.** Data on MRSA were exported from the Danish MRSA registry (Microsoft® Excel) maintained by the Staphylococcus Unit at SSI. Patients are only registered in this database the first time they are diagnosed as being infected or colonised by MRSA. Clinical information has been obtained by requesting and reviewing discharge summaries on all patients. MRSA cases were then classified as active screening (surveillance samples to detect nasal or skin colonisation), imported infection (acquired outside Denmark) infection acquired in a Danish hospital or infection acquired in the Danish primary health care. Finally, results from PFGE typing were added to the database.

Streptococcus pneumoniae. Data on susceptibility testing of *Streptococcus pneumoniae* isolates are stored as MICs in a Microsoft® Access database at the Streptococcus Unit at the Statens Serum Institut. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed with Microsoft® Excel.

Escherichia coli, coagulase*-negative staphylococci and *Streptococcus pyogenes. 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), Storstroem county (Næstved Hospital), Ribe county (Esbjerg Hospital), Vejle county (Vejle Hospital), Ringkoebing county (Herning Hospital), Aarhus county (Skejby Sygehus) and Viborg county (Viborg Hospital);
- SafirLIS Microbiology (Profdoc Lab AB, Borlänge, Sweden) for Frederiksborg County (Hillerød Hospital);
- Funen's "Green System" for Funen county (Odense University 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 number 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, only a few were able to comply with this rule. A number of laboratories removed duplicates within a window of 21 days, others submitted data on the first isolate taken from patients. 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 Fisher's 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

2003

Aarestrup FM, Wiuff C, Mølbak K, Threlfall EJ. 2003. Is it time to change the break-points for fluoroquinolones for *Salmonella* spp. Antimicrob. Agents Chemother. 47: 827-829.

Aarestrup FM, Lertworapreecha M, Evans MC, Bangtrakulnonth A, Chalermchaikit T Hendriksen RS, Wegener HC. 2003. Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. J. Antimicrob. Chemother. 52: 715-718.

Agersø Y, Sengeløv G, Jensen LB. 2003. Development of a rapid method for detection of *tet*(M) genes in soil from Danish farmland. Environ Int. 30:117-122.

Emborg H-D, Andersen JS, Seyfarth AM, Andersen SR, Boel J, Wegener HC. 2003. Relations between the occurrence of resistance to antimicrobial growth promoters among *Enterococcus faecium* isolated from broilers and broiler meat. Int. J. Food Microbiol. 84: 273-284.

Evans MC, Wegener HC. 2003. Antimicrobial growth promoters and *Salmonella* spp., *Campylobacter* spp. In poultry and swine, Denmark. Emerg. Infect. Dis. 9: 489-492

Frimodt-Møller N. 2003. Sulfamethizole versus pivmecillinam in urinary tract infections. Ugeskr. Laeger 165: 4317.

Halling-Sørensen B, Sengeløv G, Ingerslev F, Jensen LB. 2003. Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazine, streptomycin, ciprofloxacin and olaquindox due to environmental processes. Arch. Environ. Contam. Toxicol. 44: 7-16.

Iversen J, Sandvang D, Srijan A, Cam PD, Dalsgaard A. 2003. Characterization of antimicrobial resistance, plasmids, and gene cassettes in *Shigella spp.* from patients in Vietnam. Microb. Drug Resist. 1: 17-24.

Jensen LB, Willems RJ, van den Bogaard AE. 2003. Genetic characterization of glycopeptide-resistant enterococci of human and animal origin from mixed pig and poultry farms. APMIS 111: 669-672.

Kerrn MB, Frimodt-Møller N, Espersen F. 2003. Effects of sulfamethizole and amdinocillin against *Escherichia coli* strains (with various susceptibilities) in an ascending urinary tract infection mouse model. Antimicrob. Agents Chemother. 47: 1002-1009.

Knudsen JD, Odenholt I, Erlendsdottir H, Gottfredsson M, Cars O, Frimodt-Møller N, Espersen F, Kristinsson KG, Gudmundsson S. 2003. Selection of resistant *Streptococcus pneumoniae* during penicillin treatment in vitro and in three animal models. Antimicrob. Agents Chemother. 47: 2499-2506.

Kristiansen MA, Sandvang D, Rasmussen TB. 2003. In vivo development of quinolone resistance in *Salmonella enterica* serotype Typhimurium DT104. J. Clin. Microbiol. 41: 4462-4464.

Kühn I, Iversen A, Burman LG, Olsson-Liljequist B, Franklin A, Finn M, Aarestrup F, Seyfarth AM, Blanch AR, Taylor H, Caplin J, Moreno MA, Dominguez L, Herrero I, Möllby R. 2003. Aspects of the epidemiology and ecology of enterococci in animals, humans and the environment - a European study. Int. J. Food Microbiol. 88: 133-145.

Monnet DL, Sørensen TL. 2003. DANMAP 2001. EPI - NEWS, no. 1/2. Available from: http://www.ssi.dk/graphics/en/news/epinews/2003/pdf/2003_1_2.pdf

Petersen A, Aarestrup FM, Hofshagen M, Sipilä H, Franklin A, Gunnarsson E. 2003. Harmonization of antimicrobial susceptibility testing among veterinary diagnostic laboratories in the five Nordic countries. Microb. Drug Res. 9: 381-386.

