DANMAP 2005

DANMAP 2005 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark

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Danish Veterinary and Food Administration
Danish Medicines Agency
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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 2005. 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 Institut, the Danish Institute for Food and Veterinary Research, the Danish Veterinary and Food Administration and the Danish Medicines Agency. The objectives of DANMAP are:

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

The monitoring of antimicrobial resistance is based on three categories of bacteria: human and animal pathogens, zoonotic bacteria and indicator bacteria. Human and animal pathogens are included because these cause infections and they primarily reflect resistance caused by use of 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
CNS	Central Nervous System
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 ADDkg). This is an assumed average daily dose per animal, defined as the daily maintenance dose for a drug used for its main indication in a specified species. The dose is defined for a „standard animal", i.e. an animal with an estimated average weight within a specified age group. In VetStat, ADDs are calculated for each age group. Otherwise, the general principles for standardisation of dosage for animals are similar to that used by the WHO Collaborating Centre for Drug Statistics and Methodology to calculate Defined Daily Dose (DDD) in humans (Jensen VF, Jacobsen E, Bager F. 2004. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. Prev. Vet. Med. 64:201-215). The ADDkg is the ADD per kg animal. Consumption calculated in ADDkg allows summation of consumption across different age groups and animal species.

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

Antibiotikaforbruget til dyr

Det samlede forbrug af antibiotika til dyr i 2005 var uændret i forhold 2004. Der var dog ændringer i fordelingen af forbruget for de forskellige grupper af antibiotika. Der blev observeret et fald i forbruget af makrolid/tiamulin/lincosamid gruppen på 1,9 ton, mens der var en stigning på 1,3 ton i forbruget beta-laktamase følsomme penicilliner.

I 2005 faldt det totale forbrug af antibiotika til svin med 0,2 %, til 92,2 tons aktivt stof (81 % af det totale veterinære forbrug). Samtidig steg produktionen af svin med 1 %. Forbruget svarer til 47 mg/kg produceret svinekød i 2005, hvilket udgør et fald i forhold til 2004 på 1,3 %. Reduktionen skyldes primært et fald i udskrivningerne af tiamulin, aminoglykosider og makrolider til mave-tarm-infektioner. Der blev observeret en fortsat stigning i forbruget af beta-laktamase følsomme penicilliner, penicillin-streptomycin kombinationer, makrolider og tetracykliner til behandling af luftvejsinfektioner samt infektioner i led, central nervesystemet og huden. Som i de tidligere år blev der i 2005, observeret store regionale forskelle i forbruget af antibiotika. Forbruget af antibiotika var 52 % højere hos søer/smågrise og 88 % højere for slagtesvin i Jylland og på Fyn i forhold til Vestsjælland og Storstrøms amter. Flere faktorer, så som forskel i sygdomsforekomst, forskelle i produktions-systemer eller variationer i behandlingsstrategier kan være medvirkende til de regionale forskelle.

I 2005 var det estimerede antibiotikaforbrug til kvæg (inkl. malkekøer) 11 ton, svarende til 10 % af det samlede antibiotikaforbrug til dyr. Forbruget af antibiotika til kavle, kvier og ungtyre svarer til 37 mg/kg produceret kalvekød. Der er sket en stigning i udskrivning af antibiotika til behandling af luftvejsinfektioner, hvilket udgjorde 63 % af behandlingerne af kalve i 2005. Tetracykliner, makrolider og penicilliner var de antibiotika, der oftest blev udskrevet til behandling af luftvejsinfektioner. Der blev ikke anvendt fluorokinolon til køer og ungkvæg i 2005, som følge af de lovændringerne der blev implementeret i 2002.

I fjerkræproduktionen blev der anvendt 420 kg antibiotika i 2005, hvilket svarer til 0,4 % af det totale veterinære antibiotikaforbrug. Forbruget i kyllingeproduktionen var uændret fra 2004 til 2005 og svarede til 0,8 mg/kg produceret kyllingekød.

I 2005 udgjorde forbruget af antibiotika til dambrug 2406 kg, svarende til 2,1 % af det totale veterinære forbrug. Forbrug af antibiotika til fisk svarede til 29 mg/kg fisk i ferskvandsdambrug og ca. 190 mg/kg fisk i havbrug.

Antibiotikaforbruget til mennesker

Fra 2004 til 2005 steg forbruget af antibiotika til behandling af mennesker med 5,1 %, til 32,4 millioner DDD eller 16,4 DDD/1.000 indbygger-dage.

I primærsektoren steg det totale forbrug af antibiotika med 4,9 %. Forholdet mellem forbrug af de forskellige antibiotikaklasser var uændret og mere end 70 % af forbruget bestod af beta-laktamase sensitive penicilliner, penicilliner med udvidet spektrum samt makrolider. Årsager til det forsat stigende forbrug af antibiotika er ikke kendt. Mellem 1997 og 2005 har der været meget ensartede forbrugsstigninger mellem amterne, uanset niveauet for totalforbrug af antibiotika.
Den fortsatte stigning i forbrug af fluorokinoloner (fra 0,28 til 0,32 DDD/indbygger-dage fra 2004 til 2005) blev fulgt af en stigning i forekomsten af fluorokinolon-resistente *E. coli* isolater. Dette følger forventningerne beskrevet i tidligere rapporter.

Forbruget af antibiotika var forsat stigende på de danske sygehuse. Fra 1997 til 2005, steg det gennemsnitlige antibiotikaforbrug på sygehusene med 48 % til estimeret 624 DDD/sengedage.
Fra 1997 til 2005 steg antibiotikaforbruget med 12,5 % opgjort i DDD/1.000 udskrevne patienter. Det skal bemærkes, at der i perioden er sket en 8 % stigning i antallet af udskrevne patienter samt et fald i antal sengedage på 16 %. En del af stigningen i antibiotikaforbruget kan derfor, både udenfor og indenfor sygehusene, tilskrives en højere aktivitet i det danske sundhedsvæsen.
Udover stigninger i totalforbruget af antibiotika på sygehuse observeredes også markante ændringer i forbrugsmønstret. Den tidligere påpegede trend, med stigende forbrug af cephalosporiner, fluorokinoloner og carbapenemer, på bekostning af penicilliner med udvidet spektrum (undtaget pivmecillinam), aminoglykosider og makrolider, fortsatte i 2005.
I 1997 udgjorde andelen af bredspektrede antibiotika (cephalosporiner, fluorokinoloner, penicilliner med beta-laktamasehæmmere og carbapenemer) 15,4 % af

totalforbruget. Dette steg til 22,4 % i 2003 og blev estimeret til at udgøre 27,5 % i 2005. Det øgede forbrug af bredspektrede antibiotika medførte i 2005 stigende resistens over for både cephalosporiner og fluorokinoloner, hvorfor fordelen ved en bredspektret empirisk behandling måske hurtigere end ventet opvejes af stigende resistensforekomst.

Resistens i zoonotiske bakterier

Resistensniveauet blandt *Salmonella* Typhimurium isolater fra svin var uændret fra 2004 til 2005 bortset for ampicillin, hvor der var en stigning i resistensforekomsten. Fra 1999 til 2005 har der været en stigning i forekomsten af resistens overfor tetracyklin, sulfonamide og ampicillin i *S*. Typhimurium isolater fra svin. Denne stigning falder sammen med et stigende forbrug af både tetracykliner, sulfonamid/trimetroprim og bredspektrede penicilliner til behandling af svin.

Sammenlignes resistensforekomsten blandt *S*. Typhimurium isolater fra dansk svinekød med *S*. Typhimurium isolater fra importeret svinekød, var resistensforekomsten i det importerede svinekød højere for 9 antibiotika (bl.a. ampicillin og sulfonamid). Derudover var forekomsten af *S*. Typhimurium fagtyperne DT104/104b og DTU302 højere i det importerede svinekød end i det danske kød.

I *S*. Typhimurium og *S*. Enteritidis isolater fra mennesker med infektioner erhvervet i udlandet var forekomsten af resistens overfor ciprofloxacin og nalidixinsyre højere end i isolater fra infektioner erhvervet i Danmark.

To af de humane *S.* Typhimurium isolater var fænotypisk udviget-spektrum beta-laktamase (ESBL)positive. Det er første år *S.* Typhimurium isolater er blevet testet fænotypisk for ESBL- aktivitet.

Sammenlignes *C. jejuni* isolater fra dansk og importert fjerkrækød, var der højere forekomst af resistens overfor tetracyklin, nalidixinsyre og ciprofloxacin i isolater fra importeret fjerkrækød.

I *C. jejuni* isolater fra mennesker med infektioner erhvervet i udlandet, var forekomsten af resistens overfor nalidixinsyre og tetracyklin højere, end i isolater fra infektioner erhvervet i Danmark.

Resistens i indikator bakterier

Forekomsten af erythromycinresistens blandt *E. faecium* isolater fra svin faldt fra 2004 til 2005. I den samme periode blev der observeret et fald i forbruget af makrolider til behandling af svin.

I *E. coli* isolater fra svin skete der fra 2004 til 2005 et fald i resistens overfor fire antibiotika (bl.a. ampicillin).

Blandt indikatorbakterier fra raske mennesker (NorMat undersøgelsen) var der ingen ændringer i resistensforekomsten i enterokokker og *E. coli*. Der blev påvist resistens overfor gentamicin i to af 101 *E. coli* isolater og i 110 fæces-prøver blev der fundet i alt fire vancomycin-resistente enterokokker.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Antallet af methicillin resistente *Staphylococcus aureus* (MRSA) isolater steg fra 549 i 2004 til 856 i 2005 (både fra kolonisering og infektioner med MRSA, et isolat per patient). Dele af stigningen skyldes et stort hospitalsudbrud i Vejle amt, dette udbrud er nu under kontrol. Der blev også observeret en signifikant stigning i Storkøbenhavn, her var epidemiologien mere diverse, mange tilfældene var samfundserhvervede, ligeså vel som flere tilfælde var relateret til kryds-smitte imellem hospitaler og plejehjem.
I 55 % af tilfældene have personerne en infektion ved diagnosen. Tre-og halvfjers procent af de MRSA tilfælde der var erhvervet i Danmark var samfundsrelaterede. De oftes forekommende infektioner var hud- og bløddeleinfektioner, men der var også 18 tilfælde med mere seriøse infektioner, disse udgjorde 1,2 % af alle *S. aureus* infektioner. Dette var sammenligneligt med 2004, hvor MRSA for første gang i mere end 30 år udgjorde mere end 1 % af alle *S. aureus* bakterimia tilfældene.

Resistens overfor penicillin og makrolid var i 2005 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* urinisolater, således var 4,3 % af isolaterne resistente i 2005. Der blev observeret en tilsvarende stigning i forekomsten af ciprofloxacin resistente *E. coli* urinisolater på hospitalerne, således var 5,4 % af disse isolater resistente i 2005. Stigningen i ciprofloxacin resistens skete sideløbende med en stigning i forbruget af fluorokinoloner (primært ciprofloxacin) indenfor de seneste år – både i primærsektoren og på hospitalerne. I *E. coli* urin-isolater fra både primærsektoren og hospitaler er der sket en signifikant stigning i ampicillinresistens, henholdsvis 40,5 % og 38,7 % af isolaterne var resistente i 2005. I *E. coli* urin-isolater fra primærsektoren blev der endvidere observeret en signifikant stigning i forekomsten af sulfonamid-resistens, denne var 37,6 % i 2005. I *E. coli* blod-isolater steg

gentamicin og cefuroxim-resistens signifikant til henholdsvis 2,4 % og 3,5 % (sammenlignet med 2003). Disse stigninger i resistens overfor antibakterielle midler afspejler generelt tilsvarende ændringer i forbruget af antibiotika. Niveauet af antibiotikaresistens var generelt stadig lavt for de fleste antibiotika i de mest almindelige bakterier isoleret fra kliniske prøver fra inficerede patienter i Danmark. På trods heraf antyder stigningerne i antibiotikaresistens, der er blevet observeret i de seneste år, at resistens-niveauet er under forandring, og dette understreger vigtigheden af en tæt overvågning af antibiotikaresistens, både i primærsektoren og på hospitalerne.

Summary

Antimicrobial consumption in animals

The overall consumption of antimicrobial agents in food animals remained unchanged from 2004 to 2005, while changes occurred in the consumption of different antimicrobial groups. In particular, a decrease of 1.9 tonnes was seen in the macrolide/tiamulin/lincosamide group, and an increase of 1.3 tonnes was seen in beta-lactamase sensitive penicillins.

In 2005, the antimicrobial consumption in pigs decreased by 0.2% to 92.5 tonnes active compound (81% of the total antimicrobial consumption in animals), despite a 1% increase in the production of pork. The consumption in 2005 corresponded to 47 mg/kg pork produced, representing a 1.3% reduction compared to 2004. The reduction was mainly caused by reduced prescription of tiamulin, aminoglycosides and macrolides for gastrointestinal tract infections. Prescriptions of beta-lactamase sensitive penicillins, penicillin-streptomycin combinations, macrolides and tetracyclines for respiratory disease and diseases of the limbs, central nervous system or skin continued to increase. Like in the previous years, large regional differences in antimicrobial consumption occurred in 2005. The prescription of antimicrobial agents in the western regions of Denmark (Funen county and Jutland) was 52% higher in sows/ piglets and 88% higher in finishers, than in the Eastern regions (West Zealand and Storstroem counties). A number of factors e.g. differences in disease prevalence, in management systems, or in the thresholds for treatment, may explain the regional differences.

In 2005, the antimicrobial consumption in cattle (including dairy cows) was approx. 11 tonnes, corresponding to 10% of the total consumption in food animals, and representing a 5% increase compared to 2004. The consumption in calves, corresponded to 37 mg/kg veal produced. The increase was mainly related to prescription for respiratory disease in calves, heifers and steers, accounting for 63% of the treatments in 2005. Tetracyclines, macrolides, and penicillins were the most commonly prescribed antimicrobial agents for respiratory disease. Fluoroquinolones were not used in cows and heifers in 2005, due to the legal restriction imposed from 2002.

In 2005, 420 kg active compound or 0.4% of the total veterinary antimicrobial consumption was prescribed for use in poultry. The antimicrobial consumption in the broiler production remained unchanged as compared to 2004, and corresponded to 0.8 mg/kg meat produced.

The consumption in aquaculture in 2005 was 2406 kg, corresponding to 2.1 % of the total veterinary antimicrobial consumption, and representing a 1% increase compared to 2004. The total consumption in aquaculture corresponds to 29 mg/kg fish produced in fresh water and app.190 mg/kg fish produced in salt water.

Antimicrobial consumption in humans

In 2005, a marked increase of 5.1% in the use of antibacterial agents was observed compared to 2004. The total consumption of antimicrobial agents increased to 32.4 million DDD or 16.4 DDD/1,000 inhabitant-days.

In primary health care, the total consumption of antibacterial agents increased by 4.9% in 2005. There was no significant change in the usage of different classes of antibacterial agents with beta-lactamase sensitive penicillins, extended spectrum-penicillins and macrolides accounting for more than 70% of the consumption. Reasons for the steady increase in consumption of antibacterial agents have yet to be determined. Similar differences in consumption between the Danish counties from 1997 to 2005 indicate that local factors influence the consumption. The continued increase in consumption of fluoroquinolones (from 0.28 to 0.32 DDD/1,000 inhabitant-days between 2004 and 2005) has been followed by an increase in fluoroquinolone-resistant *E. coli* isolates. This is in accordance with expectations described in previous reports.

In Danish hospitals, consumption of antibacterial agents continued to increase. From 1997 to 2005, average hospital consumption increased by 48% to an estimated 624 DDD/1,000 occupied bed-days. From 1997 to 2005 the increase in consumption of antibacterial agents was 12.5% when expressed as DDD/1,000 discharges. It should be noted, however, that in the same period Denmark has seen an 8% increase in number of discharged patients and a concomitant 16% decrease in the number of bed-days. Therefore, part of the increase in antibiotic use – both in and outside hospitals – may be explained by a higher activity of the national health services. From 1997 to 2005, the increased use of antibacterial agents has been associated with marked changes in the pattern of consumption. The trend towards increased prescription of cephalosporins, fluoroquinolones and carbapenems replacing

extended spectrum-penicillins (except pivmecillinam), aminoglycosides and macrolides continued in 2005. In 1997, the broad-spectrum antibacterial agents, i.e. cephalosporins, fluoroquinolones, penicillins in combination with beta-lactamase inhibitors and carbapenems represented 15.4% of the total consumption. This increased to 22.4% in 2003 and has been estimated to represent 27.5% of total hospital consumption in 2005. With the increasing consumption of broad-spectrum antibacterial agents, further increases in the prevalence of bacterial isolates found resistant to cephalosporins and fluoroquinolones were found in 2005, suggesting that the potential advantage of empirical treatment with broad-spectrum antibacterial agents may be short-lived.

Resistance in zoonotic bacteria

From 2004 to 2005, the occurrence of resistance in *Salmonella* Typhimurium isolates from pigs remained unchanged except for a significant increase in resistance to ampicillin. From 1999 to 2005 resistance to tetracycline, sulfonamide and ampicillin increased significantly in *S*. Typhimurium isolates from pigs. This increase coincided with an increased consumption of tetracycline, sulfonamides and broad-spectrum penicillin in pigs in the same period.

In *S*. Typhimurium isolates from pork, resistance to nine antimicrobial agents (including ampicillin and sulfonamides) was significantly higher in isolates from imported pork as compared to isolates from Danish pork. The proportion of *S*. Typhimurium phage types DT104/104b and DTU302 was significantly higher among isolates from imported pork compared to Danish pork.

Resistance to ciprofloxacin and nalidixic acid in isolates of *S*. Enteritidis and *S*. Typhimurium from infections in humans was significantly higher among isolates from infections acquired abroad than among isolates from infections acquired in Denmark. Two *S.* Typhimurium isolates from human infections were tested phenotypically positive for extended-spectrum beta-lactamase (ESBL) activity. It is the first year, *S.* Typhimurium isolates are reported ESBL-positive.

In *C. jejuni* isolates obtained from Danish and imported broiler meat, resistance to tetracycline, nalidixic acid and ciprofloxacin was significantly higher in isolates from imported broiler meat compared to isolates from Danish broiler meat.

Among *C. jejuni* isolates from infections in humans, resistance to ciprofloxacin/nalidixic acid and tetracycline was significantly higher in isolates from infections acquired abroad, as compared to isolates from infections acquired domestically.

Resistance in indicator bacteria

From 2004 to 2005, erythromycin resistance in *Enterococcus faecium* from pigs decreased significantly. This coincided with a decrease in macrolide consumption in pigs in the same period.
Among indicator *Escherichia coli* isolates from pigs resistance to four of the tested antimicrobial agents (including ampicillin) decreased significantly from 2004 to 2005.

For *E. coli* and enterococcal isolates from healthy human volunteers, no significant changes in resistance were observed from 2004 to 2005. Gentamicin resistance was detected in 2 of the 101 *E. coli* isolates. This is the first isolation of gentamicin resistant *E. coli* in the surveillance of antimicrobial resistance in healthy human volunteers. Among the 110 stool samples from the healthy human volunteers, four vancomycin resistant enterococci were detected.

Resistance in bacteria from diagnostic submissions
The number of methicillin resistant *S. aureus* (MRSA) isolates continued to increase from 549 isolates in 2004 to 856 isolates in 2005 (these numbers include both colonisation and infection with MRSA, one isolate per patient). Part of the increase was due to a large hospital outbreak in Vejle County, which now seems to be under control. A significant increase was also observed in the Greater Copenhagen area, but with a different epidemiology, including cases diagnosed in the community, as well as cases related to cross-transmission in hospitals and nursing homes.

Overall, in 55% of the MRSA cases, the person had an infection at the time of diagnosis. Seventy-three percent of the MRSA infections acquired in Denmark had community onset. Skin and soft tissue infections were the most frequent, however MRSA was also responsible for serious infections such as bacteraemia. In 2005, there were 18 cases of MRSA bacteraemia, which corresponded to 1.2% of *S. aureus* bacteraemia cases. This was comparable to 2004 where MRSA represented more than 1% of *S. aureus* bacteraemia cases for the first time in 30 years.

Resistance to penicillins and macrolides in *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Group A streptococci) remained low in 2005.

Among *E. coli* urine isolates from primary health care, resistance to ciprofloxacin increased significantly, reaching 4.3% in 2005. In *E. coli* urine isolates from hospitals, ciprofloxacin resistance also increased significantly to 5.4%. These increases in ciprofloxacin resistance were consistent with parallel increases in consumption of fluoroquinolones (mainly ciprofloxacin) observed in recent years, both in primary health care and hospitals. Among *E. coli* urine isolates from primary health care and hospitals, resistance to ampicillin increased significantly in 2005 reaching 40.5% and 38.7%, respectively. In *E. coli* urine isolates from primary health care, resistance to sulphonamides also increased significantly reaching 37.6% in 2005. Among *E. coli* blood isolates, gentamicin and cefuroxime resistance increased significantly to 2.4% and 3.5%, respectively (as compared to 2003). These increases in antimicrobial resistance generally reflected similar changes in antimicrobial consumption. Although antimicrobial resistance generally remains low for most antimicrobials and most bacteria commonly isolated from clinical samples from infected patients in Denmark, the increases observed in recent years suggest that this is changing and underline the importance of close monitoring of antimicrobial resistance, both in primary health care and in hospitals.

Demographic data

Demographic data

Demographic information is presented in order to show the magnitude of animal and human populations in which antimicrobials were used during 2005.

Table 1 shows the production of food animals (including animals for live export), meat, and the population of dairy cattle. From 2004 to 2005, the production of broilers and cattle decreased by 7.8% and 13.1% respectively, while the production of pigs increased by 2.5%.

Table 2 provides information on distribution of the human population in Denmark and on the Danish health care system by county. Figure 1 shows counties in Denmark.