Sengeløv G, Agerso Y, Halling-Sorensen B, Baloda SB, Andersen JS, Jensen LB. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ. Int. 28: 587-595.

Sengeløv G, Halling-Sørensen B, Aarestrup FM. 2003. Susceptibility of *Escherichia coli* and *Enterococcus faecium* isolated from pigs and broilers to tetracycline degradation products and distribution of tetracycline resistance determinants in *E. coli* from food animals. Vet. Microbiol. 95: 91-101.

Simonsen GS, Småbrekke L, Monnet DL, Sørensen TL, Møller JK, Kristinsson KG, Lagerqvist-Widh A, Torell E, Digranes A, Harthug S, Sundsfjord A. 2003. Prevalence of resistance to ampicillin, gentamicin and vancomycin in *Enterococcus faecalis* and *Enterococcus faecium* isolates from clinical specimens and use of antimicrobials in five Nordic hospitals. J. Antimicrob. Chemother. 51: 323-331.

Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. 2003. Antibiotic resistance—the interplay between antibiotic use in animals and human beings. Lancet Infect Dis. Jan; 3(1): 47-51

Skov R, Frimodt-Møller N, Espersen F. 2003. Tentative interpretative zone diameters for fusidic acid Neosensitabs on Mueller Hinton agar and three blood containing media. Int. J. Antimicrob. Agents 22: 502-507.

Skov R, Larsen AR, Frimodt-Møller N, Espersen F. 2003. Evaluation of different disk diffusion/media combinations for detection of methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. APMIS 111: 905-914.

Skov R, Smyth R, Clausen M, Larsen AR, Frimodt-Møller N, Olsson-Liljequist B, Kahlmeter G. 2003. Evaluation of a cefoxitin 30 microg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. 52: 204-207.

Stege H, Bager F, Jacobsen E, Thougaard A.. 2003. VETSTAT-the Danish system for surveillance of the veterinary use of drugs for production animals. Prev. Vet. Med. Mar 20; 57: 105-115.

Vintov J, Aarestrup FM, Zinn CE, Olsen JE. 2003. Association between phage types and antimicrobial resistance among bovine isolates of *Staphylococcus aureus* in 10 countries. Vet. Microbiol. 95: 133-147.

Vintov J, Aarestrup FM, Zinn CE, Olsen JE. 2003. Phage types and antimicrobial resistance among Danish bovine *Staphylococcus aureus* isolates since the 1950's. Vet. Microbiol. 97: 63-72.

Wegener HC. 2003. Antibiotics in animal feed and their role in resistance development. Curr. Opinion. Microbiol. 6: 439-445.

Wegener HC. 2003. Antimicrobial resistant bacteria in the food chain. Food Australia. 55: 575-579.

Wiuff C, Lykkesfeldt J, Svendsen O, Aarestrup FM. 2003. The effects of oral and intramuscular administration and dose escalation of enrofloxacin on the selection of quinolone resistance among *Salmonella* and coliforms in pigs. Res. Vet. Sci. 75: 185-193.

Østergaard C, Yieng-Kow RV, Knudsen JD, Frimodt-Møller N, Espersen F. 2003. Evaluation of fusidic acid in therapy of experimental *Staphylococcus aureus* meningitis. J. Antimicrob. Chemother. 51: 1301-1305.

2004

Aarestrup FM. 2004. Monitoring of antimicrobial resistance among food animals: Principles and limitations. J. Vet. Med. B. 51: 380-388.

Aarestrup FM, Hasman H. 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet. Microbiol. 100: 83-89.

Aarestrup FM, Hasman H, Olesen I, Sørensen G. 2004. International spread of bla_{CMY-2} mediated cephalosporin resistance in a multiresistant *Salmonella enterica* serovar Heidelberg isolate stemming from the importation of a boar by Denmark from Canada. Antimicrob. Agents Chemother (letter). 48: 1916-1917.

Aarestrup FM, Seyfarth AM, Angen Ø. 2004. Antimicrobial susceptibility of *Haemophilus parasuis* and *Histophilus somni* from pigs and cattle in Denmark. Vet. Microbiol. 101: 143-146.

Agersø Y, Sengelov G, Jensen LB. 2004. Development of a rapid method for direct detection of tet(M) genes in soil from Danish farmland. Environ. Int. 30: 117-122.

Bangtrakulnonth A, Pornruangwong S, Pulsrikarn C, Sawanpanyalert P, Hendriksen RS, Lo Fo Wong DMA, Aarestrup FM. 2004. *Salmonella* serovars from humans and other sources in Thailand, 1993 to 2002. Emerg. Infect. Dis. 10: 131-136.

Baquero MR, Nilsson AI, Turrientes Mdel C, Sandvang D, Galan JC, Martinez JL, Frimodt-Møller N, Baquero F, Andersson DI. 2004. Polymorphic mutation frequencies in *Escherichia coli*: emergence of weak mutators in clinical isolates. J. Bacteriol. 186: 5538-5542.