DANMAP 2005

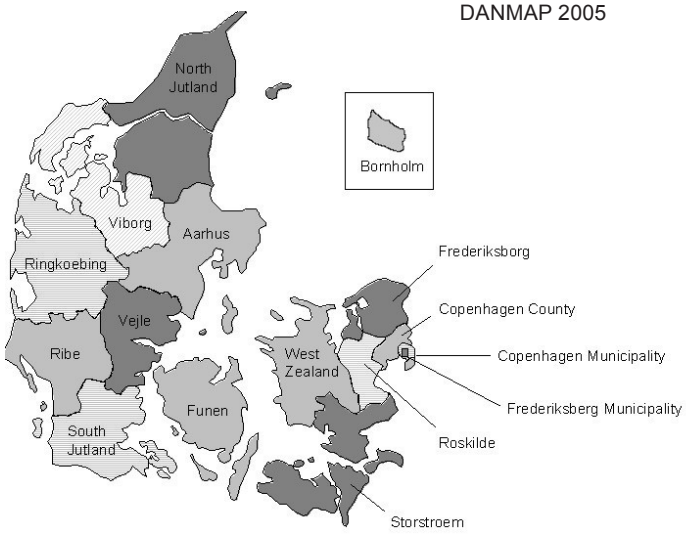

Figure 1. Counties in Denmark

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

DANMAP 2005

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs		Farmed fish	
											Fresh water	Salt water
	1,000 heads	mill.kg	1,000 heads	mill.kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	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	1,086	13	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	1,073	13	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	777	11	625	161	596	4,540	24,434	1,898	29	8
2004	130,674	181	1,086	20	632	165	563	4,434	25,141	1,965	28	7
2005	120,498	180	1,237	17	549	145	558	4,449	25,758	1,988	28	8

Source: Statistics Denmark

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

DANMAP 2005

County name	No. inhabitants (01/01/2005)	No. inh./km2 (2005)	No. inh./GP c) (2004)	No. bed-days d) (2004 provisional)	No. hospitals d) (2005)
Copenhagen Municipality a)	502,362	5,691	1,585	815,000	4
Frederiksberg Municipality a)	91,886	10,477	1,557	93,000	1
Copenhagen County b)	618,237	1,175	1,623	556,000	3
Frederiksborg	375,705	278	1,585	301,000	4
Roskilde	239,049	268	1,672	222,000	2
West Zealand	304,761	103	1,516	238,000	4
Storstroem	262,144	77	1,551	252,000	5
Bornholm	43,347	72	1,314	38,000	1
Funen	476,580	136	1,616	488,000	5
South Jutland	252,980	64	1,479	188,000	4
Ribe	224,454	72	1,570	167,000	3
Vejle	358,055	120	1,584	330,000	6
Ringkoebing	274,574	57	1,560	226,000	5
Aarhus	657,671	144	1,558	617,000	8
Viborg	234,434	57	1,553	216,000	4
North Jutland	495,068	80	1,587	468,000	8
Denmark	5,411,307	125	1,575	5,215,000	67

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

Antimicrobial consumption

In Denmark, all antimicrobials, except for those approved by the EU as feed additives, are prescription only medicines, and must either be purchased through a pharmacy (97% of the consumption) or through a feed mill (2.6% of the consumption). Antimicrobial drugs purchased at pharmacies are either administered or handed out by veterinary practitioners (13% of the purchased antimicrobials), or purchased by farmers/ animal owners according to veterinary prescription (87% of the purchased antimicrobials).

From 2001 onwards, detailed data on usage of antimicrobials in each animal species were based on the Danish register of veterinary medicines, VetStat (Please see Appendix 1 for further details on the VetStat programme). Prior to 2001, data were based on overall sales data from the pharmaceutical industry (see Table 3).

Consumption of prescribed antimicrobials (kg active compound)

Table 3 shows the trends from 1990 to 2005 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, mainly due to increased consumption in pigs. During the same period the production of pork increased by 15% (Table 1). From 2001 to 2002, the consumption was stable with a yearly increase in animal production exceeding the increase in antimicrobial consumption (Table 1). In 2003 and 2004, the yearly antimicrobial consumption in food animals increased again by 6.9% and 9.7%, respectively, due to increasing consumption in pigs. The overall consumption in food animals was unchanged in 2005 as compared to 2004, while changes occurred in the consumption of different antimicrobial groups. In particular, a decrease of 1.9 tonnes was seen in the macrolide/tiamulin/ lincosamide group, while a 1.3 tonnes increase was seen in b-lactamase sensitive penicillins.

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 before the cessation of growth promoter use.

Table 4 shows the total veterinary consumption of prescribed antimicrobial drugs in 2005 in kg active compound by animal species and age groups, including consumption in companion animals. The total veterinary consumption amounted to 114.1 tonnes in 2005, representing a small decrease (0.2%) compared to 2004. The consumption in pigs comprised 81% of the total veterinary consumption, while consumption in cattle, poultry and fish comprised 11%, 0.4% and 2% of the consumption. The remaining 5.6% was used in other animal species. These percentages include antimicrobials used in veterinary practice.

Antimicrobial consumption in pigs

In 2005, the total antimicrobial consumption in pigs decreased by 0.2%, to 92.5 tonnes, despite an increase in the production of pork of 1% (Table 1, Table 4). During January to May 2005, the antimicrobial consumption in pigs was higher as compared to the same period in 2004. Conversely, in July to December 2005, the antimicrobial use in pigs was lower than the

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

DANMAP 2005

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

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 2001-2005: VetStat. For comparability between VetStat data and previous data, see DANMAP 2000. Only veterinary drugs are included. Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded. Dermal spray with tetracyclin, used in production animals, is the only topical drug included

a) Kg active compound rounded to nearest 50 or 100

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

c) Consumption in aquaculture was not included before 2001

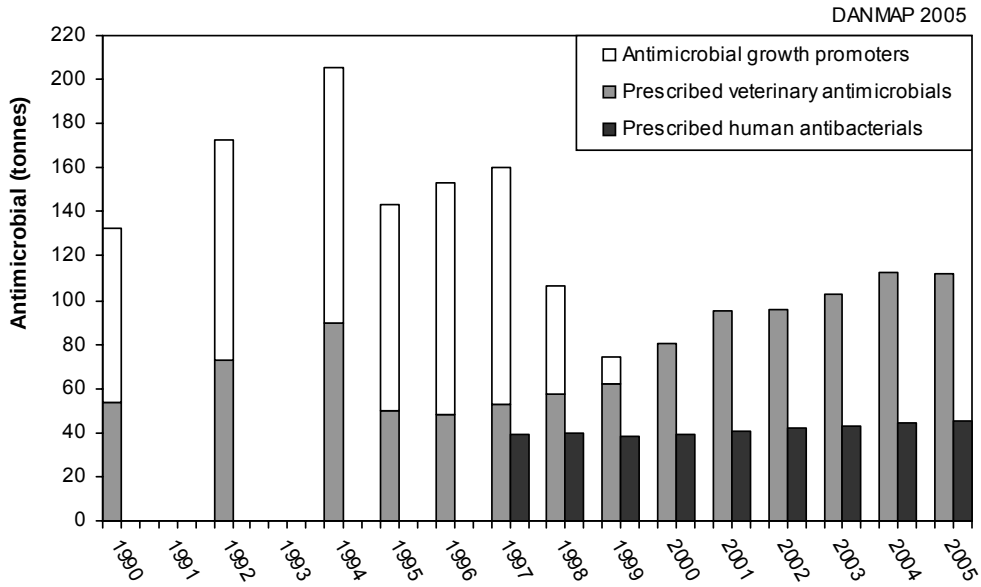

Figure 2. Consumption of prescribed antimicrobials and growth promoters in animal production and prescribed antibacterials in humans, Denmark

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). 2001-2005: Data from VetStat.

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

DANMAP 2005

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	29	2366	70	2	0	796	1025	998	8295	3357	4865	2579	23	24405
Weaners	21	5771	11	<1	0	924	5778	2634	1493	3221	2552	12268	199	34873
Finishers	26	807	7	<1	0	1374	3969	3802	5756	2428	290	12446	3	30908
Age not given	3	154	1	<1	0	82	257	271	458	233	200	680	5	2346
Cattle														
Cows and bulls	2	12	6	0	0	5	20	0	182	23	34	33	<1	317
Calves<12 months	80	215	2	<1	0	6	26	<1	280	221	335	335	2	1503
Heifers, Steers	<1	2	<1	0	0	1	2	0	5	2	3	6	<1	22
Age not given	3	27	7	<1	0	6	12	11	55	51	39	68	1	280
Poultry														
Broilers	0	10	0	6	0	5	<1	0	<1	29	3	2	0	55
Rearing, broilers	0	0	0	4	0	0	0	0	0	28	0	0	0	31
Layers, primatily rearing	0	0	0	0	0	0	0	<1	0	9	13	<1	0	23
Turkeys	0	2	0	6	0	0	0	0	0	77	4	3	0	93
Geese and ducks	0	0	0	0	0	0	<1	0	0	5	0	0	0	5
Gamebirds	0	3	0	0	0	<1	13	<1	0	18	24	5	0	64
Production category not given	0	1	0	<1	0	<1	<1	<1	0	17	11	3	<1	35
Small ruminants	0	1	<1	<1	0	<1	2	2	4	7	7	7	<1	30
Mink	<1	304	<1	1	0	63	154	<1	<1	659	186	53	<1	1420
Aquaculture	140	1	0	0	534	0		<1	<1	<1	1729	1		2406
Other production animals	<1	9	<1	0	0	4	5	<1	9	12	20	6	<1	67
Horses	<1	4	8	1	0	<1	6	<1	19	11	146	6	<1	204
Pet animals	<1	6	88	7	0	13	3	4	14	94	78	20	11	338
Farm identified d)	<1	7	<1	1	0	0	7	7	16	15	5	34	<1	93
For use in vet. practice e)														
- Small animal practice	<1	<1	225	7	0	7	4	0	55	346	62	37	20	763
-Topical drugs	<1	4	0	0	0	0	0	0	0	0	216	41	10	270
- Intramammaries	0	38	92	0	0	5	0	0	60	157	14	0	<1	367
- Micellaneous	67	1081	49	11	0	112	681	101	5609	1503	2510	1458	3	13185
Total	345	8,459	497	45	534	2,609	10,941	6,833	14,018	9,167	8,481	27,513	256	114,103

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

a) 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

year before. The decrease is succeeding a major increase of 27% from 72.9 tonnes in 2002 to 92.7 tonnes in 2004, when the production of pigs increased by only 4% (Table 1).

Taking production figures from 2005 into account (Table 1), the consumption in pigs was 46.5 mg antimicrobial per kg meat produced. This represents a 1.3% decrease compared to 2004. When measured in ADD_{kg} (Defined Animal Daily Dose for 1 kg bodyweight), the decrease was 0.4%, corresponding to a 1.4% decrease per kg meat produced.

Table 5 shows the antimicrobial consumption in pigs by route of administration (in ADD_{kg}). From 2002 to 2004, the consumption of antimicrobials for water medication increased by 25%, feed medication increased by 17%, and parenteral medication (injectables) increased by 26%. In 2005, oral medication decreased by 3.7% (in ADD_{kg}). Oral medication increased only for tetracyclines, with an 84% increase in water medication. Antimicrobials for parenteral administration increased by 8.5%. This increase was mainly in beta-lactamase sensitive penicillins.

Table 6 shows the trends in antimicrobial consumption in pigs by age group from 2001 to 2005. Antimicrobial consumption increased in sows (19%) and finishers (27%) during 2003-2004 as compared to the 2002 level. In weaners, an increase (19%) was seen only in 2004. In 2005, an increasing trend was seen in prescription for finishers, while a decreasing trend was

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

DANMAP 2005

		Oral, in water				Oral, in feed				Parenteral			
		2002	2003	2004	2005	2002	2003	2004	2005	2002	2003	2004	2005
ATC_{vet} group b)	Therapeutic group c)	ADD_{kg} (1000s)											
QJ01A	Tetracyclines	102	145	218	401	843	913	965	869	186	205	231	252
QJ01B	Amphenicols	0	0	0	0	0	0	0	0	0	4	5	4
QJ01CE	Penicillins, β-lactamase sensitive	0	0	0	0	0	0	0	0	643	726	848	915
QJ01CA/CR	Penicillins, other	290	310	383	301	4	32	66	100	183	212	229	229
QJ01DA	Cephalosporins	0	0	0	0	0	0	0	0	16	27	30	34
QJ01E/QP51	Sulfonamides, trimethoprim	0	0	0	0	83	79	105	120	187	219	239	253
QJ01FA	Macrolides	716	693	773	729	743	703	796	772	26	32	39	88
QJ01FF	Lincosamides	203	238	264	219	244	268	281	246	136	147	161	173
QJ01XX	Tiamulin	391	480	614	532	373	441	490	505	16	21	24	22
QJ01G/QA07A	Aminoglycosides	389	364	349	322	41	34	31	24	0	0	0	0
QJ01MA	Fluoroquinolones	0	0	0	0	0	0	0	0	16	5	1	1
QJ01 d)	Other antimicrobials	37	50	52	46	0	0	0	0	182	200	202	209
Total		2,127	2,279	2,653	2,551	2,330	2,470	2,734	2,637	1,593	1,796	2,009	2,180

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 a) given as Defined Animal Daily Doses (ADDs) from 2001 to 2005, Denmark

DANMAP 2005

		Pharmacies and feed mills															
Age group		Sows/piglets					Weaners				Finishers					Age not given b)	
Animal standard weight		200 kg					15 kg				50 kg					50 kg	
		2001	2002	2003	2004	2005	2001	2002	2003	2004	2005	2001	2002	2003	2004	2005	2005
ATC_{vet} group	Therapeutic group	ADD (1,000s)															
QJ01A	Tetracyclines	873	878	915	927	877	36,766	32,089	32,367	38,207	43,419	9,060	8,721	11,138	12,212	13,096	824
QJ01B	Amphenicols	<1	0	6	7	7	<1	3	84	105	71	0	0	22	32	26	3
QJ01CE	Penicillins, β–lact. sen	1,558	1,779	2,015	2,230	2,315	2,224	2,519	2,903	3,969	4,089	4,098	4,577	5,121	6,323	7,262	549
QJ01CA/CR	Penicillins, other	820	1,024	1,118	1,201	1,169	8,500	9,965	12,720	16,348	14,414	1,813	2,144	2,420	3,500	3,282	318
QJ01DA	Cephalosporins	38	60	99	113	132	59	147	254	263	267	16	36	56	60	62	10
QJ01E/QP51	Sulfonam./trimeth.	788	949	1,084	1,217	1,301	3,478	4,018	4,146	5,454	6,124	168	205	174	232	238	181
QJ01FA	Macrolides	818	809	746	773	774	50,579	46,467	41,173	52,157	50,484	11,376	11,588	12,255	12,274	12,676	881
QJ01FF	Lincosamides d)	437	567	580	588	574	14,000	17,472	19,821	22,411	19,192	3,225	3,817	4,412	4,622	4,382	330
QJ01G/A07AA	Aminoglycosides	292	254	237	220	171	27,423	23,915	22,231	21,469	19,782	261	220	194	124	237	75
QA07AA10	Colistin (local GI)	1	17	23	24	23	50	2,108	2,910	3,017	2,656	1	15	18	14	13	21
QJ01MA	Fluoroquinolones	91	49	21	3	4	521	182	11	7	3	124	67	5	3	2	<1
QJ01R	Combinations	592	642	703	669	661	1,905	2,145	2,211	3,075	3,589	291	351	423	380	369	84
QJ01X	Pleuromutilins e)	504	497	946	987	811	15,109	18,234	19,759	24,811	23,494	6,991	7,517	8,478	10,177	10,157	735
QJ51	Intramammaries	8	6	4	2	1	1	1	1	0	0	1	1	<1	1	<1	<1
QG01AA	Gynaecologic (local)	<1	<1	0	<1	<1	<1	<1	0	0	0	0	0	0	0	0	0
Total		6,821	7,531	8,498	8,958	8,820	160,615	159,264	160,590	191,295	187,585	37,424	39,257	44,717	49,956	51,802	4,009

a) Consumption in veterinary practice comprises an estimated 1-2%. These data are not included
b) Consumption of antimicrobial agents in pigs where age not given varied between 3.8 - 4.7 milionl ADD_{50} annually during 2001-2004, see DANMAP 2004
c)Beta -lactamase sensitive penicillins
d) Lincosamide/spectinomycin combinations comprise 81% of this group
e) Mainly tiamulin

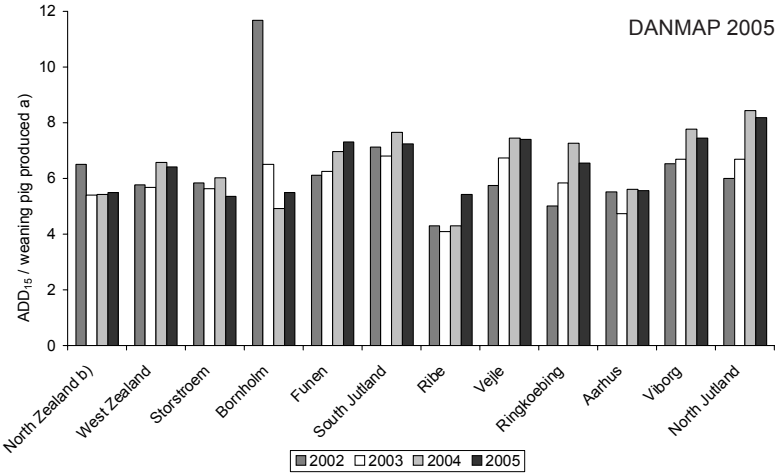

Figure 3. Antimicrobial consumption in weaning pig herds by county from 2002-2005, Denmark
a) ADD_{15} = defined daily dose for 15 kg pig
b) North Zealand defined as Roskilde and Frederiksborg counties

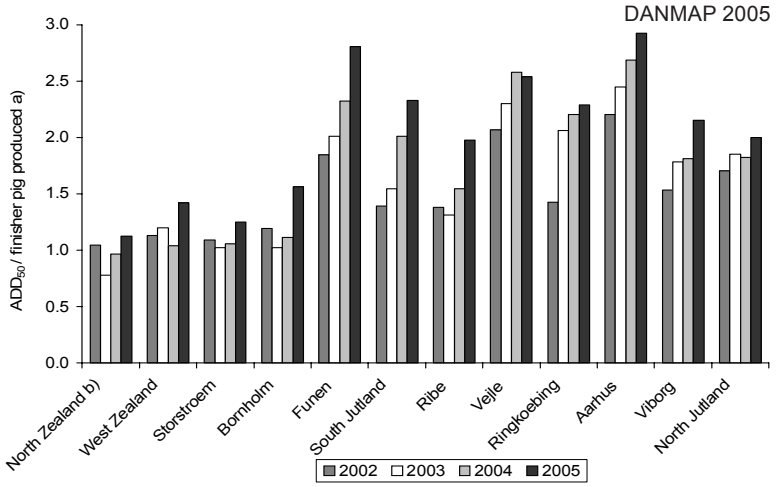

Figure 4. Antimicrobial consumption in finisher pig herds by county from 2002-2005, Denmark
a) ADD_{50} = defined daily dose for 50 kg pig
b) North Zealand defined as Roskilde and Frederiksborg counties

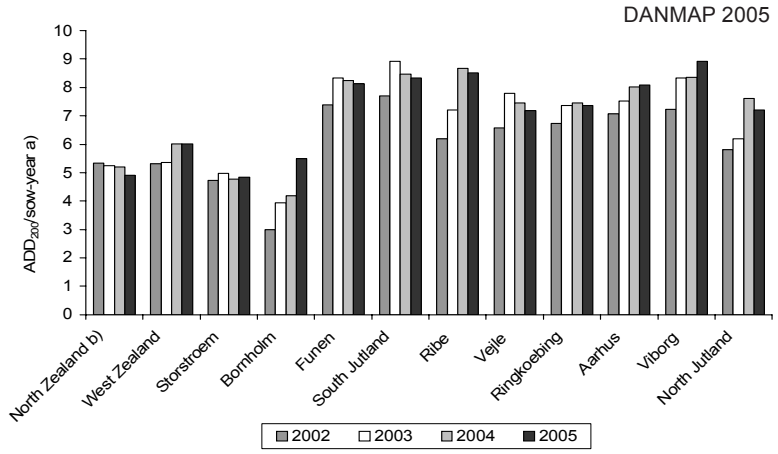

Figure 5. Antimicrobial consumption in sow herds by county from 2002-2005, Denmark
a) ADD_{200} = defined daily dose for 200 kg pig
b) North Zealand defined as Roskilde and Frederiksborg counties

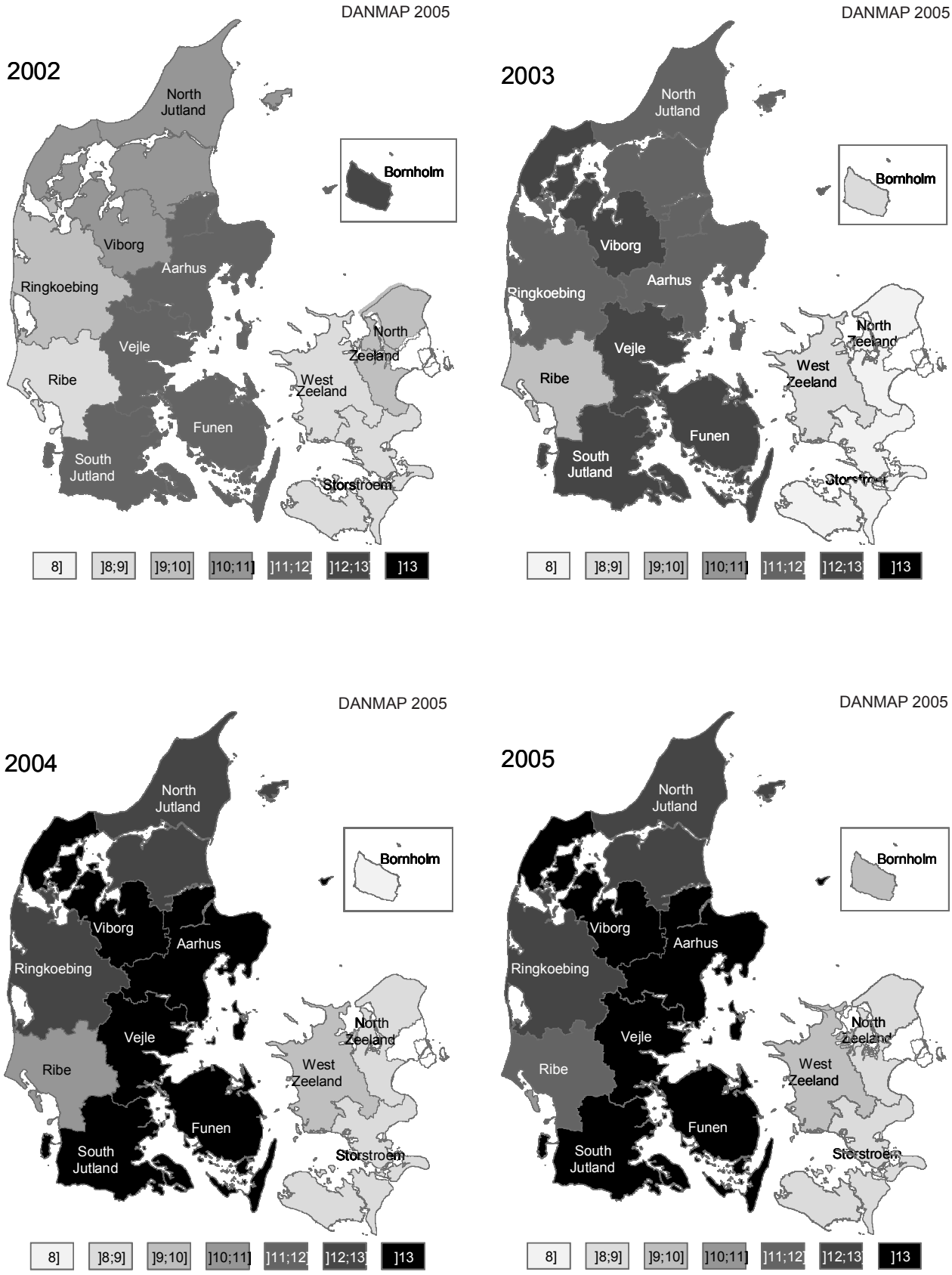

Figure 6. Antimicrobial consumption (ADD_{kg}) per kg-live-pig-year by county, Denmark
Number of pigs per county (source: Statistics Denmark, 2005)
Estimated kg-live-pig-year assuming average live weights at treatment: Weaners, 15 kg, finishers, 50 kg, sows (incl. piglets), 200 kg.
North Zealand comprises Frederiksborg and Roskilde Counties

seen in the prescription for weaners and sows.
In 2005, the consumption was 7.3 ADD/weaner and 2.0 ADD/finisher produced, including weaners and finishers for export or used in breeding. The consumption per finisher increased by 2%, while the consumption per weaner decreased by 4% as compared to 2004.