Emborg H-D, Andersen JS, Seyfarth AM, Wegener HC. 2004. Relations between the consumption of antimicrobial growth promoters and the occurrence of resistance among *Enterococcus faecium* isolated from broilers. Epidemiol. Infect. 132: 95-105.

Engberg J, Neimann J, Nielsen EM, Aarestrup FM, Fussing V. 2004. Quinolone resistant campylobacter infections in Denmark: risk factors and clinical consequences. Emerg. Infect. Dis. 10: 1056-1063.

Frimodt-Møller N. 2004. Aminoglycosides to critically ill patients—and others. Ugeskr. Laeger 166: 4496.

Frimodt-Møller N. 2004. Microbial Threat - The Copenhagen Recommendations initiative of the EU. J. Vet. Med. B. Infect. Dis. Vet. Public Health 51: 400-402.

Guardabassi L, Christensen H, Hasman H, Dalsgaard A. 2004. Members of the genera *Paenibacillus* and *Rhodococcus* harbor genes homologous to enterococcal glycopeptide resistance genes *vanA* and *vanB*. Antimicrob. Agents Chemother. 48: 4915-4918.

Hammerum AM, Lester CH, Neimann J, Porsbo LJ, Olsen KE, Jensen LB, Emborg HD, Wegener HC, Frimodt-Møller N. 2004. A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is possibly related to pig isolates. J. Antimicrob. Chemother. 53: 547-549.

Hammerum AM, Nielsen HU, Agersø Y, Ekelund K, Frimodt-Møller N. 2004. Detection of tet(M), tet(O) and tet(S) in tetracycline/minocycline-resistant *Streptococcus pyogenes* bacteraemia isolates. J. Antimicrob. Chemother. 53: 118-119.

Hutchinson JM, Patrick DM, Marra F, Ng H, Bowie WR, Heule L, Muscat M, Monnet DL. 2004. Measurement of antibiotic consumption: A practical guide to the use of the Anatomical Therapeutic Chemical classification and Defined Daily Dose system methodology in Canada. Can. J. Infect. Dis. 15: 29-35.

Jensen VF, Jacobsen E, Bager F. 2004. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. Prev. Vet. Med. 64: 201-215.

Jensen VF, Neimann J, Hammerum AM, Mølbak K, Wegener HC. 2004. Does the use of antibiotics in food animals pose a risk to human health? An unbiased review? J. Antimicrob. Chemother. 54: 274-275.

Kerrn MB, Frimodt-Møller N, Espersen F. 2004. Urinary concentrations and urine *ex-vivo* effect of mecillinam and sulphamethizole. Clin. Microbiol. Infect. 10: 54-61.

Lester CH, Frimodt-Møller N, Hammerum AM. 2004. Conjugal transfer of aminoglycoside and macrolide resistance between *Enterococcus faecium* isolates in the intestine of streptomycin-treated mice. FEMS Microbiol. Lett. 235: 385-391.

McDermott PF, Bodeis SM, Aarestrup FM, Brown S, Traczewski M, Fedorka-Cray P, Wallace M, Critchley IA, Thornsberry C, Graff R, Flamm R, Beyer J, Shortridge D, Piddock L, Ricci V, Johnson MM, Jones RN, Reller B, Mirrett S, Aldrobi J, Rennie R, Brosnikoff C, Turnbull L, Stein G, Schooley S, Hanson RA, Walker RD. 2004. Development of a standardized susceptibility test for *Campylobacter* with quality control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. Microb. Drug Resist. 10: 124-131.

Monnet DL, Frimodt-Møller N. 2004. Only percentage within species; neither incidence, nor prevalence: demographic information and representative surveillance data are urgently needed to estimate the burden of antimicrobial resistance. Int. J. Antimicrob. Agents 24: 622-623.

Monnet DL, Mölstad S, Cars O. 2004. Defined daily doses of antimicrobials reflect antimicrobial prescriptions in ambulatory care. J. Antimicrob. Chemother. 53: 1109-1111.

Müller-Pebody B, Muscat M, Pelle B, Klein BM, Brandt CT, Monnet DL. 2004. Increase and change in pattern of hospital antimicrobial use, Denmark, 1997-2001. J. Antimicrob. Chemother. 54: 1122-1126.

Muscat M, Brandt C, Frimodt-Møller N, Monnet DL. 2004. Increase in ciprofloxacin use and resistance. EPI-NEWS, no. 41. Available from: http://www.ssi.dk/graphics/en/news/epinews/2004/pdf/2004_41.pdf

Muscat M, Müller-Pebody B, Monnet DL, Frimodt-Møller N. 2004. DANMAP 2002. EPI-NEWS, no. 3. Available from: http://www.ssi.dk/graphics/en/news/epinews/2003/pdf/2004_3.pdf

Muscat M, Müller-Pebody B, Monnet DL, Frimodt-Møller N. 2004. Antimicrobial use and resistance in Denmark: a synopsis of the DANMAP 2002 report. Eurosurveillance Weekly 8(12). Available from: http://www.eurosurveillance.org/ew/2004/040318.asp

Nielsen HU, Hammerum AM, Ekelund K, Bang D, Pallesen LV, Frimodt-Møller N. 2004. Tetracycline and macrolide co-resistance in *Streptococcus pyogenes*: co-selection as a reason for increase in macrolide-resistant *S. pyogenes*? Microb. Drug Resist. 10: 231-238.