In finishers, the overall increase was related to increasing prescription for respiratory disease (11%) and disease in limbs/CNS/skin[1] (9%). Gastro-intestinal (GI) diseases, respiratory diseases and disease of the limbs/CNS/skin accounted for 59%, 22% and 18%, respectively, of the antimicrobial consumption in 2005.

In weaners, prescriptions for GI diseases accounted for 74% of the antimicrobial consumption in 2005 and prescription for respiratory disease accounted for 15%. In 2004, the corresponding proportions were 78% and 12%

In sow herds, 34% of the antimicrobials were prescribed for diseases in the limbs/CNS/skin in 2005. Thirty-seven percent were prescribed for disease in the reproductive organs and udder, and 9% were prescribed for respiratory disease. A long lasting trend (2001-2005) was seen in prescription for disease in the reproduction system and udder, explaining the increasing consumption of sulphonamide/trimethoprim in sows (Table 6)

Figure 7 presents the development in ADD_{kg} per pig slaughtered, for the most important antimicrobials used for treatment of pigs. Macrolides, tetracyclines and pleuromutilins remain the most commonly used antimicrobials in pigs, and the use of tetracyclines continued to rise in 2005. A slight decrease was seen in the use of macrolides and pleuromutilins, remaining above the 2003 level. The consumption of fluoroquinolones in pigs further decreased to 4 kg in 2005, as compared to 94 kg in 2001. The decrease from 2001 onwards was most likely due to legal restrictions on use of fluoroquinolones in food animals, implemented in 2002.

Splitting the antimicrobial consumption into indication for prescription shows that the overall decrease was caused by a decrease in prescription for the major indication, GI disease, in 2005. This decrease explains the decreasing trend in tiamulin, aminoglycosides and macrolides, of which 69%, 98%, and 79%, respectively, are used for GI disease. (Table 6). The prescription for GI disease decreased by 20% in sows/piglets and 7% in weaners in 2005 compared to 2004, but in weaners

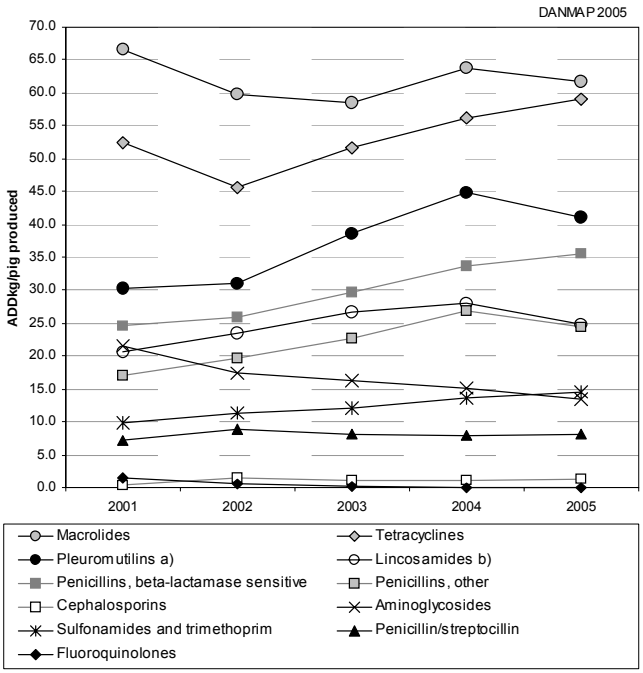

Figure 7. Trends in antimicrobial consumption (in ADD_{kg}) in pigs, 2001-2005, Denmark

Antimicrobial groups refer to ATC_{vet} groups (see Table 5). Amphenicols, colistin, intramammaries and gynaecologicals are not included in the figure. Data from veterinary practice are not included (amounts to <2%)
a) Pleuromutilins comprise primarily tiamulin
b) Lincosamide/spectinomycin combinations comprise 65% of this group

the prescription for GI disease was still 10% above the 2003 level.

The overall changes between antimicrobial groups were also related to increased prescription for respiratory disease and diseases of the limbs/CNS/skin which has continued to rise since 2002. Prescription for disease of the limbs/CNS/skin increased by 35% in sows/piglets and by 52% in finishers from 2002 to 2005. Prescription for respiratory disease increased by 59% in sows/piglets by 104% in weaners, and by 60% in finishers from 2002 to 2005. Correspondingly, the use of drugs which are mainly used for these indications increased in 2005, e.g. beta-lactamase sensitive penicillins and penicillin/streptomycin combinations, with a particularly high consumption during the first two quarters of 2005. The prescription for respiratory disease was particularly high during January-May 2005, explaining half of the increase in tetracycline (16% prescribed for respiratory disease as compared to 14% in 2004). The consumption of tetracyclines in the second half of 2005 was comparable to the level in 2004.

[1] These organ systems are merged into on disease group in VetStat

The prescription of macrolides for respiratory and limb/CNS/skin disease increased as part of the long term trend, while the prescription of macrolide for GI disease decreased in 2005.

A decrease in consumption of broad spectrum penicillins is mainly explained by decreasing prescription for respiratory disease, implying a trend towards more narrow spectrum treatment.

Lincosamides are mostly (80%) used in combination with spectinomycin, and the decrease in 2005 was the result of a gradual decreasing trend throughout 2004-2005. Lincomycin/spectinomycin is mainly used in GI disease (54% of consumption, mainly weaners) and diseases of the limbs/CNS/skin (32% of consumption, mainly in finishers).

The regional differences in antimicrobial consumption observed since 2002 were still present in 2005 (Figures 3-6). The regional differences increased during 2003-2004, with an important increase in prescription for gastrointestinal disease in finishers, restricted to the western regions (Funen County and Jutland) In 2005, the increasing consumption in finishers was nationwide. In the western regions of Denmark, the prescription of antimicrobials in 2005 was 52% higher in sows/piglets and 88% higher in finishers, as compared to the eastern regions (Zealand and Storstroem counties). Some of the difference registered in finishers may have occurred in growers (large weaners), erroneously registered as finishers at prescription by a few veterinarians.

Table 7. Consumption of antimicrobials in cattle given as Defined Animal Daily Doses (ADDs) 2003 to 2005, Denmark

DANMAP 2005

		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			100 kg		
		2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
ATC_{vet} group	Therapeutic group	ADD (1,000s)											
QJ01A	Tetracyclines	4	6	5	394	414	408	3	3	2	26	22	41
QJ01B	Amphenicols	<1	<1	<1	30	38	40	<1	<1	<1	2	2	2
QJ01CE	Penicillin, β-lact. sen. b)	7	15	24	184	151	123	1	1	1	20	15	33
QJ01CA/CR	Penicillins, other	3	2	2	188	186	157	1	2	<1	25	9	32
QJ01DA	Cephalosporins	1	2	3	24	20	14	<1	<1	<1	2	1	4
QJ01E	Sulfonamid./trimeth.	3	3	2	89	127	126	<1	<1	<1	15	8	14
QJ01FA	Macrolides	4	5	4	39	29	195	<1	<1	2	16	6	23
QJ01FF	Lincosamides c)	1	<1	1	16	14	9	<1	<1	<1	2	2	4
QJ01G/QA07AA	Aminoglycosides	1	<1	<1	66	62	52	<1	0	<1	10	3	10
QA07AA10	Colistin (local GI)	<1	0	0	5	4	4	0	0	0	0	1	2
QJ01MA	Fluoroquinolones	<1	0	0	2	3	0	0	0	0	1	0	0.3
QJ01R	Combinations	1	1	1	118	92	96	<1	<1	<1	7	5	7
QJ01X	Other antimicrobials	<1	1	0	3	1	0	<1	<1	0	15	11	12
QJ51	Intramammaries	30	49	51	<1	1	1	<1	<1	<1	0	0	1
QG01AA	Gynaecologic (local)	<1	<1	1	0	0	0	0	0	0	0	0	0
Total		55	85	94	1,158	1,142	1,225	7	9	7	143	84	183

		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	2005	2003	2004	2005	2003	2004	2005
ATC_{vet} group	Therapeutic group	ADD (1,000s)								
QJ01A	Tetracyclines	124	160	165	137	149	154	10	14	15
QJ01B	Amphenicols	<1	<1	1	10	14	24	<1	<1	0
QJ01CE	Penicillin, β-lact. sen. b)	369	456	474	52	48	42	18	26	25
QJ01CA/CR	Penicillins, other	69	90	74	59	72	65	4	6	5
QJ01DA	Cephalosporins	29	56	41	13	17	12	2	4	2
QJ01E	Sulfonamid./trimeth.	51	68	59	36	51	38	2	3	2
QJ01FA	Macrolides	69	96	80	12	17	50	4	5	4
QJ01FF	Lincosamides c)	1	3	1	7	13	8	<1	<1	0
QJ01G/QA07AA	Aminoglycosides	2	3	1	25	42	64	<1	<1	0
QA07AA10	Colistin (local GI)	<1	0	0	1	2	17	0	0	0
QJ01MA	Fluoroquinolones	<1	<1	0	2	4	1	0	<1	0
QJ01R	Combinations	13	18	20	45	45	42	2	5	2
QJ01X	Other antimicrobials	<1	1	0	<1	0	0	0	0	0
QJ51	Intramammaries	885	1,091	910	0	0	0	11	14	13
QG01AA	Gynaecologic (local)	54	119	99	0	0	0	2	4	4
Total		1,667	2,162	1,924	400	473	518	55	82	73

a) Data from veterinary practice is shown separately, because the use in cattle practice is underreported by an estimated 20% in 2003 and 2005 and estimated to 5% in 2004
b) Beta-lactamase sensitive penicillins
c) Comprises both lincomycin and lincomycin/spectinomycin combinations

The regional differences in antimicrobial consumption indicate that the increase in antimicrobial consumption during 2003-2004, was not related to the cessation of antimicrobial growth promoter usage in the late 1990s (Figure 2). This is supported by the concurrent decreasing trends in prescription for GI disease in weaners in the eastern regions and in sows nationally.

A number of factors may have caused the regional increasing trend since 2002, peaking early in 2005, and the following decrease. These may include disease prevalence, differences in management systems or variations in thresholds for treatment among farmers or veterinarians.

The viral disease Post-weaning Multisystemic Wasting Syndrome (PMWS) has been increasingly frequent during 2001-2005. The first five cases were diagnosed in 2001, and in 2002-2003 outbreaks were seen in all parts of Jutland and Funen. In 2002 the first cases were diagnosed on Zealand, but few cases were diagnosed in the following years.
In 2005, the Danish Institute for Food and Veterinary Research initiated research to investigate the relative importance of PMWS and differences between veterinarians on antimicrobial consumption. Preliminary results suggest a significant increase in antimicrobial use in sows and weaners around the diagnosis of PMWS, but the effect seems to fade out around six month after the diagnosis.

The effect of PMWS may explain some of the increase in antimicrobial use, but not the large differences between the eastern and western regions. Firstly, large differences were present already in 2001-2002. Despite the introduction of PMWS on Zealand and in Storstroem county in 2002, with increasing occurrence during 2004, antimicrobial use has not increased to the levels seen in Jutland and Funen the previous years. Finally, PMWS does not explain the large regional differences in treatment of finishers.

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

DANMAP 2005

Production type		Broilers			Rearing for broiler production			Layers, including rearing b)			Production type unknown c)		
		2003	2004	2005	2003	2004	2005	2003	2004	2005	2003	2004	2005
ATC_{vet} code	Therapeutic group	ADD_{kg} (1.000s)											
QA07AA	Aminoglycosides	0	0	0	0	0	0	0	0	0	200	300	81
QJ01A	Tetracyclines	60	2	32	0	0	0	540	2	8	91	106	56
QJ01CA	Amoxicillin	2,988	4,469	3,708	1,358	5,760	3,896	350	1,066	675	2,342	3,657	2,223
QJ01E/QP51	Sulfonamides d)	8	56	48	0	0	0	328	210	228	348	439	165
QJ01FA	Macrolides	0	29	3	0	0	0	0	0	0	186	90	3
QJ01FF	Lincosamides e)	0	20	0	0	0	0	0	8	0	0	4	40
QJ01MA	Fluoroquinolones	270	603	171	80	420	400	0	100	0	411	131	40
QJ01X	Tiamulin	0	75	0	0	0	0	3	0	3	5	3	5
Total		3,325	5,254	3,962	1,438	6,180	4,296	1,220	1,386	913	3,582	4,729	2,613

Includes data from all sources (pharmacies, feedmills and veterinary practice). Data includes delayed reporting from 2004, not included in DANMAP 2004
a) *Gallinus domesticus*
b) More than 99% is used in rearing
c) May include other species than *Gallinus domesticus*
d) Includes sulfaclozin, a coccidiostat/antibacterial on prescription
e) Lincomycin in combination with spectinomycin

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

DANMAP 2005

Production type		Turkeys			Ducks, geese			Game birds		
		2003	2004	2005	2003	2004	2005	2003	2004	2005
ATC_{vet} code	Therapeutic group	ADD_{kg} (1.000s)								
QA07AA	Aminoglycosides	0	200	100	0	0	0	100	167	100
QJ01A	Tetracyclines	0	0	60	154	14	0	128	148	94
QJ01CA	Amoxicillin	10,867	4,871	8,363	250	400	375	904	966	1,852
QJ01E/QP51	Sulfonamides b)	58	36	68	0	0	0	316	459	398
QJ01FA	Macrolides	0	7	0	0	11	12	273	113	177
QJ01FF	Lincosamides c)	0	0	100	0	0	0	0	0	14
QJ01MA	Fluoroquinolones	340	1,607	1,260	0	150	0	1	30	0
QJ01X	Tiamulin	0	0	0	0	3	0	10	18	13
Total		11,264	6,721	9,950	404	578	387	1,732	1,900	2,647

a) Poultry other than domestic fowl (*Gallinus domesticus*)
b) Includes sulfaclozin, a coccidiostat/antibcaterial on prescription
c) Lincomycin in combination with spectinomycin

Part of the increase during 2003-2004 was clearly related to the occurrence of PMWS, and the decreasing trend in antimicrobial consumption since 2005 may be partly related to decreasing effects of PMWS.

The increase in prescription for treatment of respiratory disease during 2002-2005 suggests increasing occurrence of respiratory disease. The increase in finishers was confined to Jutland and Funen county until 2004. In the other age groups, the increase in prescription for respiratory disease was not significantly different in the western and eastern parts of Denmark.

Large increases in the prescriptions for locomotor/skin diseases in finishers and sow herds from 2002-2005, suggest that part of the increase may be explained by increasing attention to welfare problems – or increasing disease occurrence.

Antimicrobial consumption in cattle

In 2005, the antimicrobial consumption in cattle was estimated at 11 tonnes, or 10% of the total consumption in food animals. The vast majority (76 % in kg active compound) of the antimicrobials were used in cows, mainly dairy cattle, and 65% of the antimicrobials for cows are prescribed for mastitis. Therefore, only the consumption in calves and heifers should be compared directly with the consumption in the other meat producing animal species. In 2005, the consumption in calves, steers and heifers was 37 mg antimicrobial per kg meat.
In 2005, the majority of the data reported by veterinarians was lacking up to 20% of the antimicrobials used, due to technical problems delaying the registration (Table 7). The consumption in ADD_{kg} in 2005 was distributed on 86% in cows, 12% in calves and 2% in heifers/steers.

From 2004 to 2005, the production of veal decreased by 10%. During the same period, the total antimicrobial consumption in calves increased by an estimated 9% (Table 7). This represents an increase from 2.5 ADD_{kg}/kg to 3.0 ADD_{kg}/kg veal produced, in 2004 and 2005 respectively. The increase was mainly (72%) related to 8-9% increase in prescription for respiratory disease, causing the large increase in macrolide consumption. The most common cause for prescription in calves is respiratory disease, accounting for 63% of the treatments in 2005 and tetracyclines, macrolides, and penicillins are most commonly used. The most frequently used antimicrobial class in calves is tetracyclines, comprising 31% of the doses applied in 2005.

The use of fluoroquinolones in cattle decreased significantly from 2001 to 2003 due to the legal restriction imposed from 2002. Fluoroquinolones were not used in cows and heifers in 2005, and the consumption in calves decreased to less than 0.06 ADD_{kg}/kg veal produced.

Consumption of antimicrobials in poultry

In 2005, 420 kg active compound or 0.4% of the total veterinary antimicrobial consumption was prescribed for use in poultry production.

The consumption of prescribed antimicrobials in broilers decreased by 25% from 2004 to 2005, but is still higher than in 2003. The consumption in broiler production was 0.8 mg per kg meat produced, half of which was used in broilers and half was used in rearing flocks.

In the broiler production, the predominant antibacterial used was amoxicillin, accounting for 84% of the doses (ADDs) used in 2005 (Table 8). Fluoroquinolones were used 12% of the treatments in broilers and rearing in 2005.

In the production of eggs for consumption, antimicrobials are almost entirely used in rearing. The use of antibacterials in rearing hens decreased by 34% in 2005 compared to the 2004 level. After a significant increase in 2004, the use of fluoroquinolones decreased to the 2003 level (Table 8).

The slaughter of turkeys in Denmark decreased by almost 50% from 2002 to 2003 but at the same time, the export for slaughter began to increase. In 2004 the production of turkeys (export and slaughter) was more than 50% higher than in 2002, and 95% were slaughtered abroad since 2004. The antibacterial consumption in turkeys decreased in 2004 to a very low level (0.3 ADD_{kg}/kg meat produced) compared to 2002 (2 ADD_{kg}/kg), and increased only marginally in 2005 to 0.6 ADD_{kg}/kg (Table 9). The consumption of fluoroquinolones increased from 0.03 ADD_{kg} per kg turkey produced in 2003, to 0.08 ADD_{kg} in 2004, and decreased to 0.04 ADD_{kg} per kg turkey in 2005.

According to the prescribing veterinarians, the major increase in fluoroquinolone use in 2004 was due to multiresistant *E. coli,* and these problems are persisting in the broiler and turkey production, although involving only a few farms.
The consumption of prescribed antimicrobials in game birds was high compared to the size of the production, with an estimated consumption of 1.1 ADD_{kg}/bird in

2004 (est. 1 mill pheasants, 0.5 mill ducks and 0.1 mill other birds), increasing further in 2005 (Table 9).

Antimicrobial consumption in fish, mink and companion animals

Antimicrobial consumption in mink increased by an estimated 40% in 2004, based on sales from pharmacies. This apparent increase should be interpreted with caution, as the figures do not include sales in veterinary practice. The increase was seen in all antimicrobials used in mink, in particular in broad-spectrum penicillins and sulfonamide-trimethoprim combinations.

In 2005, the use of antimicrobial agents in aquaculture (fish) remained at 2.4 tonnes as in 2004. The consumption corresponded to 29 mg/kg fish in fresh water fish production and app. 190 mg/kg in salt water fish production. The use of quinolones increased by 73% (204 kg), while the use of sulfonamide-trimethoprim combinations decreased by 273 kg (Table 4).

The use of antimicrobials in pet animals was an estimated 1.5 - 2 tonnes, including consumption in practice. The use of cephalosporin increased further to 313 kg, corresponding to 55% of the total veterinary use of cephalosporins. The use of fluoroquinolones in pet animals, comprised 15-20 kg, or 30-50% of the total veterinary consumption of fluoroquinolones.

Antimicrobial growth promoters

In the EU, the only antimicrobial agents alllowed for growth promotion after 1999, were flavomycin, avilamycin, salinomycin, and monensin. By 2006, these agents were also banned for use as growth promoters in the EU.

Following the official ban of virginiamycin in Denmark in 1998, the industry decided to voluntary discontinue all further use of antimicrobial growth promoters. This became effective in broilers, finishers and cattle in 1998. In weaning pigs the use of AGPs was phased out during 1999. Since 2000, there has been no reported use of AGPs for animals slaughtered in Denmark (Table 10).

Coccidiostats

Antimicrobials may be used as coccidiostats in poultry in Denmark, provided an EU approval as feed additives. This included the ionophores salinomycin and monensin, which were the most frequently used coccidiostats again in 2005. Like these ionophores, a new coccidiostat, semduramicin also has bacteriostatic effect. Use of semduramicin was reported for the first time in 2005. Salinomycin comprises 90% of the coccidiotats reported. In addition, use of monensin, narasin, lasalocid, nicarbazin and metichlorpindol/ methylbenzoat in broilers have been reported. In 2005, the reporting of coccidiostats decreased further by an estimated 20% below the level in 2004.

Antimicrobial residues

The frequency of violations of antibacterial residue limits for finishers did not exceed 0.02% from 1987 to 2002, which is extremely low compared to international levels. For that reason, the frequency of sampling has been reduced by 85% since 2002.

During 2002-2005, no positive results were found among 11,693 samples of finisher pigs. Therefore, more emphasis was put on sampling sows and boars during this period. In 2002-2003, only 133 samples of sows were tested with no positive results while in 2004-2005 a total of 3,737 sows were tested and 7 results were positive. After the industry's attention focused on the frequency of violations of antibacterial residue limits for sows, the frequency dropped from 0.3% in 2004 to 0.1% in 2005.

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

DANMAP 2005

Antimicrobial group	Growth promoter	1990	1992	1994	1996	1998	1999	2000	2001	2002	2003	2004	2005
Bacitracin	Bacitracin	3,983	5,657	13,689	8,399	3,945	63	n b)	0	0	0	0	0
Flavofosfolipols	Flavomycin	494	1,299	77	18	6	665	n	11 c)	15 c)	4 c)	0	0
Glycopeptides	Avoparcin	13,718	17,210	24,117	0	0	0	n	0	0	0	0	0
Ionophores	Monensin	2,381	3,700	4,755	4,741	935	0	n	0	0	0	5 c)	0
	Salinomycin	12	0	213	759	113	0	n	0	0	0	0	0
Macrolides	Spiramycin	0	0	95	15	0.3	0	n	0	0	0	0	0
	Tylosin	42,632	26,980	37,111	68,350	13,148	1,827	n	0	0	0	0	0
Oligosaccharides	Avilamycin	10	853	433	2,740	7	91	n	3 c)	0	0	0	0
Quinoxalines	Carbadox	850	7,221	10,012	1,985	1,803	293	n	0	0	0	0	0
	Olaquindox	11,391	21,193	22,483	13,486	28,445	9,344	n	0	0	0	0	0
Streptogramins	Virginiamycin	3,837	15,537	2,801	5,055	892	0	n	0	0	0	0	0
Total		79,308	99,650	115,786	105,548	49,294	12,283	n	14	15	4	5	0

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 presumed exported for slaughter)

In the 2005 monitoring programme, no positive results were found among 313 targeted samples of cattle, 10 sheep, 5 horses, 74 targeted samples of poultry, 62 aquaculture trout, 211 milk samples, 139 eggs, 14 samples of farmed game nor in 31 samples of honey.

Residues of coccidiostats in eggs are monitored in 140 annual samples since 2001. Very low amounts were found in 45 samples in 2001 probably due to insufficient cleaning of feedmills after production of medicated feed. The frequency dropped to 18 in 2003 and 5-8 in 2004-2005 of the 140 annual samples. In 2003-2005, only one sample contained more than 10 µg/kg.

In 2005, one positive result for the antimicrobial substance metronidazole (banned in food animals) was found among 60 targeted samples and one positive result for malachite green / leucomalachite green was found among 98 samples of aquaculture trout.

In addition to monitoring, samples are taken based on suspicion. In 2005, these samples revealed antimicrobial residues in one cow and 3 sows.

Annual reports on monitoring residues in animals and food are available on the Internet at the homepage of the Danish Veterinary and Food Administration. (www.fvst.dk/Kontrol/Kontrolresultater/Restkoncentrationer/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).

Report 1

New guidelines for veterinary practitioners prescription of antibacterials for swine

The Danish Veterinary and Food Administration (DVFA) initiated an action plan in 2005 for reduction of the use of antibacterial drugs in swine. The plan was motivated by a 25% increase from 2002 to 2004 in consumption of antibacterials in swine in Denmark, and the subsequent increased risk of antibacterial resistance and compromised treatment effect in both humans and animals. The plan consists of three elements:

1. New guidelines for practitioners prescription of antibacterials for certain common diseases of swine (www.fvst.dk/Kontrol/Laegemidler_til_dyr/Valg_af_laegemidler_til_dyr/Antibiotika_til_svin).
2. Audits of veterinary practitioners by the DVFA regarding prescription and treatment patterns and follow up of antibacterial usage for practitioners with a high usage, in order to find means of affecting these patterns by self-recognition of the veterinary practitioner and thereby reduction of the antibacterial consumption;
3. Investigation of prevalence of diseases, management, and production facilities in herds with, a high or low usage of antibiotics in swine, respectively, in order to determine risk factors for a high consumption.

The new guidelines state first and possible second priority antibacterial agents to prescribe for each disease, based on the risk profile for development of antibacterial resistance in the human sector, clinical efficiency towards the disease and the occurrence of antibacterial resistance in the relevant veterinary pathogens in Denmark. Generally, any use of antibacterial agents might select for antibacterial resistance, but there is difference in their potential for selection of resistance. Furthermore, resistance towards some antibacterial agents has a higher impact on the treatment possibilities for humans. Therefore, the objective of the guidelines is to guide veterinary practitioners into choosing antibacterial agents with a minor risk of selection for antibacterial resistance, but having the same clinical efficiency towards the relevant disease, thereby securing both animal health and welfare and treatment possibilities for humans. The guidelines were elaborated after input from the Danish Institute for Food and Veterinary Research (DFVF), Statens Serum Institut (SSI), Danish Meat (DM), the Danish Veterinary Association (DVA), the Royal Veterinary and Agricultural University (RVAU) and the Danish Animal Health Industry. DFVF and SSI assessed the risk profile for development of antibacterial resistance in the veterinary and human sector and stated the actual occurrence of antibacterial resistance in the relevant veterinary pathogens in Denmark; DVA and DM assessed the clinical efficiency and common use of antibacterial agents; RVAU assessed the pharmacological impact of introducing the antibacterial agents in the guidelines.

The guidelines are a dynamic list, which will be revised according to new information on new antibacterial agents, clinical efficiency, resistance and human health aspects.

Quinolones, macrolides and cephalosporins are excluded from the guidelines. Quinolones were excluded due to a rapid development of resistance in many bacteria. The use of fluoroquinolones in food producing animals is restricted by law in Denmark. Macrolides are excluded due to risk of cross-resistance to erythromycin and human macrolide-resistant *Campylobacter* infections are associated with increased risk of mortality and severe illness [Helms *et al.* 2005. J. Infect. Dis. 191.1050-5]. Cephalosporins are excluded as they select for beta-lactamase activity and penicillinase-resistant penicillins are used in therapy of human *Staphylococcus aureus* infections. Moreover, cephalosporins are used in therapy of *Salmonella* infections in children.

Since summer 2005 there has been much debate about the new guidelines among veterinary practitioners, mostly concerning the exclusion of macrolides. Interestingly, macrolide consumption has decreased by 6% in 2005 and consumption of beta-lactamase-sensitive penicillins has increased, which is in line with the goal of the new guidelines. Whether the changes in consumption are influenced by the DVFAs actions or related to a decrease in disease prevalence might be clarified by DVFAs planned investigation of herds.

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Antimicrobial consumption in humans

Overall

In 2005, the overall consumption of antibacterials for systemic use (ATC group J01, 2006 definition) in humans in Denmark increased to 32.4 million DDDs or 16.4 DDD/1,000 inhabitant-days representing an increase of 5.1% compared to 2004. The percentage of DDDs prescribed in the primary health care sector remained stable at around 90%. The distribution of different classes of antibacterials used in primary health care and in hospitals is shown in Figure 8.

To follow overall changes in the consumption of antibacterials and to allow comparison with consumption of antibacterials in animals, total human consumption is presented in kilograms (Table 11). In 2005, 45.6 tonnes of antibacterials for systemic use were used in humans in Denmark, representing an increase of 3.5% as compared to 2004 and of 17.8% compared to 1997.

Primary health care sector

The consumption of antibacterials for systemic use in primary health care is presented in Table 12 as a number of DDD per 1,000 inhabitant-days and in Table 13 as a number of packages per 1,000 inhabitants. In 2005, the overall consumption of antibacterials for systemic use in the primary health care sector was 14.8 DDD/1,000 inhabitant-days and 649 packages/ 1,000 inhabitants. Beta-lactamase sensitive penicillins still represented 35.8% of the total consumption of antibacterials followed by penicillins with extended spectrum (18.8%) and macrolides (16.3%). This distribution was similar to previous years.

Total consumption expressed in DDD/1,000 inhabitant-days increased by 4.9% as compared to 2004. Since 2000, there has been a small but steady increase in antibacterial consumption in DDD/1,000 inhabitant-days ranging from 2 to 5% yearly. Overall, antibacterial consumption increased by 20.5% between 2000 and 2005. More than 76% of the increase between 2004 and 2005 was due to an increased consumption of macrolides (+0.18 DDD/1,000 inhabitant-days), penicillins with extended spectrum (+0.16 DDD/1,000 inhabitant-days), tetracyclines (+0.10 DDD/1,000 inhabitant-days) and beta-lactamase sensitive penicillins (+0.08 DDD/1,000 inhabitant-days).The increase in consumption of penicillins with extended

Table 11. Consumption of antibacterials for systemic use in humans (kg active compound), Denmark. These data include data from both primary health care and hospitals and have been re-calculated from original data expressed as a number of DDDs. For monitoring in human primary health care and hospitals, the recommended way of expressing consumption is DDD per 1,000 inhabitant-days and DDDs per 1,000 bed-days, respectively (see Tables 12 and 13)

DANMAP 2005

ATC group a)	Therapeutic group	Year									(lowest cal.limit - highest cal.limit) b)
		1997	1998	1999	2000	2001	2002	2003	2004	2005	
J01AA	Tetracyclines	1,519	1,486	1,383	1,486	1,475	1,501	1,542	1,636	1,747	
J01B	Amphenicols	1	1	0	0	1	0	0	0	0	
J01CA	Penicillins with extended spectrum	5,525	5,477	5,202	5,141	5,385	5,356	5,295	5,346	5,561	
J01CE	Beta-lactamase sensitive penicillins	18,840	19,969	18,825	19,749	20,730	21,263	21,630	22,230	22,520	
J01CF	Beta-lactamase resistant penicillins	1,919	2,120	2,425	2,655	3,230	3,738	4,075	4,377	4,564	
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	49	56	52	93	146	249	336	480	533	
J01D	Cephalosporins and related substances	626	614	650	692	739	811	830	894	1,475 d)	
J01EA	Trimethoprim and derivatives	245	256	258	262	280	293	307	334	351	
J01EB	Short-acting sulfonamides	3,503	3,497	3,296	3,142	3,113	3,092	3,064	3,067	2,987	
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	350	330	286	291	289	288	273	185	208	
J01FA	Macrolides	3,093	3,332	3,075	2,962	3,020	3,069	2,889	2,834	2,881	(2,869 - 2,893)
J01FF	Lincosamides	25	34	29	29	37	40	45	53	52 d)	
J01G	Aminoglycosides	61	35	42	32	30	31	28	31	32	
J01MA	Fluoroquinolones	384	405	383	344	398	451	611	722	866 d)	
J01MB	Other quinolones	15	17	16	0	0	0	0	0	0	
J01XA	Glycopeptides	25	27	33	37	36	42	43	46	51	
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70	59	59	58	52	62	
J01XD	Imidazoles	129	129	142	155	168	179	191	195	206	
J01XE	Nitrofuran derivatives (nitrofurantoin)	141	144	145	151	155	163	166	171	177	
J01XX05	Methenamine	2,234	2,132	1,956	1,788	1,637	1,662	1,590	1,473	1,383	(1,107 - 1,660)
J01XX08	Linezolid	0	0	0	0	0	3	4	5	10	
J01	Antibacterials for systemic use (total) c)	38,760	40,133	38,276	39,080	40,927	42,293	42,978	44,130	45,682	(45,393 - 45,970)

a) From the 2005 edition of the ATC classification system

b) cal.limit = calculated limit. When two different DDDs of an antimicrobial existed for different presentations, e.g. for erythromycin, methenamine, an average DDD was used. Estimates using the lowest and the highest DDD, are given in parentheses

c) Does not include polymyxins

d) In 2005, the kg active compound was estimated taking into account the DDD for each route of administration, e.g. cefuroxime parenteral DDD=3 g and cefuroxime oral DDD=0.5 g. From 1997 to 2004, it was estimated with a DDD corresponding to an average for the various routes, e.g. for cefuroxime: 1.75 g

spectrum was essentially due to pivmecillinam, which use increased from 0.85 to 0.97 DDD/1,000 inhabitant-days between 2004 and 2005.

All the Danish counties were concerned by the increase in antibacterial consumption and showed – with the exception of Bornholm - similar variations between 1997 and 2005 (Figure 9). This suggests that the determinants behind these variations were shared by most counties, however these determinants are presently not known. Inversely, Bornholm County, which had the lowest consumption in 1997 showed the sharpest increase during 1997-2005 and now has one of the highest in Denmark (Figure 9). The reasons behind this rapid increase presently are unknown. Among other counties, the difference between the county with the lowest and the highest consumption remained about 1.3 times and the ranking of counties according to the consumption was the same in 1997 and in 2005 (Spearman's rho= 0.67, *P*=0.007). While some counties always had a high consumption during 1997-2005, e.g. Ribe County (second highest in 1997 and the highest in 2005), others always had a low consumption, e.g. Aarhus County (second lowest in 1997 and the lowest in 2005). This suggests the existence of county-specific determinants that influence the prescription of antibacterials in Danish counties.

Table 12. Consumption of antibacterials for systemic use in human primary health care (DDD/1,000 inhabitant-days), Denmark

DANMAP 2005

ATC group a)	Therapeutic group	Year								
		1997	1998	1999	2000	2001	2002	2003	2004	2005
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	0.99	1.04	1.07	1.17	1.27
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	2.47	2.51	2.52	2.63	2.78
J01CE	Beta-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	4.91	5.00	5.07	5.20	5.28
J01CF	Beta-lactamase resistant penicillins	0.34	0.40	0.48	0.52	0.65	0.77	0.85	0.92	0.97
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.02	0.03	0.02	0.02	0.03	0.04	0.05	0.06	0.07
J01D	Cephalosporins and related substances	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.02	0.03
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	0.35	0.36	0.38	0.41	0.44
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	0.36	0.36	0.36	0.36	0.35
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	0.08	0.04	0.03	0.03	0.04	0.03	0.03	0.00	0.00
J01FA	Macrolides	2.03	2.28	2.17	2.02	2.10	2.15	2.13	2.23	2.41
J01FF	Lincosamides	0.01	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	0.01
J01MA	Fluoroquinolones	0.22	0.23	0.20	0.15	0.17	0.18	0.25	0.28	0.32
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	0.39	0.41	0.42	0.43	0.45
J01XX05	Methenamine	0.46	0.43	0.40	0.36	0.33	0.34	0.32	0.30	0.28
J01XX08	Linezolid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01	Antibacterials for systemic use (total)	12.24	12.76	12.14	12.24	12.86	13.26	13.53	14.06	14.75

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

Table 13. Consumption of antibacterials for systemic use in human primary health care (No. packages/1,000 inhabitants), Denmark

DANMAP 2005

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

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

The increase in antibacterial consumption in primary health care was more important for some classes than others. Figure 10 shows the changes in consumption for selected classes of antibacterials for 1997-2005. The consumption of these selected classes was relatively stable between 1997 and 2000. However, the consumption of combinations of penicillins, including beta-lactamase inhibitors and of fluoroquinolones more than doubled since 2000.

Consumption of fluoroquinolones (primarily ciprofloxacin) continued to increase, from 0.28 DDD/1,000 inhabitant-days in 2004 to 0.32 in 2005. The most likely explanation for this is a markedly reduced price per DDD due to the opening of the market to generic ciprofloxacin (EPI-NEWS 2004, no. 41: http://www.ssi.dk/sw18090.asp). Price is an important issue when prescribing drugs, however, the choice of antibiotic treatment should be based on recommendations rather than on price. Ciprofloxacin, as well as other fluoroquinolones, are potent antibiotics which should be reserved for treatment of serious infections, primarily in hospitals. It is thus essential that fluoroquinolones do not replace narrow spectrum antibiotics in the treatment of uncomplicated infections. The main indications for prescribing ciprofloxacin are complicated and recurrent urinary tract infections, infections caused by bacteria resistant to other

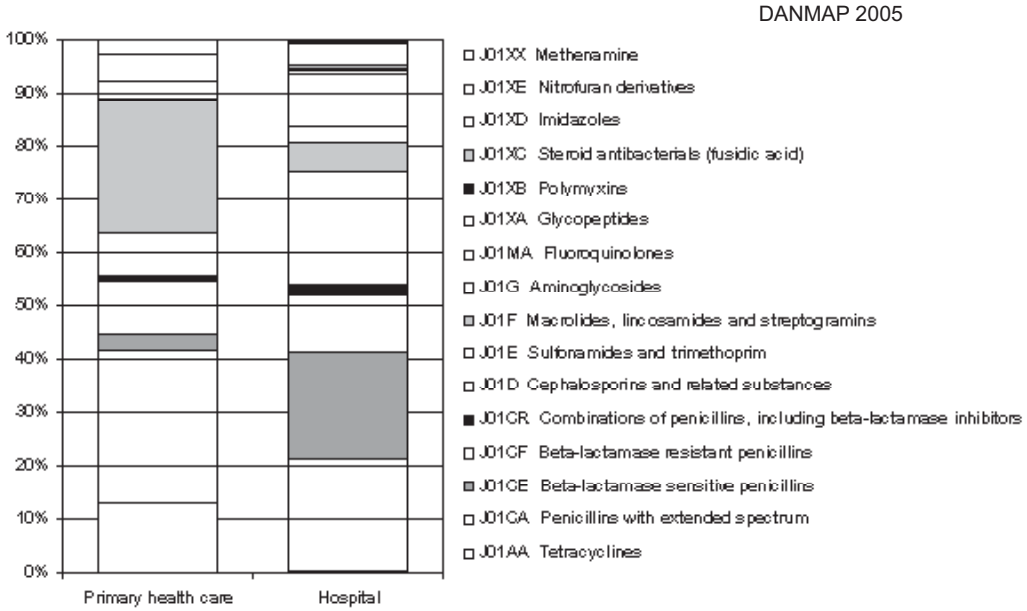

Figure 8. Distribution of antibacterials used in primary health care and in hospitals in 2005, measured in DDDs, Denmark

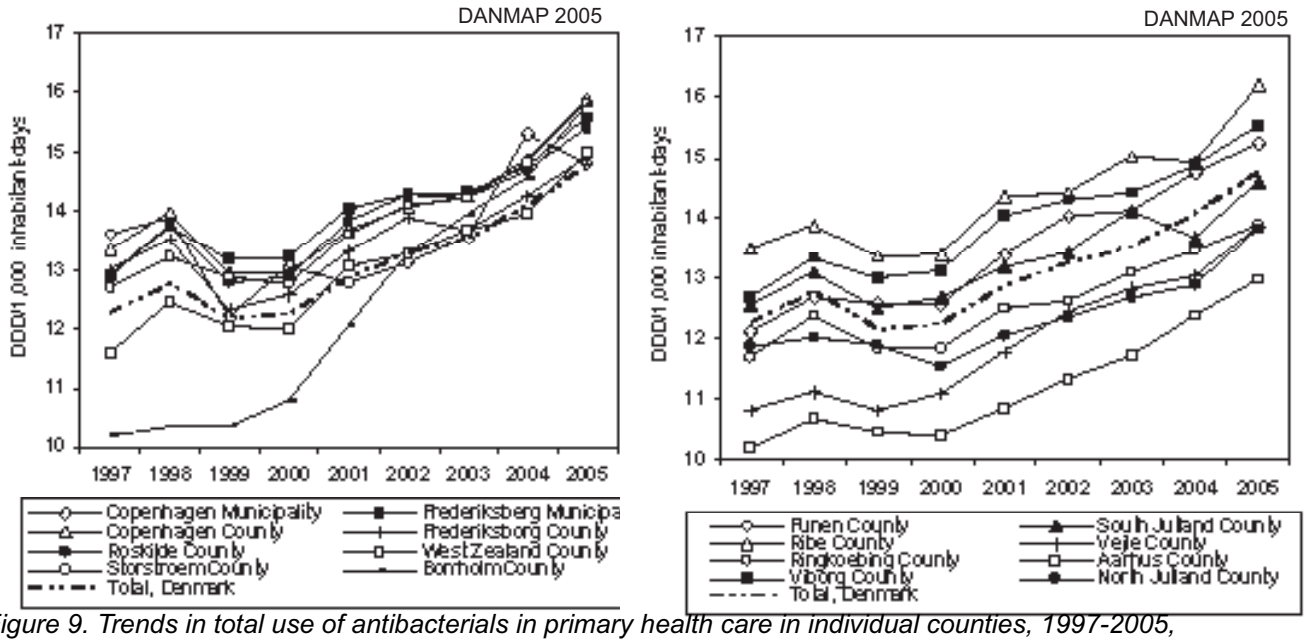

Figure 9. Trends in total use of antibacterials in primary health care in individual counties, 1997-2005, Denmark

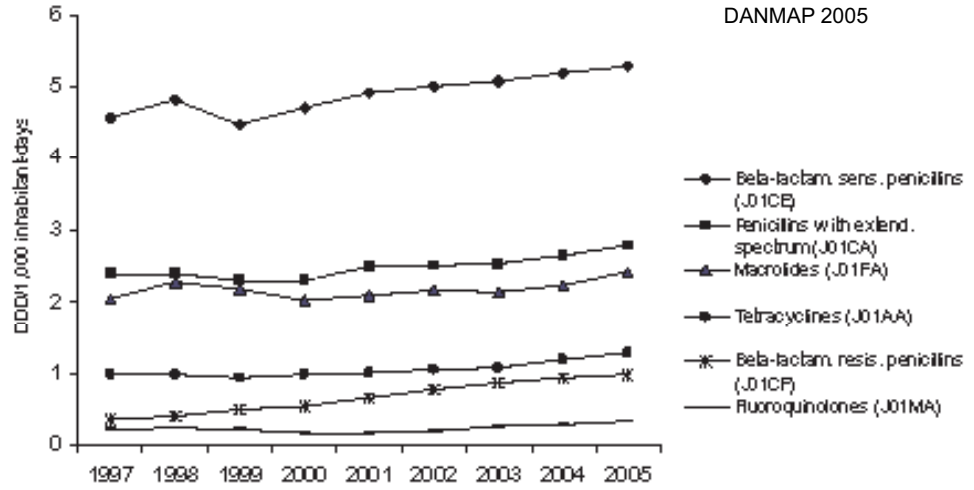

Figure 10. Consumption of selected antibacterials for systemic use in primary health care, Denmark

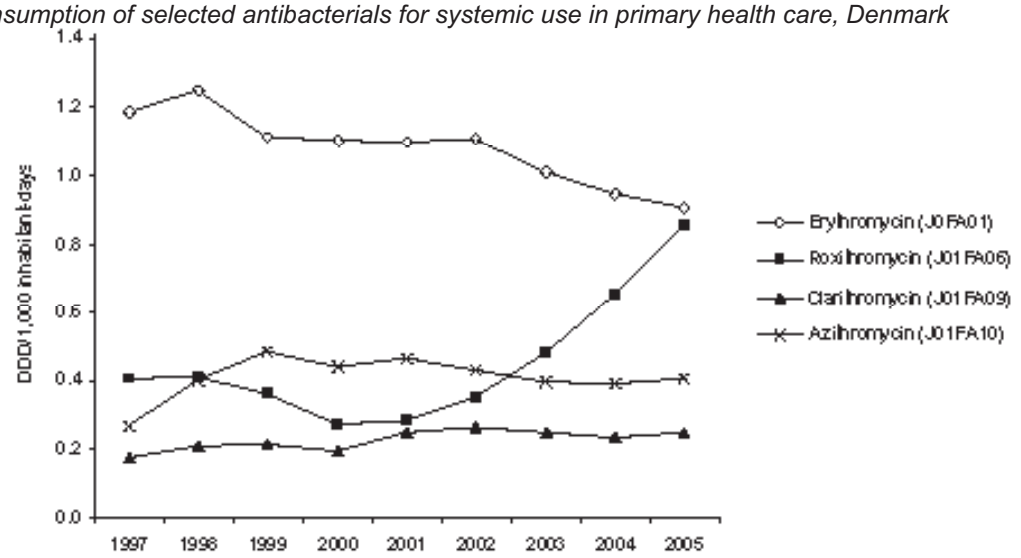

Figure 11. Consumption of macrolides in primary heath care, Denmark

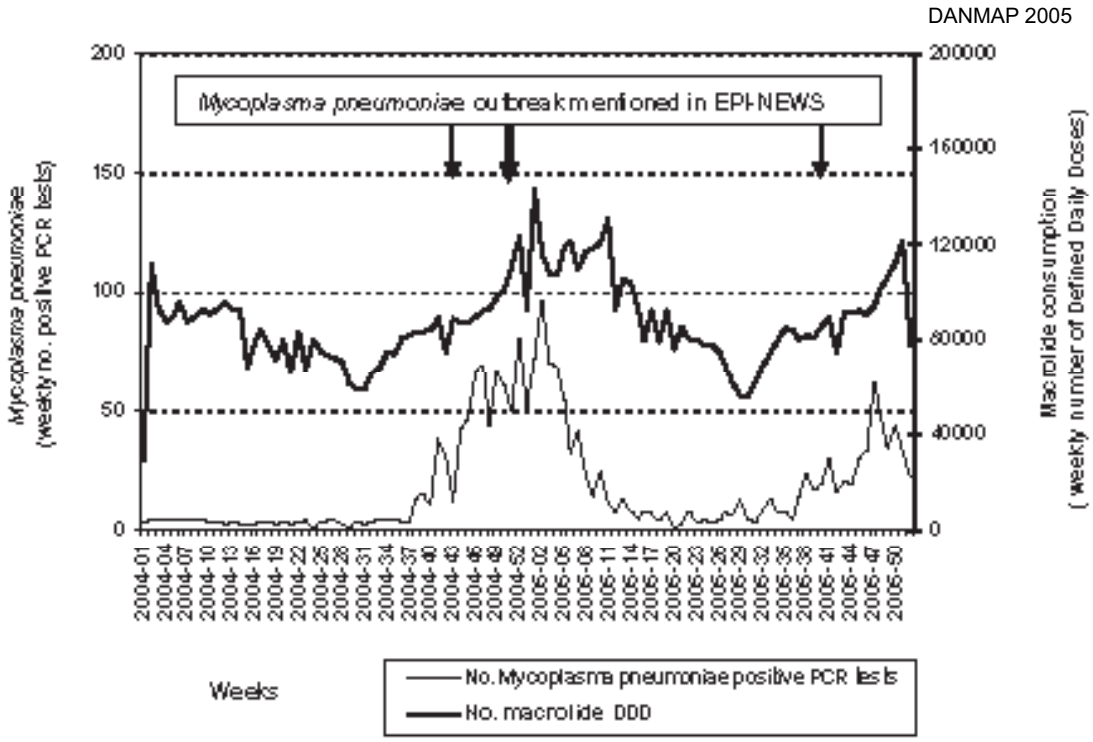

Figure 12. Percent positive Mycoplasma pneumoniae *PCR tests and macrolide consumption, Denmark*

antibiotics, pyelonephritis, and certain gastrointestinal infections. In the case of serious infections caused by *Pseudomonas* or mycobacteria, fluoroquinolones should be used in combination with another antibiotic to prevent emergence of resistance. In general, the advice of a clinical microbiologist is recommended before using fluoroquinolones for the treatment of complicated infections.