Nielsen HU, Konradsen HB, Lous J, Frimodt-Møller N. 2004. Nasopharyngeal pathogens in children with acute otitis media in a low-antibiotic use country. Int. J. Pediatr. Otorhinolaryngol. 68: 1149-1155.

Olesen I, Hasman H, Aarestrup FM. 2004. Prevalence of β-lactamases among ampicillin resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. Microb. Drug Resist. 10: 334-340.

Patrick DM, Marra F, Hutchinson J, Monnet DL, Ng H, Bowie WR. 2004. Per capita antibiotic consumption: how does a North American jurisdiction compare with Europe? Clin. Infect. Dis. 39: 11-17.

Petersen, AH. and LB. Jensen 2004. Analysis of *gyrA* and *parC* mutations in enterococci from environmental samples with reduced susceptibility to ciprofloxacin. FEMS Microbiology Letters. 231:73-76.

Sahly H, Aucken H, Benedi VJ, Forestier C, Fussing V, Hansen DS, Ofek I, Podschun R, Sirot D, Tomas JM, Sandvang D, Ullmann U. 2004. Increased serum resistance in *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases. Antimicrob. Agents Chemother. 48: 3477-3482.

Sahly H, Aucken H, Benedi VJ, Forestier C, Fussing V, Hansen DS, Ofek I, Podschun R, Sirot D, Sandvang D, Tomas JM, Ullmann U. 2004. Impairment of respiratory burst in polymorphonuclear leukocytes by extended-spectrum beta-lactamase-producing strains of *Klebsiella pneumoniae*. Eur. J. Clin. Microbiol. Infect. Dis. 23: 20-26.

Tian Y, Aarestrup FM, Lu CP. 2004. Characterizations of *Streptococcus suis* serotype 7 isolates from diseased pigs in Denmark. Vet. Microbiol. 103:55-62.

Tollefson L, Kruse H, Wegener HC. 2004. Public health consequences of macrolide use in food animals: a deterministic risk assessment, a comment on: J. Food Prot. 67: 980-992. J Food Prot. 67: 2368-2369.

2005

Aarestrup FM. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. 96: 271-281.

Agersø Y, Guardabassi L. 2005. Identification of Tet 39, a novel class of tetracycline resistance determinant in *Acinetobacter* spp. of environmental and clinical origin. J. Antimicrob. Chemother. 55: 566-569.

Engberg J, Bang DD, Aabenhus R, Aarestrup FM, Fussing V, Gerner-Smidt P. 2005. *Campylobacter concisus*: an evaluation of certain phenotypic and genotypic characteristics. Clin. Microbiol. Infect. 11: 288-295.

Faria NA, Oliveira DC, Westh H, Monnet DL, Larsen AR, Skov R, de Lencastre H. 2005. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. J. Clin. Microbiol. 43: 1836-1842.

Hasman H, Aarestrup FM. 2005. Relationship between copper, glycopeptide and macrolide resistance among *Enterococcus faecium* strains isolated from pigs in Denmark between 1997 and 2003. Antimicrob. Agents Chemother. 49: 454-456.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. 2005. Beta-lactamases among amoxicillin-resistant *Escherichia coli* from veal calves and *Salmonella* from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother. (in press).

Hasman H, Villadsen AG, Aarestrup FM. 2005. Diversity and stability of plasmids from glycopeptide resistant *Enterococcus faecium* (GRE) isolated from Pigs in Denmark. Microb. Drug Resist.(in press).

Heuer OE, Jensen VF, Hammerum AM. 2005. Antimicrobial drug consumption in companion animals. Emerg. Infect. Dis. 11: 344-345.

Jensen LB, Aarestrup FM. 2005. Regulation of the *erm*(C) gene in staphylococci from reservoir with different usage of macrolides. Acta Veterinaria Scandinavica (in press).

Kerrn MB, Struve C, Blom J, Frimodt-Møller N, Krogfelt KA. 2005. Intracellular persistence of *Escherichia coli* in urinary bladders from mecillinam-treated mice. J. Antimicrob. Chemother. 55: 383-386.

Monnet DL. 2005. Antibiotic development and the changing role of the pharmaceutical industry. The International Journal of Risk and Safety in Medicine (in press).

Monnet DL, Brandt CT, Kaltoft MS, Bagger-Skjøt L, Sørensen TL, Nielsen HUK, Frimodt-Møller N. 2005. High prevalence of macrolide resistance: not in every country! J. Antimicrob. Chemother. (in press).

Monnet DL, Ferech M, Frimodt-Møller N, Goossens H. 2005. The more antibacterial-drug trade names, the more consumption: a European study. Clin. Infect. Dis. 41:114-117.

Monnet DL, MacKenzie FM, Skov R, Jensen ET, Gould IM, Frimodt-Møller N. 2005.
Fighting MRSA in hospitals: time to restrict the broad use of specific antimicrobial classes? J. Hosp. Infect. (in press).