The level of resistance to fluoroquinolones in *E. coli* is still low in Denmark, but the recent increase in fluoroquinolone consumption has already resulted in increasing resistance to fluoroquinolones, e.g. in *Escherichia coli* isolates from community acquired infections (see page 65). This rapid increase in resistance is worrisome and prescribers should again be alerted on the ecological risks of a continued increase in fluoroquinolone consumption. Rational prescribing of ciprofloxacin and other fluoroquinolones is required to avoid unnecessary use and development of resistance, thus preserving the unique role of fluoroquinolones in the treatment of complicated infections.

The increase in the consumption of macrolides (8.1% between 2004 and 2005) was essentially due to an increased consumption of roxithromycin, whereas consumption of erythromycin decreased (Figure 11). The most likely explanation for this increase in roxithromycin consumption is an outbreak of *Mycoplasma pneumoniae* started at the end of 2004 and continued during the following year, though with a trough during the spring of 2005 (Figure 12). This outbreak was reported several times in the Danish weekly epidemiological newsletter (EPI-NEWS 2004, no. 42/43, http://www.ssi.dk/sw18611.asp; EPI-NEWS 2004, no. 51, http://www.ssi.dk/sw22168.asp; EPI-NEWS 2005, no. 40, http://www.ssi.dk/sw33183.asp), although only one of these weekly issues of the journal, i.e. 2004, week 51, specifically focused on this topic. In Denmark, roxithromycin is the recommended macrolide to treat *M. pneumoniae* infections in adults. Additionally, and similarly to ciprofloxacin, its price per DDD has dramatically decreased due to the introduction of generic roxithromycin on the Danish market. Increased awareness of general practioners about the *M. pneumoniae* outbreak combined with availability of a cheap generic macrolide to treat these infections probably both have contributed to the increase in macrolide consumption in 2005.

Hospitals

The consumption of antibacterials for systemic use in hospitals is presented in Table 14 as a number of DDD per 1,000 occupied bed-days and in Table 15 as a number of DDD per 1,000 discharged patients. Data on the number of hospital bed-days from the National Board of Health has been updated and corrected for 2003 and 2004. This update has led to only minor changes in the reported consumption.

From 1997 to 2004, total consumption in hospitals increased by 38.9% from 421 to 585 DDD/1,000 occupied bed-days. This increase in consumption was due to a 17.3% increase in the number of DDDs of antibacterials registered by hospital pharmacies (from 2.6 million DDDs in 1997 to 3.1 million DDDs in 2004), while there was a concurrent 13.5% decrease in the total number of hospital bed-days registered in Denmark in the same period. When expressed as a number of DDD per 1,000 discharged patients the total consumption in hospitals increased by 10% from 2,412 to 2,656 DDD/1,000 discharged patients in 2004. Between 2004 and 2005, antibacterial use in hospitals continued to increase whether it was expressed as a number of DDDs, in DDD/1,000 occupied bed-days or in DDD/1,000 discharged patients with increases of 3.8%, 6.6% and 1.9%, respectively. However, the data for 2005 should be interpreted with caution because they are calculated with estimated numbers of occupied bed-days and of discharged patients based on the variation observed previously between 2003 and 2004.

Figures 13 and 14 show how the increase in consumption in DDD/1,000 bed-days and in DDD/1,000 discharged patients was due to an increase in the number of DDDs of antibacterials while there was a decrease in the total number of hospital bed-days and an increase in the total number of discharged patients registered in Denmark in the same period. The increase in the number of DDDs of antibacterials used in Danish hospitals could be explained by an increase in the number of antibacterial treatments because of the admission of patients who more frequently required an antibiotic, e.g. for peri-operative antibiotic prophylaxis, by an increase in the daily dosage or by an increase in the frequency of combination therapies prescribed in hospitals. However, more detailed data on the quality of antibacterial prescriptions including information on the indication for treatment, the dosage and the duration of treatment, are necessary to verify these hypotheses and interpret these changes in consumption.

Table 14. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 occupied bed-days), Denmark. Data represented 97.5% of the total DDDs used in Danish hospitals in 2005. Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were excluded

DANMAP 2005

ATC group a)	Therapeutic group	Year								
		1997	1998	1999	2000	2001	2002	2003	2004	2005 b)
J01AA	Tetracyclines	3.4	3.3	2.8	2.9	2.8	3.2	3.1	3.5	3.3
J01CA	Penicillins with extended spectrum	112.1	113.4	112.7	115.7	116.1	115.2	119.2	118.5	130.0
J01CE	Beta-lactamase sensitive penicillins	80.2	89.2	95.3	100.3	106.5	114.3	121.2	123.7	123.1
J01CF	Beta-lactamase resistant penicillins	44.4	45.8	48.3	53.5	60.2	62.8	66.8	70.2	67.7
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.3	0.4	0.4	0.9	1.7	3.1	5.0	8.5	11.7
J01DB	First-generation cephalosporins	1.3	1.0	1.2	1.0	1.2	1.4	1.4	1.7	1.5
J01DC	Second-generation cephalosporins	39.9	41.9	44.0	47.4	52.1	58.5	63.9	71.0	79.2
J01DD	Third-generation cephalosporins	5.0	5.4	6.4	6.7	6.5	6.5	6.7	6.8	7.5
J01DF	Monobactams	0.6	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.0
J01DH	Carbapenems	3.6	2.4	3.2	3.9	4.2	6.0	6.9	8.6	11.2
J01EA	Trimethoprim and derivatives	4.2	4.4	3.8	3.7	4.3	4.2	4.4	4.2	4.1
J01EB	Short-acting sulfonamides	12.9	13.3	12.9	12.3	12.5	12.4	11.8	10.8	9.9
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	4.4	13.9	13.7	14.0	13.4	14.6	15.4	18.4	21.2
J01FA	Macrolides	34.5	35.3	33.5	32.8	32.6	32.3	30.9	29.5	29.1
J01FF	Lincosamides	1.3	1.8	1.5	1.6	1.7	1.9	1.9	2.3	2.4
J01GB	Aminoglycosides	33.8	23.6	27.6	21.3	18.5	17.7	17.4	20.4	19.6
J01MA	Fluoroquinolones	14.6	15.5	18.8	23.1	28.4	35.2	39.6	50.1	61.8
J01XA	Glycopeptides	2.1	2.3	2.8	3.3	3.2	3.7	4.2	4.7	5.2
J01XB	Polymyxins	0.4	0.2	0.3	0.4	0.3	0.3	0.3	0.6	1.2
J01XC	Steroid antibacterials (fusidic acid)	2.5	2.5	2.6	2.3	2.0	1.9	2.2	2.3	2.5
J01XD	Imidazoles	14.2	14.4	16.2	17.9	19.6	21.1	23.7	24.8	26.4
J01XE	Nitrofuran derivatives	3.7	3.5	3.0	2.9	2.9	2.8	2.8	2.8	2.9
J01XX05	Methenamine	1.8	1.8	1.6	1.4	1.3	1.2	0.8	1.0	0.8
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.7	1.5
J01	Antibacterials for systemic use (total)	421.3	435.3	452.9	469.5	492.1	521.0	550.0	585.2	624.2

a) From the 2006 edition of the ATC classification system
b) Estimate calculated with the actual number of DDDs and the estimated number of occupied bed-days based on the variation observed previously between 2003 and 2004

Table 15. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 discharged patients), Denmark. Private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices were excluded

DANMAP 2005

ATC group a)	Therapeutic group	Year								
		1997	1998	1999	2000	2001	2002	2003	2004	2005 b)
J01AA	Tetracyclines	19.3	18.2	15.2	15.4	14.8	16.3	14.8	15.8	14.5
J01CA	Penicillins with extended spectrum	641.8	634.4	608.7	610.5	604.9	578.0	566.2	538.0	564.3
J01CE	Beta-lactamase sensitive penicillins	459.3	498.9	514.6	529.1	555.0	573.5	575.9	561.4	534.1
J01CF	Beta-lactamase resistant penicillins	254.4	256.3	260.9	282.5	313.7	314.9	317.3	318.6	294.0
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	1.8	2.1	2.4	4.9	8.9	15.6	23.6	38.7	50.7
J01DB	First-generation cephalosporins	7.4	5.4	6.7	5.2	6.1	7.2	6.8	7.7	6.7
J01DC	Second-generation cephalosporins	228.7	234.2	237.7	250.2	271.5	293.6	303.6	322.2	343.6
J01DD	Third-generation cephalosporins	28.6	29.9	34.8	35.6	34.0	32.5	32.0	31.1	32.6
J01DF	Monobactams	3.3	0.7	0.8	0.9	0.5	0.2	0.2	0.2	0.2
J01DH	Carbapenems	20.6	13.5	17.2	20.6	21.9	29.9	32.7	39.0	48.7
J01EA	Trimethoprim and derivatives	24.0	24.6	20.7	19.5	22.6	21.0	21.0	19.1	17.8
J01EB	Short-acting sulfonamides	73.9	74.4	69.7	64.9	64.9	62.2	55.8	49.2	43.0
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	25.1	77.5	74.1	73.6	70.0	73.2	73.1	83.7	91.8
J01FA	Macrolides	197.6	197.3	180.8	173.1	170.1	161.9	146.7	134.0	126.1
J01FF	Lincosamides	7.6	10.0	8.1	8.5	9.0	9.5	9.0	10.4	10.4
J01GB	Aminoglycosides	193.6	131.9	149.0	112.5	96.4	88.6	82.8	92.4	85.3
J01MA	Fluoroquinolones	83.4	86.8	101.4	121.8	148.1	176.7	188.0	227.2	268.2
J01XA	Glycopeptides	12.3	13.1	15.3	17.2	16.6	18.8	19.9	21.3	22.7
J01XB	Polymyxins	2.5	1.4	1.8	2.1	1.5	1.7	1.5	2.7	5.3
J01XC	Steroid antibacterials (fusidic acid)	14.4	14.1	14.2	12.1	10.2	9.7	10.5	10.2	11.0
J01XD	Imidazoles	81.5	80.6	87.6	94.5	102.0	106.0	112.5	112.7	114.4
J01XE	Nitrofuran derivatives	21.3	19.5	16.3	15.5	15.0	14.2	13.1	12.8	12.8
J01XX05	Methenamine	10.2	10.3	8.6	7.5	6.7	6.1	3.9	4.6	3.6
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	2.2	2.1	3.4	6.4
J01	Antibacterials for systemic use (total)	2,412.6	2,435.2	2,446.2	2,477.6	2,564.6	2,613.3	2,613.0	2,656.4	2,709.0

a) From the 2006 edition of the ATC classification system
b) Estimate calculated with the actual number of DDDs and the estimated number of discharged patients based on the variation observed previously between 2003 and 2004

In 2005, penicillins still represented more than 53% of hospital antibacterial use in DDDs followed by cephalosporins and related substances (15.9%) and fluoroquinolones (9.9%). Since 1997, there has been a progressive switch towards newer antibacterial classes. The percentage of "broad-spectrum" antibacterials, i.e. cephalosporins, fluoroquinolones, combination of penicillins including beta-lactamase inhibitors and carbapenems, which represented 15.4% of the hospital antibacterial consumption in 1997, increased to 22.4% in 2003 and then to 27.5% in 2005. Glycopeptides (mainly vancomycin) still represented less than 1% of total hospital use in 2005 but increased from 3.3 to 5.2 DDD/1,000 occupied bed-days and 17.2 to 22.7 DDD/1,000 discharged patients between 2000 and 2005. This could be related to an increased frequency of patients infected with methicillin resistant staphylococci in Danish hospitals (see page 65 and 66).

Figure 15 illustrates this steady shift towards increasing consumption of newer, broad-spectrum antibacterials in Danish hospitals. In 1997, consumption of penicillins with extended spectrum still represented 26.6% of total hospital antibacterial consumption in Denmark, but this percentage decreased to 20.8% in 2005. The decrease mainly concerned amoxicillin whereas consumption of pivmecillinam increased. Consumption of macrolides and aminoglycosides continued to decrease and these classes represented less than 4.7% and 3.2% of the total hospital consumption in 2005, respectively. The consequences of these changes in pattern of antibacterial use could be a better coverage by empirical treatment of bacteria responsible for infection. However, this potential gain seems to be rapidly counterbalanced by the emergence of resistance towards newer classes of antibacterials.

DANMAP 2005

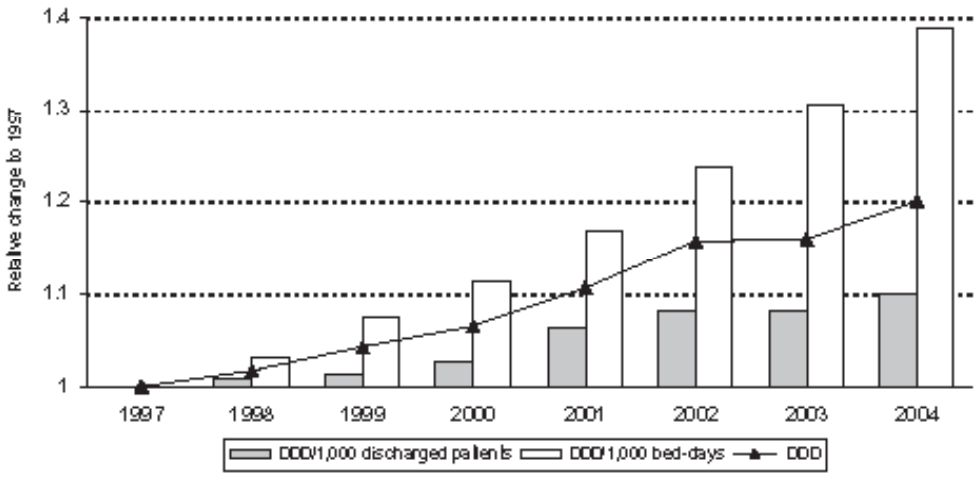

Figure 13. Trends in relative consumption of antibacterials for systemic use in hospitals relative to 1997, Denmark

DANMAP 2005

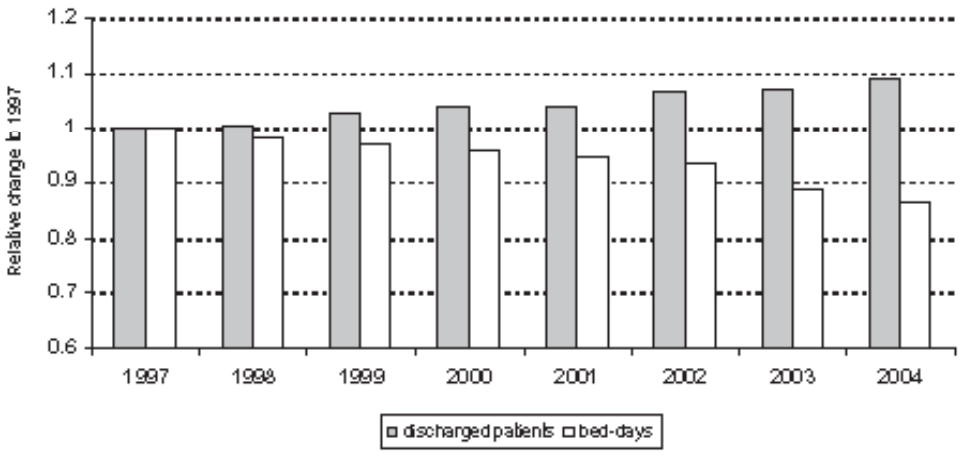

Figure 14. Changes in hospital discharged patients and occupied bed-days relative to 1997 used as reference year, Denmark

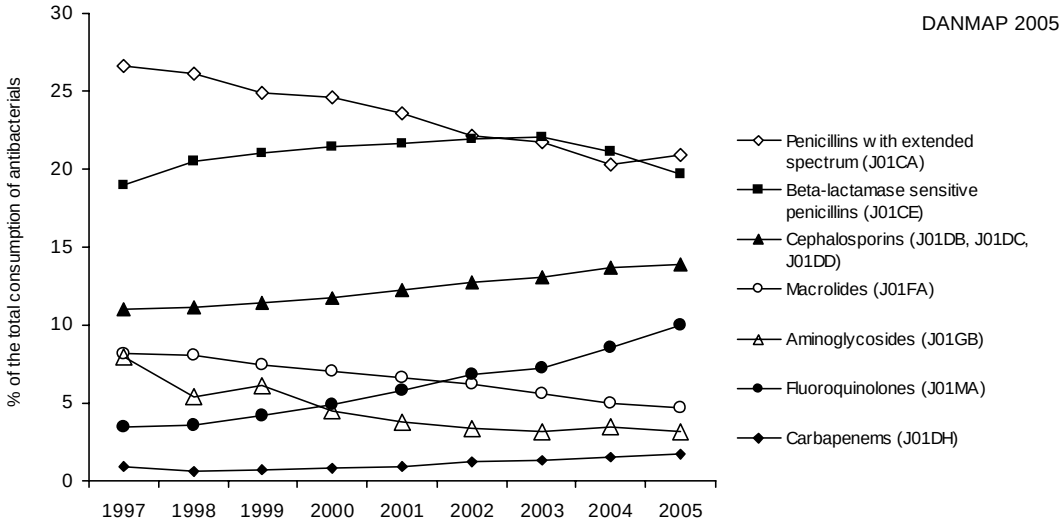

Figure 15. Changes in hospital consumption of selected antibacterials for systemic use, 1997-2005, Denmark

Resistance in zoonotic bacteria

Salmonella

Table 16 shows the *Salmonella* serotype distribution of isolates from food animals, foods and humans in 2005. The phage type distributions of *Salmonella* Typhimurium and *Salmonella* Enteritidis are presented in Tables 17 and 18.

Salmonella from food animals

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

Table 16. Distribution (%) of Salmonella *serotypes isolated from food animals, foods and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2005

Serotypes	Poultry a)	Broiler meat b)	Cattle a)	Beef	Pigs a)	Pork c)	Humans
	%	%	%	%	%	%	%
Agona					<1	1	1
Derby	6				10	8	<1
Dublin			63	15			1
Enteritidis	22	40			<1		36
Hadar		18					1
Indiana	11	8	2				<1
Infantis	17	6		15	3	2	2
Newport							2
Stanley					<1	<1	2
Typhimurium	14	11	28	45	80	66	32
Virchow	<1						2
Others including non-typeable	29	16	8	25	6	21	20
Number of isolates	95	62	65	20	923	220	1,767

a) Only one isolate per serotype per farm
b) All isolates originated from imported meat
c) 142 isolates originated from Danish pork and 78 from imported pork

Table 17. Distribution (%) of Salmonella Typhimurium *phage types from food animals, foods and human cases acquired domestically or associated with travel abroad among the isolates selected for susceptibility testing, Denmark*

DANMAP 2005

Phage type	Poultry	Cattle	Pigs	Pork		Humans a)	
	Danish	Danish	Danish	Danish	Imported	Domestic b)	Travel abroad
	%	%	%	%	%	%	%
1						1	3
3			<1	1	2	<1	
12		6	25	15	2	14	
17	8	11	4	3	2	1	
41						1	3
66	8		3			<1	
104/104b		23	6	2	17	22	51
110	8		<1				
120	8	11	16	21	13	16	9
135	15		<1	1		1	
170	15	11	13	17		3	
193	15		6	8	12	9	3
U302			<1	1	12	4	
Others including non-typeable	23	28	26	31	40	28	31
Number of isolates	13	18	734	94	52	501	35

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

Table 18. Distribution (%) of Salmonella Enteritidis *phage types from broiler meat and human cases acquired domestically or associated with travel abroad among the isolates selected for susceptibility testing, Denmark*

DANMAP 2005

Phage type	Broiler meat a)	Humans b)	
	%	Domestic c) %	Travel abroad %
1	4	11	19
4	24	15	21
6		2	2
6a	4	3	2
6b	16	<1	2
8		35	10
14b		5	2
21/21b	24	12	25
Others including non-typeable	28	16	17
Number of isolates	25	298	48

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

Table 19 shows the MIC distributions and the occurrence of antimicrobial resistance in *S.* Typhimurium from poultry, cattle and pigs in 2005. Twenty-one *S.* Enteritidis isolates from poultry were collected. These isolates were susceptible to all antimicrobial agents in the test panel except for six isolates (28.6%) that were resistant to the fluoroquinolones nalidixic acid and ciprofloxacin (data not shown).

From 1999 to 2005 a significant increase in resistance to tetracycline (*P*<0.0001), chloramphenicol (*P*<0.001), ampicillin (*P*<0.0001), and sulfonamide (*P*<0.0001) has been observed among *S*. Typhimurium from pigs (Figure 16). This increase was also significant for the occurrence of ampicillin resistance (*P*<0.02) among *S*. Typhimurium from pigs from 2004 to 2005. The increases coincide with an increased consumption of tetracyclines, penicillins with extended spectrum and sulfonamide/trimethoprim in pigs in the same period i.e.1999 to 2005.

Figure 17 shows the significant increase in concurrent resistance to at least the three antimicrobial agents streptomycin, sulfonamide and tetracycline (*P*<0.0001), as well as an increase in concurrent resistance to at least ampicillin, streptomycin, sulfonamide and tetracycline (*P*<0.0001) from 1999 to 2005. The proportion of fully sensitive isolates decreased significantly (*P*<0.0001) in the same.

DANMAP 2005

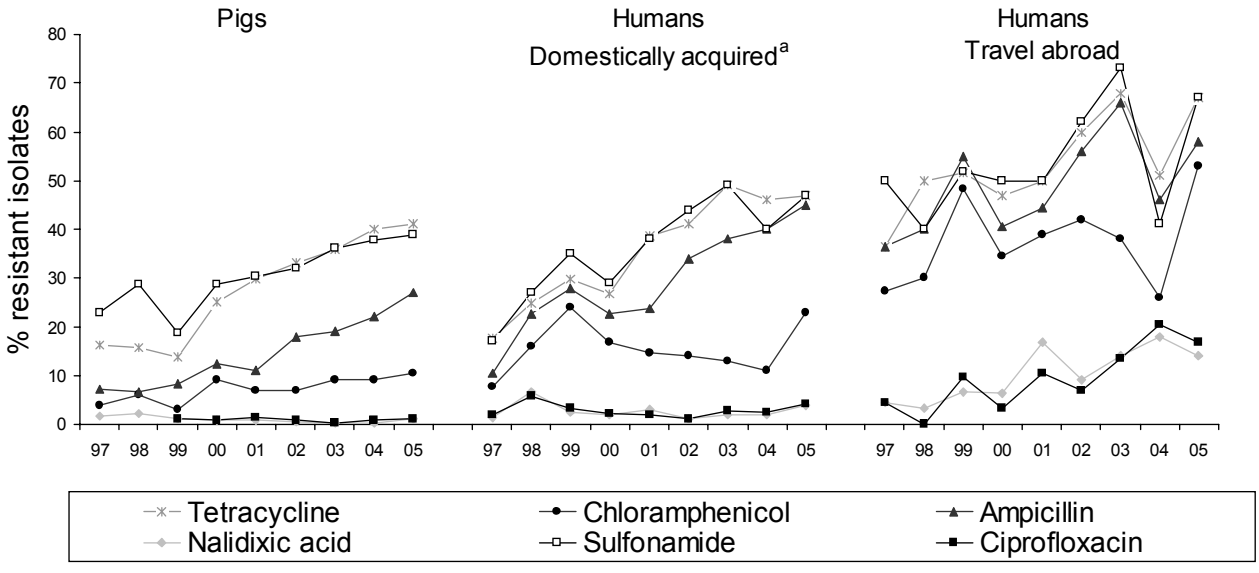

Figure 16. Trends in resistance to selected antimicrobials among Salmonella Typhimurium *isolated from poultry and pigs and from human cases, Denmark*

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

Table 19. Distribution of MICs and occurence of resistance among Salmonella Typhimurium *from poultry (broilers and layers) (n=13), cattle (n=17), and pigs (n=734), Denmark*

DANMAP 2005

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	Poultry	8	[0.2-36.0]							92.3					7.7					
	Cattle	29	[10.3-56.0]							70.6				23.5	5.9					
	Pigs	41	[37.2-44.4]							58.3	1.0		0.3	5.2	35.3					
Chloramphenicol	Poultry	0	[0.0-24.7]								53.9	46.2								
	Cattle	24	[6.8-50.0]								64.7	11.8				23.5				
	Pigs	10	[8.4-12.9]							0.8	70.6	17.4	0.7	0.4	1.5	8.6				
Florfenicol	Poultry	0	[0.0-24.7]							7.7	84.6	7.7								
	Cattle	24	[6.8-50.0]								70.6	5.9		17.7		5.9				
	Pigs	4	[3.0-6.1]							3.3	82.3	7.8	2.3	3.5	0.3	0.5				
Ampicillin	Poultry	0	[0.0-24.7]						69.2	30.8										
	Cattle	35	[14.2-61.7]						52.9	5.9	5.9				35.3					
	Pigs	27	[23.8-30.3]						55.3	16.2	1.5			0.1	26.8					
Amoxicillin/clavulanic acid a)	Poultry	0	[0.0-24.7]							100										
	Cattle	0	[0.0-19.5]							64.7		11.8	23.5							
	Pigs	0	[0.0-0.5]							73.2	7.8	15.8	3.3							
Cephalothin	Poultry	0	[0.0-24.7]								92.3	7.7								
	Cattle	0	[0.0-19.5]								82.3	17.7								
	Pigs	<1	[0.2-1.4]								80.3	15.7	3.5	0.4	0.1					
Ceftiofur	Poultry	0	[0.0-24.7]					61.5	38.5											
	Cattle	0	[0.0-19.5]					82.4	11.8	5.9										
	Pigs	0	[0.0-0.5]					54.8	42.6	2.6										
Cepodoxime b)	Poultry	0	[0.0-24.7]				60.0	40.0												
	Cattle	0	[0.0-19.5]				66.7	25.0	8.3											
	Pigs	0	[0.0-0.5]			0.9	73.6	22.4	2.1											
Sulfonamide	Poultry	8	[0.2-36.0]												92.3					7.7
	Cattle	35	[14.2-61.7]												64.7					35.3
	Pigs	39	[35.4-42.6]												60.8	0.1	0.1		1.5	37.5
Trimethoprim	Poultry	0	[0.0-24.7]								100									
	Cattle	0	[0.0-19.5]								100									
	Pigs	8	[6.4-10.6]								91.7				8.3					
Apramycin	Poultry	0	[0.0-24.7]								92.3	7.7								
	Cattle	0	[0.0-19.5]								100									
	Pigs	1	[0.7-2.5]								97.0	1.6			1.4					
Gentamicin	Poultry	0	[0.0-24.7]						100											
	Cattle	0	[0.0-19.5]						100											
	Pigs	1	[0.7-2.5]						98.0	0.5	0.1		0.5	0.7	0.1					
Neomycin	Poultry	0	[0.0-24.7]							100										
	Cattle	0	[0.0-19.5]							100										
	Pigs	8	[6.1-10.5]							90.5	1.2	0.4		0.4	7.5					
Spectinomycin	Poultry	0	[0.0-24.7]										7.7	38.5	53.9					
	Cattle	41	[18.4-67.1]											58.8			41.2			
	Pigs	15	[12.4-17.6]										3.1	78.2	3.8	2.6	12.2			
Streptomycin	Poultry	15	[1.9-45.5]								7.7	38.5	38.5	7.7		7.7				
	Cattle	47	[23.0-72.2]									47.1	5.9		29.4	17.7				
	Pigs	38	[34.9-42.1]								2.0	49.5	10.1	2.6	3.1	32.7				
Ciprofloxacin	Poultry	0	[0.0-24.7]	92.3	7.7															
	Cattle	0	[0.0-19.5]	94.1	5.9															
	Pigs	1	[0.6-2.3]	92.8	6.0		0.7		0.3	0.3										
Nalidixic acid	Poultry	0	[0.0-24.7]									100								
	Cattle	0	[0.0-19.5]									100								
	Pigs	1	[0.6-2.3]									97.8	1.0			1.2				
Colistin	Poultry	0	[0.0-24.7]								100									
	Cattle	0	[0.0-19.5]								100									
	Pigs	0	[0.0-0.5]								100									

Vertical lines indicate breakpoints for resistance

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

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

b) For cefpodoxime the number of tested isolates was n=10 for poultry, n=12 for cattle and n=434 for pigs

In 2005 multi-resistance (resistance to ≥4 of 8 antimicrobials; ampicillin, chloramphenicol, gentamicin, naldixic acid, streptomycin, sulfonamide, tetracycline, trimethoprim) was found in 24.6% of the isolates. This proportion has increased significantly (P<0.0001) since 1999, where 6.3% of the isolates were multi-resistant. Even though 21.4% of the isolates were resistant to at least ampicillin, streptomycin, sulfonamide and tetracycline, this could not be explained by the presence of *S.* Typhimurium phage types DT104/104b or DTU302, which represented only 6.5% of the isolates in 2005. Figure 17 shows that from 1999 to 2005, a significant increase in concurrent resistance to the three antimicrobial agents streptomycin, sulfonamide and tetracycline (P<0.0001) occurred, as well as an increase in concurrent resistance to the combination ampicillin, streptomycin, sulfonamide and tetracycline (P<0.0001) (Figure 17).
Only a low number of *S*. Typhimurium isolates were available from poultry and cattle in 2004 and 2005, which makes it difficult to detect differences in the occurrence of resistance from year to year.

Salmonella from foods

In 2005, *Salmonella* isolates from food were obtained from Danish and imported broiler meat, beef and pork sold at wholesale and retail outlets. A total of 146 *S*. Typhimurium isolates were obtained from pork, 94 isolates from Danish pork and 52 isolates from imported pork. In addition, seven *S*. Typhimurium isolates were obtained from imported broiler meat and eight from imported beef. The results of the susceptibility testing are shown in Table 20. Twenty-five *S*. Enteritidis isolates were obtained from imported broiler meat and no isolates were obtained from Danish broiler meat. The results of the susceptibility testing of the 25 isolates from imported broiler meat are shown in Table 21. For further discussion of the occurrence of resistance in food isolates please see „*Salmonella* from farm to table“.

Salmonella in humans

In 2005, 1,775 cases of human salmonellosis occurring in Denmark were reported to the Statens Serum Institut. This represents an increase in incidence from 28 cases per 100,000 inhabitants in 2004 to 33 cases per 100,000 inhabitants in 2005. This increase is seen after a long period of steady decline in the number of *Salmonella* infections (EPI-NEWS 2006, no. 9: http://www.ssi.dk/sw38271.asp), which had been obtained owing to the implementation of measures to control *Salmonella* in eggs, poultry and pigs. The 2005 increase is presumably due to a number of factors, including transmission from

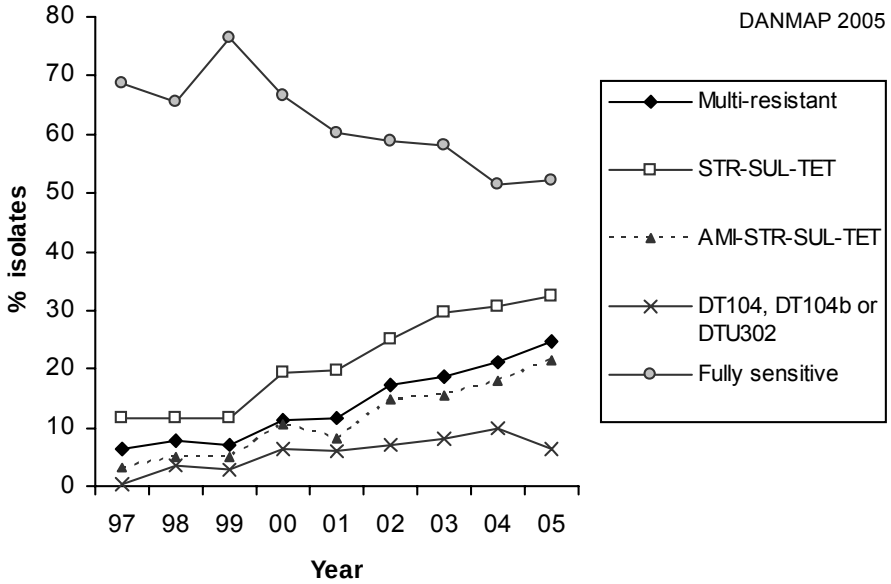

Figure 17. Trends in multi-resistance andselected resistance patterns among Salmonella Typhimurium *isolated from pigs in Denmark*

Multi-resistance defined as isolates resistant to ≥4 of 8 antimicrobial agents (ampicillin, chloramphenicol, gentamicin, nalidixic acid, sulfonamides, streptomycin, tetracycline or trimethoprim); STR-SUL-TET, at least resistant to streptomycin, sulfonamides and tetracyclines; AMP-STR-SUL-TET, at least resistant to ampicillin, streptomycin, sulfonamides and tetracycline; Fully susceptible isolates which were susceptible to all 8 antimicrobial agents

Table 20. Distribution of MICs and occurence of resistance in Salmonella Typhimurium *from broiler meat (imported n=7), beef (imported n=8) and pork (Danish n=94; imported n=52), Denmark* DANMAP 2005

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	86	[41.1-99.6]							14.3				28.6	57.1					
	Beef	Imported	100	[63.1-100]											75.0	25.0					
	Pork	Danish	38	[28.5-48.9]							60.6	1.1			2.1	36.2					
		Imported	81	[67.5-90.4]							19.2				26.9	53.9					
Chloramphenicol	Broiler meat	Imported	29	[3.7-71.0]								28.6	42.9				28.6				
	Beef	Imported	75	[34.9-96.8]								25.0					75.0				
	Pork	Danish	5	[1.8-12.0]							1.1	79.8	13.8				5.3				
		Imported	40	[27.0-54.9]								51.9	7.7		1.9	5.8	32.7				
Florfenicol	Broiler meat	Imported	29	[3.7-71.0]								71.4				28.6					
	Beef	Imported	75	[34.9-96.8]								25.0			75.0						
	Pork	Danish	3	[0.7-9.0]							1.1	89.4	6.4		2.1		1.1				
		Imported	27	[15.6-41.0]								55.8	5.8	11.5	19.2	3.9	3.9				
Ampicillin	Broiler meat	Imported	86	[41.1-99.6]							14.3					85.7					
	Beef	Imported	87	[47.4-99.7]							12.5					87.5					
	Pork	Danish	21	[13.5-30.9]						52.1	24.5	2.1				21.3					
		Imported	73	[58.9-84.4]						13.5	13.5					73.1					
Amoxicillin/ clavulanic acid a)	Broiler meat	Imported	0	[0.0-41.0]							14.3		57.1	28.6							
	Beef	Imported	0	[0.0-36.9]							12.5		50.0	37.5							
	Pork	Danish	0	[0.0-3.9]							77.7	9.6	11.7	1.1							
		Imported	0	[0.0-6.9]							26.9	3.9	40.4	28.9							
Cephalothin	Broiler meat	Imported	0	[0.0-41.0]								28.6	71.4								
	Beef	Imported	0	[0.0-36.9]								100									
	Pork	Danish	1	[0.03-5.8]								83.0	13.8	2.1	1.1						
		Imported	2	[0.05-10.3]								59.7	26.9	11.5	1.9						
Ceftiofur	Broiler meat	Imported	0	[0-41.0]					28.6	71.4											
	Beef	Imported	0	[0.0-36.9]					75.0	25.0											
	Pork	Danish	0	[0.0-3.9]					46.8	50.0	3.2										
		Imported	0	[0.0-6.9]					46.2	50.0	3.9										
Cefpodoxime b)	Broiler meat	Imported	0	[0.0-41.0]			50.0		50.0												
	Beef	Imported	0	[0.0-36.9]			62.5		25.0	12.5											
	Pork	Danish	0	[0.0-6.9]			58.8	3.9	31.4	5.9											
		Imported	0	[0.0-11.6]			83.3		16.7												
Sulfonamide	Broiler meat	Imported	86	[41.1-99.6]												14.3					85.7
	Beef	Imported	100	[63.1-100]																	100
	Pork	Danish	36	[26.5-46.7]												63.8					36.2
		Imported	75	[61.1-86.0]												25.0				3.9	71.2
Trimethoprim	Broiler meat	Imported	57	[18.4-90.1]								42.9				57.1					
	Beef	Imported	13	[0.3-52.7]								87.5				12.5					
	Pork	Danish	3	[0.7-9.0]								96.8				3.2					
		Imported	37	[23.6-51.0]								63.5				36.5					
Apramycin	Broiler meat	Imported	0	[0.0-41.0]								100									
	Beef	Imported	0	[0.0-36.9]								100									
	Pork	Danish	0	[0.0-3.9]								98.9	1.1								
		Imported	4	[0.5-13.2]								92.3	3.9			3.6					
Gentamicin	Broiler meat	Imported	0	[0.0-41.0]						100											
	Beef	Imported	0	[0.0-36.9]						100											
	Pork	Danish	0	[0.0-3.9]						100											
		Imported	2	[0.05-10.3]						94.2	1.9		1.9	1.9							
Neomycin	Broiler meat	Imported	43	[9.9-81.6]							57.1					42.9					
	Beef	Imported	0	[0.0-36.9]							100										
	Pork	Danish	5	[0.0-3.9]							94.7					5.3					
		Imported	19	[9.6-32.5]							76.9	3.9		1.9		17.3					
Spectinomycin	Broiler meat	Imported	71	[29.0-96.3]											28.6		42.9	28.6			
	Beef	Imported	75	[34.9-96.8]											25.0			75.0			
	Pork	Danish	6	[1.8-12.0]										1.1	86.2	6.4	1.1	5.3			
		Imported	50	[35.8-64.2]											40.4	9.6	9.6	40.4			
Streptomycin	Broiler meat	Imported	86	[41.1-99.6]										14.3		28.6	57.1				
	Beef	Imported	88	[47.4-99.7]									12.5			62.5	25.0				
	Pork	Danish	35	[2.4-13.4]									54.3	10.6	2.1		33.0				
		Imported	67	[52.9-79.7]									21.2	11.5	3.9	13.5	50.0				
Ciprofloxacin	Broiler meat	Imported	29	[3.7-71.0]	71.4		28.6														
	Beef	Imported	0	[0.0-36.9]	100																
	Pork	Danish	1	[25.5-45.6]	92.6	6.4	1.1														
		Imported	2	[0.05-10.3]	94.2	3.9	1.9														
Nalidixic acid	Broiler meat	Imported	29	[3.7-71.0]									71.4				28.6				
	Beef	Imported	0	[0.0-36.9]									100								
	Pork	Danish	2	[0.03-5.8]									97.9		1.1		1.1				
		Imported	2	[0.05-10.3]									98.1				1.9				
Colistin	Broiler meat	Imported	0	[0.0-41.0]								100									
	Beef	Imported	0	[0.0-36.9]								100									
	Pork	Danish	0	[0.0-3.9]								100									
		Imported	0	[0.0-6.9]								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) Contration of amoxicillin given, tested with clavulanic acid in concentration ratio 2:1

b) For cefpodoxime the number of tested isolates was n=4 for broiler meat, n=8 for beef, n=51 for Danish pork and n=30 for imported pork

infected Danish eggs, poultry and meat products and from imported foods. The proportion of *Salmonella* infections reported as acquired abroad was 12%. 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 16. Susceptibility testing was performed for 57% of *S.* Enteritidis isolates and for >99% of the *S.* Typhimurium isolates. Tables 22 and 23 present the MIC distributions and occurrence of antimicrobial resistance among *S.* Enteritidis and *S.* Typhimurium from humans by origin of infection in 2005.

Resistance was generally low in domestically acquired *S.* Enteritidis isolates as well as in those acquired abroad, except for ciprofloxacin and nalidixic acid (Tables 22 and 24). Resistance to fosfomycin and mecillinam was 0% in both *S.* Enteritidis isolates acquired abroad and in Denmark. In isolates acquired from infections in Denmark and abroad, resistance to ciprofloxacin and nalidixic acid was at the same level as in 2004. However, in 2005 resistance to ciprofloxacin (P=0.001) and nalidixic acid (P=0.001) was significantly higher in *S.* Enteritidis isolates from infections acquired abroad than from infections acquired in Denmark.

In *S.* Typhimurium isolates, resistance to tetracycline (P=0.03), chloramphenicol (P<0.0001), florfenicol (P<0.0001), sulfonamide (P=0.02), spectinomycin (P<0.0001), streptomycin (P=0.03), ciprofloxacin (P<0.0001) and nalidixic acid (P=0.02) was significantly higher in cases where the infection was acquired abroad, compared to cases where the infection was acquired in Denmark (Tables 23 and 25). Among *S.* Typhimurium isolates from cases with infection acquired in Denmark, a significant increase occurred from 2004 to 2005 in resistance to chloramphenicol (P<0.0001), florfenicol (P=0.0004), sulfonamide (P=0.03), apramycin (P=0.02), spectinomycin (P<0.0001) and ciprofloxacin (P=0.009). However, a significant decrease in resistance to trimethoprim (P=0.01) occurred. In *S.* Typhimurium isolates from infections acquired abroad, a significant increase in resistance to chloramphenicol (P=0.02), florfenicol (P=0.04), sulfonamide (P=0.03), and spectinomycin (P=0.03) was observed in 2005. Resistance to fosfomycin and mecillinam was 0% in both *S.* Typhimurium isolates acquired abroad and in Denmark. Two *S.* Typhimurium isolates were resistant to both cefpodoxime and ceftiofur (Table 23). When tested

Table 21. Distribution of MICs and occurrence of resistance in Salmonella Enteritidis *from broiler meat (imported n=25), Denmark*

DANMAP 2005

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	20	[6.8-40.7]							80.0					20.0					
Chloramphenicol	0	[0.0-13.7]								100									
Florfenicol	0	[0.0-13.7]								100									
Ampicillin	0	[0.0-13.7]						92.0	8.0										
Amoxicillin/ clavulanic acid[a]	0	[0.0-13.7]							100										
Cephalothin	0	[0.0-13.7]								100									
Ceftiofur	0	[0.0-13.7]					24.0	76.0											
Sulfonamide	0	[0.0-13.7]												100					
Trimethoprim	0	[0.0-13.7]								100									
Apramycin	0	[0.0-13.7]								96.0	4.0								
Gentamicin	0	[0.0-13.7]						100											
Neomycin	0	[0.0-13.7]							100										
Spectinomycin	0	[0.0-13.7]										68.0	32.0						
Streptomycin	0	[0.0-13.7]								88.0	12.0								
Ciprofloxacin	40	[21.1-61.3]	60.0			32.0	8.0												
Nalidixic acid	40	[21.1-61.3]									60.0			40.0					
Colistin	0	[0.0-13.7]								100									

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

phenotypically for extended-spectrum beta-lactamase (ESBL) activity using the Etest both isolates were confirmed ESBL-positive (Etests cefotaxime and ceftazidime with and without clavulanic acid, according to Etest technical guide 3B, Gram-negative aerobic specific EAS 004, from AB BIODISK, Solna, Sweden).