Peirano G, Agersø Y, Aarestrup FM, Rodrigues DP. 2005. Occurrence of Integrons and resistance genes among *Shigella* spp from Brazil. J. Antimicrob. Chemother. 55: 301-305.

Skov R, Frimodt-Møller N, Menday P, Espersen F. 2005. Susceptibility testing of urinary isolates of *Escherichia coli* to mecillinam using NCCLS methodology. Int. J. Antimicrob. Agents 25: 198-204.

Skov R, Smyth R, Larsen AR, Frimodt-Møller N, Kahlmeter G. 2005. Evaluation of cefoxitin 5 and 10 microg discs for the detection of methicillin resistance in staphylococci. J. Antimicrob. Chemother. 55: 157-161.

Sompolinsky D, Nitzan Y, Tetry S, Wolk M, Vulikh I, Kerrn MB, Sandvang D, Hershkovits G, Katcoff DJ. 2005. Integron-mediated ESBL resistance in rare serotypes of *Escherichia coli* causing infections in an elderly population of Israel. J. Antimicrob. Chemother. 55: 119-122.

Summary research reports

Report 3

Conjugal transfer of aminoglycoside and macrolide resistance among *Enterococcus faecium* demonstrated *in vitro* and *in vivo* in the intestine of mice

Enterococcus spp. are commonly isolated from clinical specimens and are known to harbour different genetic elements such as conjugative transposons and different types of plasmids with resistance genes. A hot spot for gene transfer could be the gut of different animal species, as well as humans since transient colonization of enterococci in the human gut easily takes place after ingestion of food containing such bacteria [Sørensen *et al*. (2001) N. Engl. J. Med. 345:1161-1166]. *aadE, aphA-3,* and *erm*(B) encoding resistance to streptomycin, kanamycin and erythromycin, respectively, can be found on the *erm*(B)-Tn*5405*-like element in *Enterococcus faecium* [Werner *et al.* (2003) Microb. Drug Resist. 9:9-16].

This study investigated the presence of the *erm*(B)-Tn*5405*-like element in a multi-resistant human *E. faecium* isolate together with the presence of *aac(6')-Ie-aph(2'')-Ia*. The transfer of these genes was also studied both *in vitro* and *in vivo* in the intestine of streptomycin-treated mice [Lester *et al*. (2004) FEMS Microbiol. Lett. 235:385-391]. Strain *E. faecium* 160/00 of human origin was used as donor. This strain harbours the *erm*(B), *aac(6')-Ie-aph(2'')-Ia*, *aad*E, and *sat4* resistance genes. The strain used as recipient was *E. faecium* 64/3, which is resistant to streptomycin, rifampin, and fusidic acid. Conjugation *in vitro* was performed by the filter mating procedure. *In vivo* transfer experiments were carried out in the intestine of streptomycin treated mice.
Strain 160/00 harboured the *erm*(B)-Tn*5405*-like element. Co-transfer of *erm*(B), *aad*E and *aphA-3* genes indicated transfer of this element and this was shown in all *in vitro* and *in vivo* experiments.

Additionally, co-transfer of *aac(6')-Ie-aph(2'')-Ia* was obtained in 26 out of 30 *in vitro* transconjugants tested and in all *in vivo* transconjugants recovered from faeces. The frequency of *in vitro* transfer was approximately 2×10^{-5} transconjugants/donor. *In vivo* transconjugants harbouring the *erm*(B)-Tn*5405*-like element and *aac(6')-Ie-aph(2'')-Ia* could already be recovered 24 hours after mouse inoculation (Figure 1). Throughout the experiment, high numbers of transconjugants approaching 10^6 CFU/g of faeces were observed (Figure 1). Plasmid profiles and Southern blots showed that the *erm*(B)-Tn*5405*-like element and *aac(6')-Ie-aph(2'')-Ia* were both placed on the same large plasmid (>150 kb).
In conclusion, several resistance genes were transferred at the same time both in *in vitro* and *in vivo* experiments. This was done without selective pressure. These results suggest that the intestine is a hot spot for transfer of resistance genes.

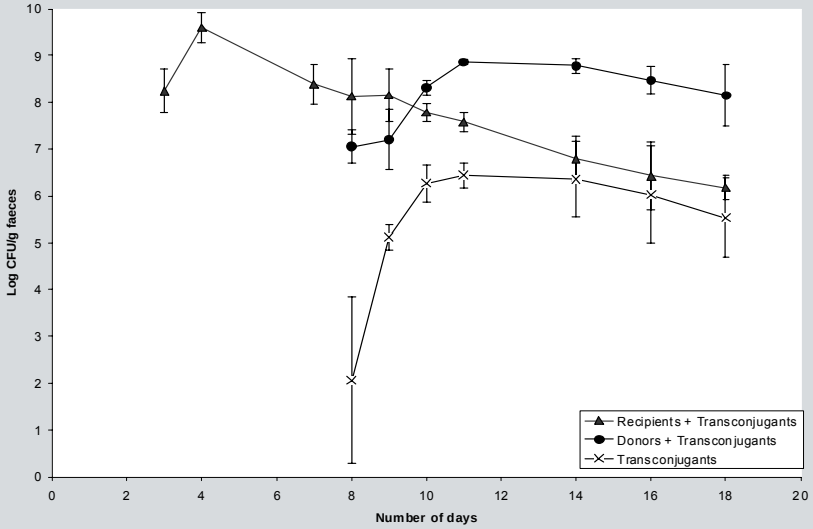

Figure 1. Concentrations of recipients, donors and transconjugants in faecal samples from mice. The recipient was introduced on day 2 and the donor was introduced on day 7