Table 26 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 increased from 15% in 2004 to 27% in 2005 (*P*<0.0001). Among *S.* Typhimurium isolates other than phage types DT104 and related phage types, resistance to trimethoprim (*P*=0.004), gentamicin (*P*=0.003), and ciprofloxacin (*P*=0.03) was significantly higher in cases where the infection was acquired abroad, compared to cases where the infection was acquired in Denmark. Among isolates from cases with infection acquired in Denmark, a significant decrease in resistance to tetracycline (*P*=0.02) and trimethoprim (*P*=0.02) occurred from 2004 to 2005.

The increase from 2004 to 2005 in resistance towards several antimicrobials among *S.* Typhimurium isolates is coincident with a significant increase in the proportion of *S.* Typhimurium DT104 and related phage types. The increase in resistance might partly be explained by the change in phage type distribution (Tables 25 and 26).

Table 22. Distribution of MICs and occurrence of resistance among Salmonella Enteritidis *from human cases acquired domestically (n=310) or associated with travel abroad (n=53) b), Denmark*

DANMAP 2005

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	1	[0.4-3.3]							97.7	1.0				1.3					
	Travel abroad	0	[0.0-6.7]							100										
Chloramphenicol	Domestic	0	[0.0-1.2]							2.6	93.9	3.5								
	Travel abroad	0	[0.0-6.7]							5.7	88.7	5.7								
Florfenicol	Domestic	0	[0.0-1.2]							6.1	92.6	1.3								
	Travel abroad	0	[0.0-6.7]							5.7	94.3									
Ampicillin	Domestic	3	[1.3-5.4]						52.6	43.2	1.3				2.9					
	Travel abroad	9	[3.1-20.7]						52.8	37.7					9.4					
Amoxicillin/	Domestic	0	[0.0-1.2]							96.8	0.3	2.9								
clavulanic acid a)	Travel abroad	0	[0.0-6.7]							90.6		7.5	1.9							
Cephalothin	Domestic	0	[0.0-1.2]								96.8	2.6	0.6							
	Travel abroad	0	[0.0-6.7]								96.2	3.8								
Cefpodoxime b)	Domestic	0	[0.0-1.5]			5.9	89.9	2.5	1.7											
	Travel abroad	0	[0.0-8.0]			13.6	81.8	4.5												
Ceftiofur	Domestic	0	[0.0-1.2]					93.2	6.8											
	Travel abroad	0	[0.0-6.7]					86.8	13.2											
Sulfonamide	Domestic	<1	[0.2-2.8]												99.0					1.0
	Travel abroad	2	[0.1-10.1]												98.1					1.9
Trimethoprim	Domestic	<1	[0.1-2.3]								99.4				0.6					
	Travel abroad	2	[0.1-10.1]								98.1				1.9					
Apramycin	Domestic	0	[0.0-1.2]								100									
	Travel abroad	0	[0.0-6.7]								100									
Gentamicin	Domestic	<1	[0.0-1.8]						98.7	1.0			0.3							
	Travel abroad	0	[0.0-6.7]						98.1	1.9										
Neomycin	Domestic	0	[0.0-1.2]							99.7	0.3									
	Travel abroad	2	[0.1-10.1]							98.1					1.9					
Spectinomycin	Domestic	<1	[0.2-2.8]										65.5	33.5		0.3	0.6			
	Travel abroad	2	[0.1-10.1]										71.7	26.4			1.9			
Streptomycin	Domestic	<1	[0.0-1.8]								97.7	1.9			0.3					
	Travel abroad	4	[0.5-13.0]								96.2					3.8				
Ciprofloxacin	Domestic	13	[9.4-17.2]	86.1	1.0	9.0	3.5	0.3												
	Travel abroad	30	[18.3-44.3]	67.9	1.9	18.9	9.4	1.9												
Nalidixic acid	Domestic	13	[9.4-17.2]									86.5	0.6			12.9				
	Travel abroad	30	[18.3-44.3]									67.9	1.9		1.9	28.3				
Colistin	Domestic	0	[0.0-1.2]								100									
	Travel abroad	0	[0.0-6.7]								100									

Vertical lines indicate breakpoints for resistance

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

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

b) For cefpodoxime the number of tested isolates was n=238 for human cases acquired domestically and n=44 for cases associated with travel abroad

Salmonella from farm to table

The occurrence of resistance among *S*. Enteritidis isolates from imported broiler meat and human cases acquired domestically or abroad are compared in Table 24, while the occurrence of resistance in *S*. Typhimurium isolates from Danish food animals, imported and Danish pork and human cases acquired domestically or abroad, is compared in Table 25.

In 2005, no *S*. Typhimurium isolates were available from Danish broiler meat and only seven isolates were obtained from imported poultry meat. From beef, only one isolate was obtained from Danish beef and seven S. Typhimurium were obtained from imported beef. The prevalence of antimicrobial resistance in S. Typhimurium isolates from imported pork was significantly higher than the occurrence of resistance in isolates from Danish pork, for the following nine antimicrobial agents: tetracycline (P<0.0001), chloramphenicol (P<0.0001), florfenicol (P<0.0001), ampicillin (P<0.0001), sulfonamide (P<0.0001), trimethoprim (P<0.0001), neomycin (P=0.008), spectinomycin (P<0.0001) and streptomycin (P=0.002) (Table 20).

The proportion of multi-resistant *S*. Typhimurium isolates can have a strong influence on the total frequency of resistance. Therefore, resistance among *S*. Typhimurium other than phage types DT104/104b and DTU302 are presented in Table 26. The proportion of *S*. Typhimurium phage types DT104/104b and DTU302 was significantly higher (P=0.002) among isolates from imported pork (17.3%) compared to Danish pork (2.1%).

Table 23. Distribution of MICs and occurrence of resistance among Salmonella Typhimurium *from human cases acquired domestically (n=518) or associated with travel abroad (n=36), Denmark*

DANMAP 2005

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	47	[43.1-51.9]							51.7	0.6	0.2	2.9	15.1	29.5					
	Travel abroad	67	[49.0-81.4]							33.3			2.8	38.9	25.0					
Chloramphenicol	Domestic	23	[19.4-26.8]							2.1	61.6	13.1	0.2	0.8	4.1	18.1				
	Travel abroad	53	[35.5-69.6]							2.8	27.8	16.7			16.7	36.1				
Florfenicol	Domestic	10	[7.8-13.2]							9.7	67.4	2.7	10.0	7.9	1.9	0.4				
	Travel abroad	36	[20.8-53.8]							8.3	38.9	2.8	13.9	19.4	11.1	5.6				
Ampicillin	Domestic	45	[40.6-49.4]						46.7	7.5	0.8				45.0					
	Travel abroad	58	[40.8-74.5]						22.2	16.7	2.8				58.3					
Amoxicillin/	Domestic	<1	[0.1-1.4]							54.2	7.3	20.3	17.8	0.2	0.2					
clavulanic acid a)	Travel abroad	3	[0.1-14.5]							41.7		13.9	41.7	2.8						
Cephalothin	Domestic	<1	[0.1-1.7]								85.9	12.4	1.2	0.2	0.4					
	Travel abroad	0	[0.0-9.7]								88.9	8.3	2.8							
Cefpodoxime b)	Domestic	1	[0.1-3.7]			8.4	88.0	1.6	1.0			1.0								
	Travel abroad	0	[0.0-9.7]			11.1	66.7	22.2												
Ceftiofur	Domestic	<1	[0.1-1.4]					89.8	9.5	0.4			0.4							
	Travel abroad	0	[0.0-9.7]					80.6	19.4											
Sulfonamide	Domestic	47	[43.1-51.9]												50.8	1.5	0.2	0.2	47.3	
	Travel abroad	67	[49.0-81.4]												33.3				66.7	
Trimethoprim	Domestic	4	[2.4-5.9]								96.1				3.9					
	Travel abroad	11	[3.1-26.1]								88.9				11.1					
Apramycin	Domestic	1	[0.5-2.8]								98.1	0.4	0.2		1.4					
	Travel abroad	0	[0.0-9.7]								97.2	2.8								
Gentamicin	Domestic	2	[0.9-3.5]						97.5		0.6	1.0	0.2	0.4	0.4					
	Travel abroad	8	[1.8-2.5]						91.7					5.6	2.8					
Neomycin	Domestic	2	[0.9-3.5]							97.9	0.2			0.8	1.2					
	Travel abroad	3	[0.1-14.5]							97.2				2.8						
Spectinomycin	Domestic	25	[21.2-28.9]										11.2	63.1	0.8	0.4	24.5			
	Travel abroad	56	[38.1-72.1]										5.6	36.1	2.8		55.6			
Streptomycin	Domestic	45	[40.8-49.6]								15.1	36.5	3.3	7.3	14.1	23.7				
	Travel abroad	64	[46.2-79.2]								13.9	19.4	2.8	8.3	41.7	13.9				
Ciprofloxacin	Domestic	4	[2.7-6.4]	94.8	1.0	2.3	1.2	0.4				0.4								
	Travel abroad	17	[6.4-32.8]	80.6	2.8		11.1	2.8				2.8								
Nalidixic acid	Domestic	4	[2.4-5.9]									95.8	0.4	0.2		3.7				
	Travel abroad	14	[4.7-29.5]									83.3	2.8			13.9				
Colistin	Domestic	0	[0.0-0.7]								99.6	0.4								
	Travel abroad	0	[0.0-9.7]								100									

Vertical lines indicate breakpoints for resistance

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

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

b) For cefpodoxime the number of tested isolates was n=191 for human cases acquired domestically and n=9 for cases associated with travel abroad

In 2005, *S*. Enteritidis was rare in layers and broilers in Denmark, therefore no isolates were obtained from Danish animals. Table 24 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 tetracycline, ciprofloxacin and nalidixic acid, few *S*. Enteritidis isolates were resistant to the antimicrobial agents in the test panel. The occurrence of ciprofloxacin ($P<0.001$), nalidixic acid ($P<0.001$) and tetracycline ($P<0.0001$) resistance was significantly higher in isolates from imported broiler meat compared to isolates from human cases acquired domestically. The high prevalence of ciprofloxacin and nalidixic acid resistance among *S.* Enteritidis isolates from imported broiler meat was not significantly different as compared to 2004, while the prevalence of tetracycline resistance was significantly higher in 2005 than in 2004 ($P=0.008$). The national estimates of sources of human salmonellosis (Annual Report on Zoonoses in Denmark 2005) showed that imported chicken accounted for 8.6 to 13.4% of human cases of salmonellosis in 2005, while table eggs accounted for 10.2 to 14% of human cases (Report no. 2).

Table 24. Comparison of resistance (%) among Salmonella Enteritidis *from imported broiler meat and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2005

Compound	Broiler meat	Humans	
	Imported	Domestic a)	Travel abroad
	%	%	%
Tetracycline	20	1	0
Chloramphenicol	0	0	0
Florfenicol	0	0	0
Ampicillin	0	3	9
Amoxicillin/clavulanic acid	0	0	0
Cephalothin	0	0	0
Cefpodoxime b)	0	0	0
Ceftiofur	0	0	0
Sulfonamide	0	<1	2
Trimethoprim	0	<1	2
Apramycin	0	0	0
Gentamicin	0	<1	0
Neomycin	0	0	2
Spectinomycin	0	<1	2
Streptomycin	0	<1	4
Ciprofloxacin	40	13	30
Nalidixic acid	40	13	30
Colistin	0	0	0
Number of isolates b)	25	310	53

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

b) For cefpodoxime the number of tested isolates was n = 238 for human cases acquired domestically and n = 44 for cases associated with travel abroad

Table 25. Comparison of resistance (%) among Salmonella Typhimurium *from food animals, imported pork and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2005

Compound	Poultry	Cattle	Pigs	Pork		Humans	
	Danish	Danish	Danish	Danish	Imported	Domestic a)	Travel abroad
	%	%	%	%	%	%	%
Tetracycline	8	29	41	38	81	47	67
Chloramphenicol	0	24	10	5	40	23	53
Florfenicol	0	24	4	3	27	10	36
Ampicillin	0	35	27	21	73	45	58
Amoxicillin/clavulanic acid	0	0	0	0	0	<1	3
Cephalothin	0	0	<1	1	2	<1	0
Cefpodoxime b)	0	0	0	0	0	1	0
Ceftiofur	0	0	0	0	0	<1	0
Sulfonamide	8	35	39	36	75	47	67
Trimethoprim	0	0	8	3	37	4	11
Apramycin	0	0	1	0	4	1	0
Gentamicin	0	0	1	0	2	2	8
Neomycin	0	0	8	5	19	2	3
Spectinomycin	0	41	15	6	50	25	56
Streptomycin	15	47	38	35	67	45	64
Ciprofloxacin	0	0	1	1	2	4	17
Nalidixic acid	0	0	1	2	2	4	14
Colistin	0	0	0	0	0	0	0
Number of isolates b)	13	17	734	94	52	518	36

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

a) For cefpodoxime the number of isolates was n=10 for poultry, n=12 for cattle, n=434 for pigs, n=51 for Danish pork, n=30 for imported pork, n=191 for human cases acquired domestically and n=9 for humancases associated with travel abroad

Table 26. 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 2005

Compound	Poultry	Cattle	Pigs	Pork		Humans	
				Danish	Imported	Domestic a)	Travel abroad
	%	%	%	%	%	%	%
Tetracycline	8	8	39	38	77	36	35
Chloramphenicol	0	0	7	4	28	6	6
Florfenicol	0	0	1	2	12	<1	0
Ampicillin	0	17	25	21	67	32	18
Amoxicillin/clavulanic acid	0	0	0	0	0	0	0
Cephalothin	0	0	1	1	2	<1	0
Cefpodoxime b)	0	0	0	0	0	<1	0
Ceftiofur	0	0	0	0	0	<1	0
Sulfonamide	8	8	37	36	70	34	35
Trimethoprim	0	0	9	3	40	3	24
Apramycin	0	0	1	0	2	1	0
Gentamicin	0	0	1	0	2	1	18
Neomycin	0	0	8	5	23	2	6
Spectinomycin	0	17	12	5	40	7	12
Streptomycin	15	25	37	35	60	33	29
Ciprofloxacin	0	0	1	1	0	3	18
Nalidixic acid	0	0	1	2	0	3	12
Colistin	0	0	0	0	0	0	0
Number of isolates	13	12	686	92	43	374	17

a) Includes cases where origin of infection is not documented and may therefore include some isolates acquired abroad but not documented as such
b) For cefpodoxime the number of tested isolates was n=10 for poultry, n=10 for cattle, n=411 for pigs, n=51 for Danish pork, n=24 for imported pork, n=131 for human cases acquired domestically and n=6 for human cases associated with travel abroad

Report 2

Annual report on Zoonoses in Denmark 2005

The Annual Report on Zoonoses presents a summary of the trends and sources of zoonotic infections in humans and animals, as well as the occurrence of zoonotic agents in food and feeding stuffs in Denmark in 2005. The report is based on data compiled according to the zoonoses directive 03/99/EEC, supplemented by data obtained from national surveillance and control programmes and data from relevant research projects provided by institutions contributing to the report.

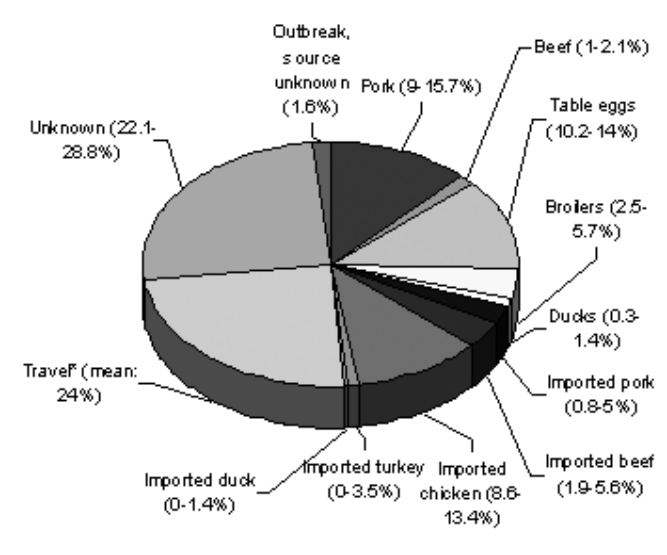

Figure 1. Estimated sources on 1,775 cases of human salmonellosis in Denmark, 2005. Estimates of travel related cases should be interpreted carefully, since availability of travel history data was very poor in 2005.
Source: Danish Zoonosis Centre

Profile of the year

In 2005, the number of human *Salmonella* infections increased for the first time since 2001, to approximately the same level as in 2003. A total of 1,775 cases were reported representing a 17% increase compared to 2004. The increase was primarily attributed to an increase in the number of *S.* Enteritidis cases and the number of *S.* Typhimurium cases. The increase in the number of human *Salmonella* cases is mainly explained by an increased number of cases attributable to Danish produced food, particularly pork (9-15% of cases) and table eggs (7-11% of cases). Overall, 28% of all *Salmonella* cases were attributed to Danish produced food of animal origin, whereas 16% were associated with the consumption of imported meat and meat products. Thirty-two percent of *Salmonella* cases were estimated to be travel related. The remaining approximately 24% of cases could not be associated with any source (Figure 1).The number of human *Campylobacter* cases remained at the same level as in 2004. A total of 3,671 cases was reported. The prevalence of *Campylobacter* in the broiler flocks increased slightly from 27% in 2004 to 30% in 2005. This is still, however, a significant decrease compared to the years prior to the implementation of the voluntary intervention strategy. Consumption and handling of fresh poultry and poultry products is believed to be the major source of human campylobacteriosis in Denmark, though other sources also exist. Tables 1 and 2 show the occurrence of *Salmonella* spp. and *Campylobacter* spp. in Danish meat production and occurrence of *Salmonella* from imported meat found in the import control.

Table 1. Occurrence of Salmonella *and* Campylobacter *in pig, cattle and broiler production in Denmark, 2005*

	Pigs				Cattle				Broilers			
	Primary herds		At Slaughter samples		Primary herds		At slaughter samples		Primary flocks		At slaughter samples	
	N	Positive	N	Positive	N	Positive	N	Positive	N	Positive	N	Positive
Salmonella	11,676	41.0%	30,809	1.0%	-	-	9,832	0.6%	4,083	2.1%	1,174	2.3%
Campylobacter	185	85.0%	-	-	73	42.0%	-	-	4,918	30.0%	-	-

Table 2. Occurrence of Salmonella *in batches of imported meat, 2005*

	No. of batches examined	Positive batches (%)
Pork	285	23%
Beef	311	3%
Chicken/hen	226	26%

For further information: Please contact the Danish Zoonosis Centre (dzc@dzc.dk). The report is available from www.dfvf.dk, or can be ordered from the Danish Zoonosis Centre.

Campylobacter

Campylobacter from food animals

Table 27 presents the MIC distributions and occurrence of antimicrobial resistance among *C. jejuni* from broilers and cattle in 2005 and Table 28 presents data for *C. coli* from pigs in 2005. Trends in resistance to selected antimicrobial agents among *C. jejuni* and *C. coli* from 1996 to 2005 are presented in Figures 18 and 19, respectively. Among *C. jejuni* isolates from broilers and cattle, few resistant isolates were observed. With the exception of resistance to nalidixic acid ($P<0.0001$) and ciprofloxacin ($P<0.0001$) in *C. jejuni* isolates from cattle, no significant changes in the occurrence of antimicrobial resistance were observed from 2004 to 2005.

The observed increase in resistance to nalidixic acid and ciprofloxacin in cattle was unexpected and could not be explained by changes in antimicrobial consumption on the farms where the cattle was raised.

Among *C. coli* isolates from pigs the occurrence of resistance remained unchanged from 2004 to 2005, except for resistance to tetracycline, which increased from 0% in 2004 to 6% in 2005 ($P=0.02$). The occurrence of resistance to ciprofloxacin and nalidixic acid increased from 3% in 2003 to 16% in 2004, and has remained at the same level in 2005 (Figure 19). The consumption of tetracycline in pigs has increased steadily from 2002 to 2005. However, from 2002 to 2004 resistance in *C. coli* remained low (0-1%) .

Campylobacter from foods

In 2005, a total of 162 *C. jejuni* isolates and 29 *C. coli* isolates obtained from broiler meat samples collected at retail outlets were subjected to susceptibility testing. The results are presented in Tables 29 and 30. Five out of 29 *C. coli* isolates originated from Danish broiler meat products, while 76 out of 162 *C. jejuni* isolates originated from Danish products. The remaining isolates originated from imported broiler meat products.

The occurrence of resistance to tetracycline ($P<0.0001$), nalidixic acid ($P<0.0001$) and ciprofloxacin ($P<0.0001$) was significantly higher in *C. jejuni* isolates from imported broiler meat compared to *C. jejuni* isolates from broiler meat of Danish origin (Table 29). Similar differences between *C. jejuni* isolates from imported and Danish broiler meat were observed in the previous year i.e. 2004. Among *C. coli* isolates from broiler meat, comparison between Danish and imported meat was hampered by low sample size, and no significant differences were observed (Table 30).

Campylobacter in humans

In 2005, there were 3,671 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 68 per 100,000 inhabitants and reflects a small decrease in the number of cases by 1% over the previous year. The number of infections has remained relatively stable during the past three years (EPI-NEWS 2006, no. 9: http://www.ssi.dk/sw38271.asp). About 12% 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 126 (3%) of all *Campylobacter* isolates reported to the Unit of Gastrointestinal Infections at the Statens Serum Institut; all of these were *C. jejuni*.

Tables 31 and 32 show 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 18.

Isolates of *C. jejuni* from infections in humans were generally susceptible to chloramphenicol, erythromycin, gentamicin and streptomycin (Table 31). Resistance to ciprofloxacin ($P=0.002$), nalidixic acid ($P=0.002$) and tetracycline ($P=0.02$) was significantly higher in *C. jejuni* isolates from infections acquired abroad, as compared to isolates from infection acquired in Denmark (Tables 31 and 32). 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 when *Campylobacter* infections are acquired outside Denmark. Among *C. jejuni* isolates from cases acquired abroad and in Denmark in 2005, resistance to tetracycline, erythromycin, nalidixic acid, and ciprofloxacin was at the same level as in 2004 (Figure 18).

Farm to table

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.

As in previous years a low level of resistance was observed in *C. jejuni* isolates from Danish broilers and Danish broiler meat (Table 32). Poultry meat is regarded as an important source of *Campylobacter* infections in humans, however the occurrence of resistance to tetracycline (*P*=0.006), ciprofloxacin (*P*=0.0002) and nalidixic acid (*P*=0.0002) was significantly higher in *C. jejuni* isolates from domestically acquired human cases compared with the occurrence in isolates from Danish broiler meat (Table 32). At the same time a high level of resistance to tetracycline, ciprofloxacin and nalidixic acid was observed in *C. jejuni* isolates from imported broiler meat. As the consumption of imported broiler meat is increasing in Denmark, the observation of high levels of resistance to these antimicrobials in isolates from domestically acquired human cases, may indicate imported broiler meat as a likely source of resistant *Campylobacter* isolates associated with domestically acquired infections in humans.