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Report 4

Identification of Tet 39, a novel class of tetracycline resistance determinant in *Acinetobacter* spp. of environmental and clinical origin

Three classes of genes encoding tetracycline resistance by specific active efflux have been described in *Acinetobacter* spp.: *tet*(A), *tet*(B) and *tet*(H) [Guardabassi *et al*. 2000. J. Med. Microb. 49. 929-36; Miranda *et al*. 2003. Antimicrob. Agents Chemother 47. 883-8]. Other tetracycline resistance genes occasionally reported in clinical *Acinetobacter baumannii* isolates include *tet*(M), a widespread gene encoding tetracycline resistance by ribosomal protection, and *adeB*, which confers multi-drug resistance by an unspecific efflux pump mechanism [Ribera *et al*. 2003. Antimicrob. Agents. Chemother. 47. 2310-2; Magnet *et al*. 2001. Antimicrob. Agents Chemother. 45. 3375-80].

The genetic basis of tetracycline resistance in *Acinetobacter* strains that do not carry known resistance determinants were determined [Agersø and Guardabassi. 2005. J. Antimicrob. Chemother. 55: 566-69]. Cloning of the tetracycline resistance gene in one of the strains revealed a novel tetracycline resistance determinant named Tet 39. Sequencing of a 3,727 bp insert revealed the presence of an open reading frame named *tetA*(39) (1188 bp) having 50% predicted amino acid identity to TetA(C) in *Aeromonas salmonicida* (GenBank accession no. AY043298). *tetA*(39) was flanked upstream by a reverse complementary open reading frame named *tetR*(39) (642 bp) encoding a putative repressor protein with 41% identity over 202 amino acids to TetR(B) from the transposon Tn*10* (GenBank accession no. AP000342). Downstream from *tetA*(39), there were two reverse complementary open reading frames named Orf1 (188 amino acids) and Orf2 (307 amino acids).

The predicted secondary structure of TetA(39) revealed a putative transmembrane protein consisting of twelve transmembrane α-helices (TMS) as seen for other tetracycline efflux pumps (has either 12- or 14-TMS) belonging to the major facilitator superfamily (MFS). Moreover, the amino acid sequence contained the highly conserved motifs A, B, C and D2 found in 12-TMS family of MFS [Paulsen et al.1996, Microbiol. Mol. Biol. Rev. 60. 575-608]. The phylogenetic relationship of *tetA*(39) was revealed by comparing the gene with representative genes of each class of 12-TMS tetracycline efflux pumps. *tetA*(39) showed closest relationship to *tetA*(30) found in *Agrobacterium tumefaciens* originating from soil. The two genes formed a separate branch indicating the closest evolutionary relationship between these genes.

Eleven of fifteen strains harbored Tet 39, the strains originated from the environment of Danish freshwater trout farms (n=4), from sewage collected in Denmark (n=6), and from a clinical specimen of urine collected in the Netherlands in 1986. This indicates that this novel tetracycline resistance determinant occurs in different reservoirs and geographical areas. Tet 39 was in most cases located on transferable plasmids varying in size from approx. 25 to 50 kb.

Tet 39, a 12-TMS tetracycline efflux pump, seems to be more frequent in *Acinetobacter* spp. from the aquatic environment than in clinical isolates. The finding of Tet 39 was found in *Acinetobacter* from geographically distinct areas and reservoirs and in a clinical strain isolated 18 years ago. This suggests that the gene is widespread among *Acinetobacter* isolates and possibly also other Gram-negative bacteria.

GenBank accession no. AY743590

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Report 5

International spread of multiple resistant *Salmonella* Schwarzengrund from Thailand to Denmark through trade of poultry products

Salmonella enterica is one of the most common causes of human gastroenteritis worldwide. The infection is primarily caused by improper handling and ingestion of undercooked food. A large number of different food animal sources have been identified as reservoirs for *Salmonella*. More than 2,500 different serovars of *Salmonella enterica* have been identified and most of them have been described as the cause of human infections, but only a limited number of serovars are of public health importance. Most reports have mentioned *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis as the most common causes of human salmonellosis worldwide. However, in some regions other serovars have been reported to be of greater importance. In addition, changes in the relative importance of different serovars over time seem to occur. The distribution of different serovars in one country can be of global importance because of trade with breeding animals and food products worldwide and travel.

The importance of *Salmonella enterica* serovar Schwarzengrund has recently increased in Thailand. In humans, the prevalence of this serovar increased from 0% in 1992 to 2.4% in 2001. This increase was followed by a simultaneous *S.* Schwarzengrund increase in chicken meat. The importance of this serovar has also increased in USA and in Denmark it emerged into top 40 causes of gastro-intestinal infections in humans in 2003 (www.germ.dk). The simultaneous emergence of *S.* Schwarzengrund among chicken meat and humans in Thailand could point to broilers as the source of infections in this country, while the simultaneous emergence also in other regions such as USA and Denmark could indicate a common source for several countries.

We compared 159 *S.* Schwarzengrund isolates from patients and food animal sources in Thailand and Denmark by *Xba*I PFGE typing, *Pst*I ribotyping and antimicrobial susceptibility profile. Antimicrobial resistance was very frequent in Thai patient and in Danish and Thai chicken meat isolates, it was medium in Danish patient isolates, whereas all isolates from turkeys, pigs and pork in Denmark were susceptible (fluoroquinolones resistance example is shown in Table 1). Four ribotypes were found with 139 isolates (87%) belonging to the most common ribotype. Sixty-two PFGE-types were observed, with 44 (28%) belonging to the most common type. Six of the 14 Danish patient isolates belonged to types also found in food products. Thirty (64%) of the Thai patient and 26 (59%) of the chicken meat isolates belonged to common types. Four of the PFGE-types found among patients and chicken meat in Denmark were also observed among patients and chicken meat isolates in Thailand.

The results of the antimicrobial susceptibility testing and typing indicates a spread of multiple resistant *S.* Schwarzengrund from chickens to humans in Thailand and from chickens produced in Thailand through imported chicken meat to humans in Denmark. In addition, pigs and pork products seem also to be a source of susceptible *S.* Schwarzengrund isolates for humans in Denmark.

Table 1. Occurrence of fluoroquinolone resistance among Salmonella *Schwarzengrund from different sources in Denmark and Thailand*

Country	Origin	No. isolates	Percentage quinolone resistance
Thailand	Chicken meat	44	89
	Patients	47	91
Denmark	Chicken meat	19	79
	Patients	14	57
	Pigs, pork and turkey	35	0

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Report 6

Occurrence of integrons and resistance genes among sulphonamide-resistant *Shigella* spp. from Brazil

Shigellosis is endemic throughout the world. There are ~165 million cases worldwide per year, of which 163 million occur in developing countries and 1.5 million in industrialized countries [WHO/IVR. 2003. www.who.int]. Each year 1.1 million people are estimated to die from *Shigella* infections and nearly 580,000 cases of shigellosis are reported among travellers from industrialized countries.

Knowledge on the epidemiology and molecular mechanisms of antimicrobial resistance in this important pathogen is essential to implement intervention strategies. This study was conducted to investigate the occurrence of sulphonamide and additional resistance genes, and class 1 and 2 integrons as mediators of antimicrobial resistance among *Shigella* spp. isolated from humans in Brazil [Peirano et al. 2005. J. Antimicrob. Agents Chemother. 55: 301-305].

A total of 62 sulphonamide resistant *Shigella* (*S. flexneri,* n=47 and *S. sonnei*, n=15) from various regions of Brazil, were tested against 21 antimicrobial agents. The presence of integron classes 1 and 2 and antimicrobial resistance genes were investigated by PCR using specific primers. The results are shown in Tables 1 and 2, respectively.

Table 1. Number of Shigella *strains resistant to antimicrobials*[a]

	No. tested	AMP	AMX/CLA[b]	CHL	SPT	STR	SMX	TET	TMP
S.flexneri	47	46	30	41	46	47	47	44	47
S. sonnei	15	3	0	1	13	15	15	13	15
Total	62	49	30	42	59	62	62	57	62

[a]Based on MIC test: AMP (ampicillin), AMX/CLA (amoxicillin/clavulanic acid), CHL (chloramphenicol), SPT (spectinomycin), STR (streptomycin), SMX (sulfamethoxazole), TET (tetracycline), and TMP (trimethoprim)
[b]The 30 isolates were AMX/CLA intermediate with MIC 16/8 mg/L

Table 2. Distribution of integrons and antimicrobial resistance genes among Shigella *spp.*

	sul1[a]	*sul2*	*intI1*	*intI2*	bla_{OXA}	bla_{TEM}	*catA1*	*tet*(A)	*tet*(B)
S. flexneri	1	47	1	44[b]	43	3	41	1[c]	43
S. sonnei	1	15	1	13	-	3	1	1[c]	12
Total	2	62	2	57	43	6	42	2	55

[a]Detected as part of class 1 integron
[b]Including one isolate positive for both classes of integrons
[c]*tet*(A) + *tet*(B). No isolate was positive for *sul3, tet*(C), and *tet*(D)

A total of eight antimicrobial resistant profiles were identified. The most common profile in *S. flexneri* were resistance to AMP, SMX, TMP, SPT, STR, TET, CHL (*n*=38) and among *S. sonnei* the resistance profile SMX, TMP, SPT, STR, TET (*n*=12) was the most common. Class 2 integron positive strains harbored a gene cassette array analogous to that found in Tn7 (accession no. M63169).
In summary, all isolates were phenotypically resistant to the antimicrobials accounted by the resistance genes encoded not only within the gene cassettes of class 1 and 2 integrons but also by integron-independent genes.

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Report 7

Tetracycline and macrolide co-resistance in *Streptococcus pyogenes*: co-selection as a reason for increase in macrolide resistant *S. pyogenes*?

Due to the increasing number of reports on emerging macrolide resistance in *S. pyogenes*, DANMAP included *S. pyogenes* for surveillance beginning January 2001. Collection of macrolide resistant *S. pyogenes* (MRSP) from clinical microbiological laboratories was initiated to analyze the genetic background for macrolide resistance. Furthermore, we investigated the correlation between macrolide and tetracycline use and macrolide resistance using international data [Nielsen *et al.* 2004. Microb. Drug Resist. 10:231-8].

In 2001, macrolide susceptibility results were reported by microbiological laboratories for 4,875 *S. pyogenes* isolates. Of these, 133 were MRSP and were submitted to Statens Serum Institut. Macrolide resistance was confirmed and susceptibility testing for tetracycline was performed. Additionally, MRSP isolates were examined for macrolide and tetracycline resistance genes by PCR, and T-typed.
The frequency of macrolide resistance was 2.7% (133 of 4,875 isolates). *erm*(B) was found in 46%, *erm*(A) in 18% and *mef*(A) in 32% of the 133 MRSP isolates, respectively. Tetracycline resistance was found in 69 (52%) of the 133 MRSP isolates, due to either *tet*(M) or *tet*(O). *erm*(B) and *mef*(A) were associated with *tet*(M). The T-type distribution suggested only few, minor clones.

Analysis of the importance of antibiotic use for development of macrolide resistance in *S. pyogenes* showed no correlation with macrolide use alone (P=0.15), but a significant correlation (P=0.03) for the combination of macrolide and tetracycline use.

The frequency of macrolide resistance in Danish *S. pyogenes* was low and mainly due to *erm* genes. A high frequency of tetracycline resistance in MRSP is found in many countries including Denmark. Tetracycline use should therefore be considered a possible cause for selection of MRSP [Nielsen *et al.* 2004. Microb. Drug Resist. 10:231-8].

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Report 8

TRAINAU – Training Risk Assessment In Non-human Antibiotic Usage

TRAINAU is a multidisciplinary Marie Curie Early Stage Training site on identification, characterisation, and assessment of public health risks associated with non-human use of antimicrobials. TRAINAU will bring together early-stage researchers with different backgrounds and provide them with the scientific and technological competences necessary to perform high-level research and obtain substantial progress in this field.

The Early Stage Training site builds on the experience gained in Denmark. The country has established a fully integrated surveillance programme on antimicrobial usage and antimicrobial resistance in animals, food, and humans. The Early Stage Training site consists of six inter-related research groups located at two universities: The Royal Veterinary and Agricultural University (KVL) and The Danish University of Pharmaceutical Science (DFU) and two national reference laboratories: Statens Serum Institut (SSI) and the Danish Institute for Food and Veterinary Research (DFVF), with The Royal Veterinary and Agricultural University being the co-ordinator institution.

TRAINAU will contribute to coordination of research training in the area of microbiological risk assessment and enable dissemination of principles and methods for surveillance of antimicrobial usage and resistance to other European countries. TRAINAU will also contribute to reinforce the capacity of emerging research groups through enhancing the scientific capacities of the fellows.

Students will primarily be enrolled at the Royal Veterinary and Agricultural University, but the research activities will also be carried out at one or more of the TRAINAU institutions all situated in Copenhagen, Denmark. Fellows will have the opportunity to join research groups of an international standard within their field of research and to receive interesting and stimulating research training. All fellows will have an individual training plan that takes into consideration training needs in terms of courses, specific techniques and project development. Fellows will follow the standard PhD programme and rules of Danish universities, which comprise a three-year period of full-time studies and includes completion of a research project, various approved PhD courses, experience in teaching and communication, submission/ publication of scientific articles in international peer-reviewed journals, preparation of a thesis and the public defence of this thesis.

Mobility will play an essential role in TRAINAU. Although spending a significant part of their training period at the host institute, fellows will have the opportunity of training periods at European universities and research institutions. The numerous research networks of the TRAINAU partner institutions will ensure international collaboration and participation of teachers from foreign countries. Intersectorial aspects of the proposed training include collaboration with private sector. Relevant research schools are hosted by the two TRAINAU universities, providing possibilities for the fellows to follow various advanced PhD courses at different universities in Copenhagen.

The seven PhD projects will be within the following areas:

- Transmission of resistant bacteria and antimicrobial resistance genes by the food chain,
- Transmission of resistant bacteria and antimicrobial resistance genes by contact with animals,
- Development of *in vivo* and *in vitro* models for the investigation of selective pressure of different veterinary treatment strategies,
- Associations between non-human antimicrobial usage and resistance in animals and food,
- Effects of antimicrobial resistance on incidence and severity of human infections and treatment outcomes investigated by animal models,
- Development of mathematical models of selection and spread of resistant bacteria and antimicrobial resistance genes in the food chain,
- The effects of antimicrobial drug metabolites on selection of resistant bacteria

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