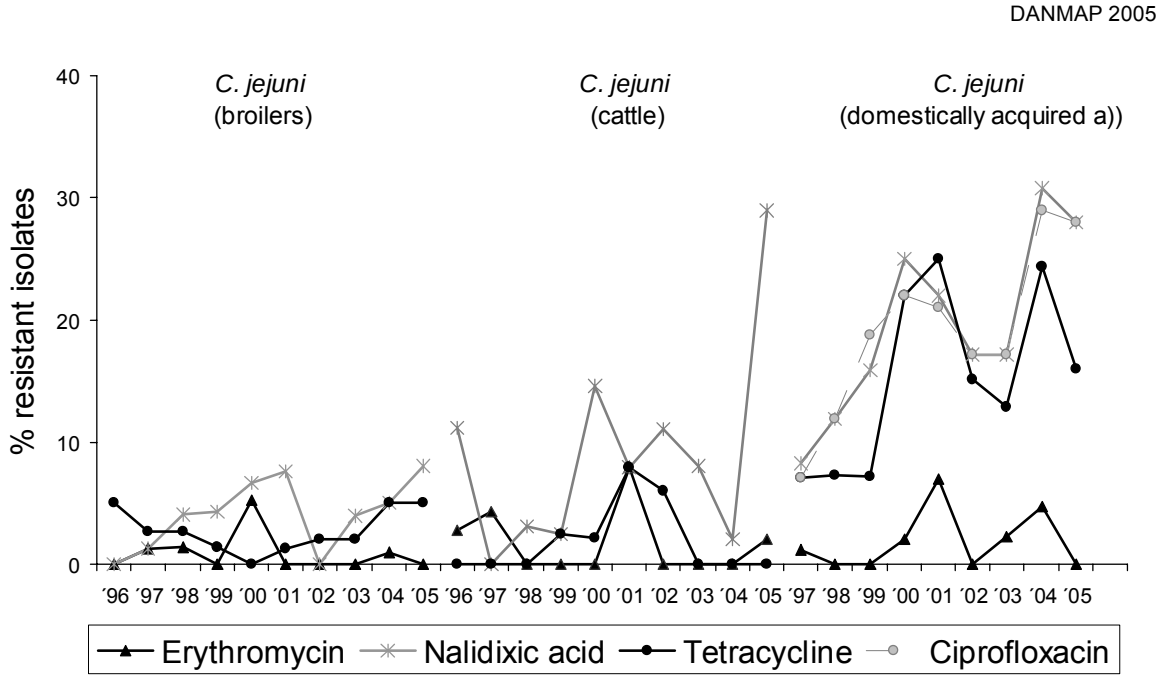

Figure 18. Trends in resistance to selected antimicrobials among Campylobacter jejuni *isolates from broilers, cattle and human domestic cases, Denmark*

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

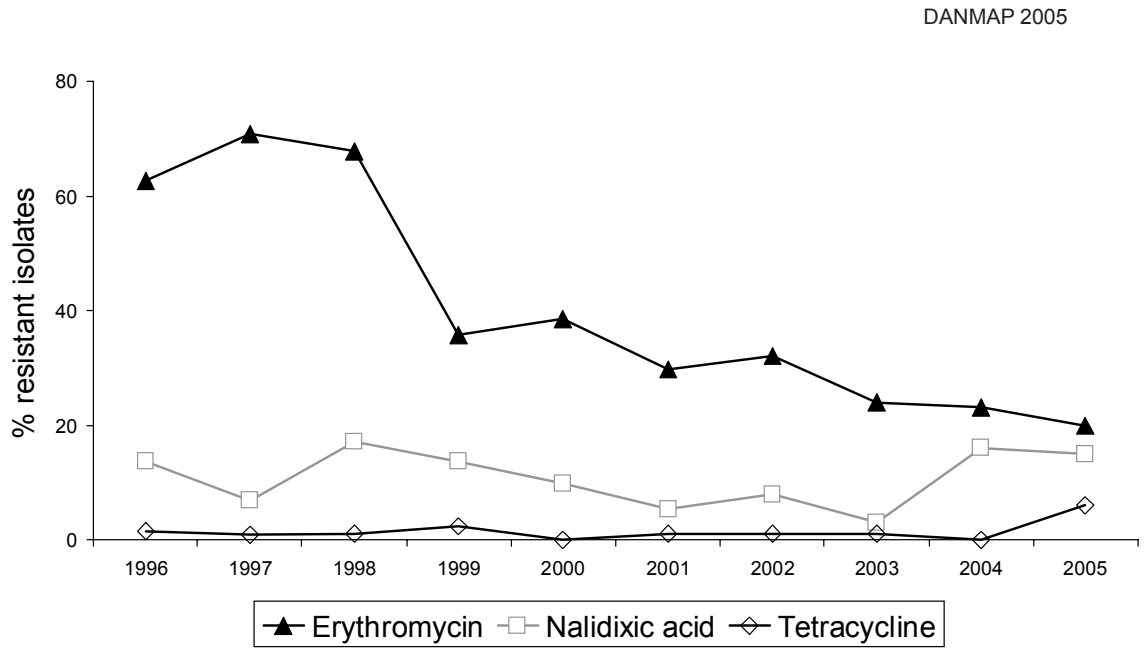

Figure 19. Trends in resistance to selected antimicrobials among Campylobacter coli *isolates from pigs, Denmark*

Table 27. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *isolates from broilers (n=76) and cattle (n=41), Denmark*

DANMAP 2005

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs												
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	Broilers	5	[1.5-12.9]				71.1	18.4	4.0	1.3				5.3		
	Cattle	0	[0.0-8.6]				92.7	7.3								
Chloramphenicol	Broilers	0	[0.0-4.7]							76.3	17.1	5.3	1.3			
	Cattle	0	[0.0-8.6]							100.0						
Erythromycin	Broilers	0	[0.0-4.7]					36.8	51.3	11.8						
	Cattle	2	[0.1-12.9]					63.4	34.2					2.4		
Gentamicin	Broilers	0	[0.0-4.7]			51.3	47.4	1.3								
	Cattle	0	[0.0-8.6]			70.7	26.8	2.4								
Streptomycin	Broilers	1	[0.0-6.1]							98.7				1.3		
	Cattle	0	[0.0-8.6]							100.0						
Ciprofloxacin	Broilers	8	[3.0-16.4]	4.0	31.6	44.7	9.2	1.3	1.3			7.9				
	Cattle	29	[16.1-45.5]	2.4	34.1	34.2						29.3				
Nalidixic acid	Broilers	8	[3.0-16.4]							7.9	57.9	23.7	2.6			7.9
	Cattle	29	[16.1-45.5]							9.8	53.6	7.3				29.3

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 28. Distribution of MICs and occurrence of resistance among Campylobacter coli *isolates from pigs (n=105), Denmark*

DANMAP 2005

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs												
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	6	[2.1-12.0]				36.2	35.2	19.1	3.8			1.0	4.8		
Chloramphenicol	1	[0.0-5.2]							11.4	49.5	32.4	5.7	1.0		
Erythromycin	20	[12.8-28.9]					16.2	18.1	29.5	13.3	2.9			20.0	
Gentamicin	0	[0.0-3.5]			8.6	49.5	41.9								
Streptomycin	48	[37.8-57.6]							50.5	1.0	1.0		47.6		
Ciprofloxacin	14	[8.2-22.5]	8.6	28.6	33.3	12.4	2.9			4.8	9.5				
Nalidixic acid	15	[9.0-23.6]							3.8	12.4	40.0	27.6	1.0	1.9	13.3

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 29. Distribution of MICs and occurrence of resistance in Campylobacter jejuni *from broiler meat (Danish n=76; imported n=86), Denmark*

DANMAP 2005

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	>64
Tetracycline	Danish	3	[0.3-2.9]				60.5	29.0	5.3	1.3		1.3		2.6		
	Imported	42	[31.3-53.0]				20.9	23.3	7.0	7.0				41.9		
Chloramphenicol	Danish	0	[0.0-4.7]							61.8	35.5	2.6				
	Imported	0	[0.0-4.2]							32.6	44.2	15.1	8.1			
Erythromycin	Danish	1	[0.0-7.1]					31.6	40.8	22.4	4.0				1.3	
	Imported	2	[0.3-8.1]				20.9	43.0	29.1	4.7					2.3	
Gentamicin	Danish	0	[0.0-4.7]			60.5	36.8	2.6								
	Imported	0	[0.0-4.2]			48.8	45.4	4.7			1.2					
Streptomycin	Danish	4	[0.8-11.1]							96.1			2.6	1.3		
	Imported	1	[0.0-6.3]							98.8				1.2		
Ciprofloxacin	Danish	5	[1.5-12.9]		31.6	50.0	11.8	1.3			1.3	4.0				
	Imported	41	[30.2-51.8]	3.5	7.0	27.9	17.4	3.5				40.7				
Nalidixic acid	Danish	5	[1.5-12.9]							10.5	60.5	18.4	2.6	2.6		5.3
	Imported	41	[30.2-51.8]							7.0	27.9	17.4	5.8	1.2	1.2	39.5

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

Table 30. Distribution of MICs and occurrence of resistance among Campylobacter coli *from broiler meat (Danish n=5; imported n=24), Denmark*

DANMAP 2005

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	>64
Tetracycline	Danish	20	[0.5-71.6]				60.0		20.0					20.0		
	Imported	54	[32.8-74.4]				20.8	12.5		8.3	4.2			54.2		
Chloramphenicol	Danish	0	[0.0-52.2]							60.0	40.0					
	Imported	0	[0.0-14.2]							29.2	45.8	16.7	8.3			
Erythromycin	Danish	0	[0.0-52.2]					20.0	40.0	40.0						
	Imported	17	[4.7-37.4]					20.8	29.2	12.5	20.8				16.7	
Gentamicin	Danish	0	[0.0-52.2]			40.0	40.0	20.0								
	Imported	0	[0.0-14.2]			45.8	41.7	4.2			8.3					
Streptomycin	Danish	0	[0.0-52.2]							100.0						
	Imported	13	[2.6-32.4]							87.5				12.5		
Ciprofloxacin	Danish	40	[5.3-85.3]	20.0	20.0	20.0					20.0	20.0				
	Imported	58	[36.6-77.9]		4.2	33.3	4.2				4.2	54.2				
Nalidixic acid	Danish	40	[5.3-85.3]							20.0	40.0					40.0
	Imported	58	[36.6-77.9]							4.2	25.0	8.3	4.2		4.2	54.2

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

Table 31. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *from human cases acquired domestically (n=116) or associated with travel abroad (n=10), Denmark*

DANMAP 2005

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	>64
Tetracycline	Domestic	16	[10.2-24.4]				66.4	10.3	6.0	0.9				16.4		
	Travel abroad	50	[18.7-81.3]				50.0							50.0		
Chloramphenicol	Domestic	0	[0.0-3.1]							44.8	44.0	9.5	1.7			
	Travel abroad	0	[0.0-30.9]							50.0	50.0					
Erythromycin	Domestic	0	[0.0-3.1]					22.4	57.8	15.5	4.3					
	Travel abroad	0	[0.0-30.9]					40.0	60.0							
Gentamicin	Domestic	0	[0.0-3.1]			18.1	73.3	8.6								
	Travel abroad	0	[0.0-30.9]			40.0	50.0	10.0								
Streptomycin	Domestic	4	[1.4-9.8]							95.7				4.3		
	Travel abroad	0	[0.0-30.9]							100						
Ciprofloxacin	Domestic	28	[19.7-36.7]	0.9	25.8	33.6	9.5	2.6			0.9	26.7				
	Travel abroad	80	[44.4-97.5]			20.0					10.0	70.0				
Nalidixic acid	Domestic	28	[19.7-36.7]							3.5	54.3	12.9	1.7		0.9	26.7
	Travel abroad	80	[44.4-97.5]								20.0					80.0

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

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 2005

Compound	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestically acquired a)	Travel abroad
	%	%	%	%	%	%
Tetracycline	0	5	3	42	16	50
Chloramphenicol	0	0	0	0	0	0
Erythromycin	2	0	1	2	0	0
Gentamicin	0	0	0	0	0	0
Streptomycin	0	1	4	1	4	0
Ciprofloxacin	29	8	5	41	28	80
Nalidixic acid	29	8	5	41	28	80
Number of isolates	41	76	76	86	116	10

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. Enterococci from cattle were not collected in 2005.

The MIC distribution and the occurrence of resistance among enterococci from food animals are shown in Tables 33 and 34. Trends in resistance among *E. faecium* isolates from broilers and pigs to antimicrobial growth promoters and tetracycline are presented in Figures 20 - 24**.**

From 2004 to 2005, erythromycin resistance decreased significantly from 50% to 35% among *E. faecium* isolates from pigs (Figure 22). This coincides with a decrease in macrolide consumption in pigs in the same period. 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.

Resistance to penicillin among *E. faecium* isolates from broilers has remained at a relatively high level (50-60%) from 1999 to 2003. From 2003 to 2004, a significant decrease (P<0.0001) in penicillin resistance from 54% to 35% was observed, and in 2005 a further decrease to 26% was observed.

Finally, the occurrence of resistance in *E. faecalis* isolates from broilers and pigs remained unchanged from 2004 to 2005.

Enterococci from food

Results from susceptibility testing of enterococci from food were not available for analysis before the current version of this report was published.

Enterococci from healthy human volunteers

In 2005, stool samples from 110 healthy human volunteers were collected. In total 50 *E. faecium* isolates and 50 *E. faecalis* isolates were obtained.

The MIC distributions and occurrence of antimicrobial resistance among enterococci from humans are shown in Tables 35 and 36.

Table 33. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broilers (n=131) and pigs (n=105), Denmark*

DANMAP 2005

Compound		% Resistant	[95% Confidence interval]	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	7	[3.2-12.6]							93.1					2.3	4.6						
	Pigs	59	[49.0-68.5]							38.1		1.0	1.9	1.9	17.1	40.0						
Tigecycline[a)]	Broilers	0	[0.0-3.9]		16.1	72	10.8	1.1														
	Pigs	0	[0.0-6.4]	1.8	17.9	75	3.6	1.8														
Chloramphenicol	Broilers	2	[0.2-5.4]								7.6	28.2	62.6		1.5							
	Pigs	1	[0.0-5.2]								3.8	40.0	54.3	1.0	1.0							
Florfenicol	Broilers	0	[0.0-2.8]								16.8	83.2										
	Pigs	0	[0.0-3.5]								30.5	69.5										
Penicillin[b)]	Broilers	26	[13.4-43.1]								42.1	26.3	5.3	18.4	5.3		2.6					
	Pigs	18	[8.8-32.0]								40.8	6.1	34.7	14.3	4.1							
Erythromycin	Broilers	23	[16.7-31.9]						22.1	3.1	14.5	36.6	9.9	6.1	1.5	6.1						
	Pigs	35	[26.2-45.2]						8.6	5.7	23.8	26.7	4.8	1.9		28.6						
Gentamicin	Broilers	0	[0.0-2.8]														100					
	Pigs	0	[0.0-3.5]														100					
Kanamycin	Broilers	3	[0.8-7.6]														36.6	34.4	21.4	4.6	1.5	1.5
	Pigs	28	[19.3-37.2]														16.2	40.0	12.4	3.8		27.6
Streptomycin	Broilers	5	[1.7-9.7]														94.7	0.8			0.8	3.8
	Pigs	29	[20.2-38.2]														65.7	1.0		4.8	10.5	18.1
Vancomycin	Broilers	2	[0.5-6.5]								96.2	1.5				2.3						
	Pigs	2	[0.2-6.7]								98.1					1.9						
Quinupristin/dalfopristin	Broilers	13	[7.7-20.0]						46.6	11.5	29.0	10.7	2.3									
	Pigs	16	[9.7-24.7]						17.1	8.6	58.1	15.2	1.0									
Avilamycin	Broilers	2	[0.5-6.5]								43.5	53.4	0.8	1.5	0.8							
	Pigs	0	[0.0-3.5]								98.1	1.9										
Salinomycin	Broilers	0	[0.0-2.8]								10.7	19.1	70.2									
	Pigs	0	[0.0-3.5]								100											
Linezolid	Broilers	0	[0.0-2.8]							13.7	85.5	0.8										
	Pigs	0	[0.0-3.5]							13.3	86.7											

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
a) For tigecycline, 93 isolates from broilers and 56 isolates from pigs were tested
b) For penicillin, 38 isolates from broilers and 49 isolates from pigs were tested

As in 2004, resistance towards quinupristin/dalfopristin (Q/D) (54%) was most common among *E. faecium* isolates. All Q/D resistant isolates had MIC = 4 and tested PCR-negative for the presence of *vat*(D) and *vat*(E) genes (encoding resistance to streptogramin A). These isolates might not be resistant either due to a low breakpoint or the low level resistance might be encoded by an unknown mechanism (see also comment on Q/D in the performance test Appendix 1, page 80). One vancomycin resistant *E. faecium* isolate was detected with the non-selective method (Table 35) and another vancomycin resistant *E. faecium* isolate was detected using a selective method (see Appendix 1 for details).

Resistance to tetracycline (30%) was most common among *E. faecalis* isolates in 2005 (Table 36). Resistance to "high-level" gentamicin was detected in a single *E. faecalis* isolate. Two vancomycin resistant *E. faecalis* isolates were detected using a selective method. These had not been detected in the previous years.
No significant changes in resistance were observed between 2004 and 2005.

Comparison of resistance in enterococci from farm and healthy human volunteers

A comparison of resistance among enterococci from Danish food animals and humans is presented in Tables 37 and 38 and in Figures 20 - 24.

Differences between levels of resistance were observed between *E. faecium* isolates from pigs and healthy humans, where resistance to tetracycline (P<0.0001), kanamycin (P=0.001) and streptomycin (P=0.003) was significantly higher in isolates from pigs (Table 38). The resistance levels for *E. faecium* isolates were similar for broilers and healthy humans.

Enterococcus faecalis isolates from pigs and healthy humans, were comparable for most antimicrobials except for tetracycline (P<0.0001) and erythromycin (P=0.0003) where the resistance level was significantly higher in isolates from pigs.
The resistance levels were similar for *E. faecalis* isolates from broiler and healthy humans (Table 38).

Table 34. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broilers (n=54) and pigs (n=119), Denmark*

DANMAP 2006

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	39	[25.9.7-53.1]							57.4			3.7	5.6	22.2	11.1						
	Pigs	82	[74.3-88.7]							16.8			0.8	10.9	16.0	55.5						
Tigecycline[a)]	Broilers	0	[0.0-8.4]	4.8	42.9	45.2	7.1															
	Pigs	0	[0.0-4.9]	1.4	15.1	26.0	57.5															
Chloramphenicol	Broilers	0	[0.0-6.6]								1.9	44.4	53.7									
	Pigs	1	[0.0-4.6]								2.5	26.9	65.6	4.2			0.8					
Florfenicol	Broilers	0	[0.0-6.6]								16.7	83.3										
	Pigs	0	[0.0-3.1]								15.1	84.9										
Penicillin[b)]	Broilers	0	[0.0-6.6]									100.0										
	Pigs	0	[0.0-3.1]									50.0	50.0									
Erythromycin	Broilers	13	[5.4-24.9]						31.5	24.1	16.7	14.8	1.9		1.9	9.3						
	Pigs	42	[33.0-51.4]						27.7	23.5	6.7					42.0						
Gentamicin	Broilers	0	[0.0-6.6]														100.0					
	Pigs	7	[2.9-12.8]														92.4		0.8	1.7	1.7	3.4
Kanamycin	Broilers	0	[0.0-6.6]														100.0					
	Pigs	22	[14.8-30.4]														77.3		0.8		0.8	21.0
Streptomycin	Broilers	2	[0.0-9.9]														83.3	14.8			1.9	
	Pigs	30	[22.2-39.3]														61.3	6.7	0.8	0.8	3.4	26.9
Vancomycin	Broilers	0	[0.0-6.6]								100.0											
	Pigs	0	[0.0-3.1]								100.0											
Avilamycin	Broilers	0	[0.0-6.6]								100.0											
	Pigs	0	[0.2-4.6]								99.2	0.8										
Flavomycin	Broilers	0	[0.0-3.1]									100										
	Pigs	0	[0.02-3.6]									97.5	2.5									
Salinomycin	Broilers	0	[0.0-6.6]								74.1	24.1	1.9									
	Pigs	0	[0.0-3.1]								100.0											
Linezolid	Broilers	0	[0.0-6.6]							31.5	68.5											
	Pigs	0	[0.0-3.1]							24.4	75.6											

Vertical lines indicate breakpoints for resistance
Virginiamycin and quinupristin/dalfopristin are not listed since *Enterococcus faecalis* is naturally resistant
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest
a) For tigecycline, 42 isolates from broilers and 73 isolates from pigs were teste
b) For penicillin, 12 isolates from broilers and 46 isolates from pigs were tested

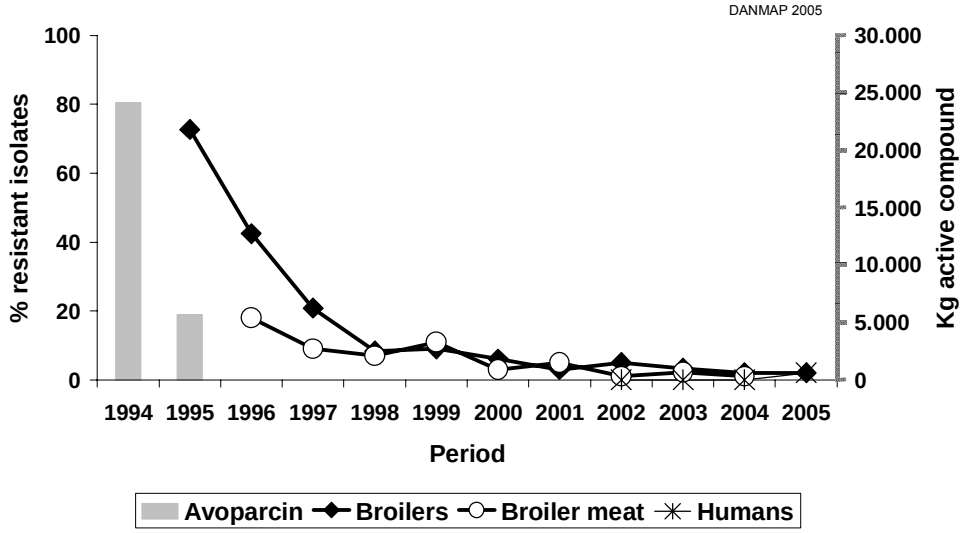

Figure 20.Trends in glycopeptide resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avoparcin in animals, Denmark*

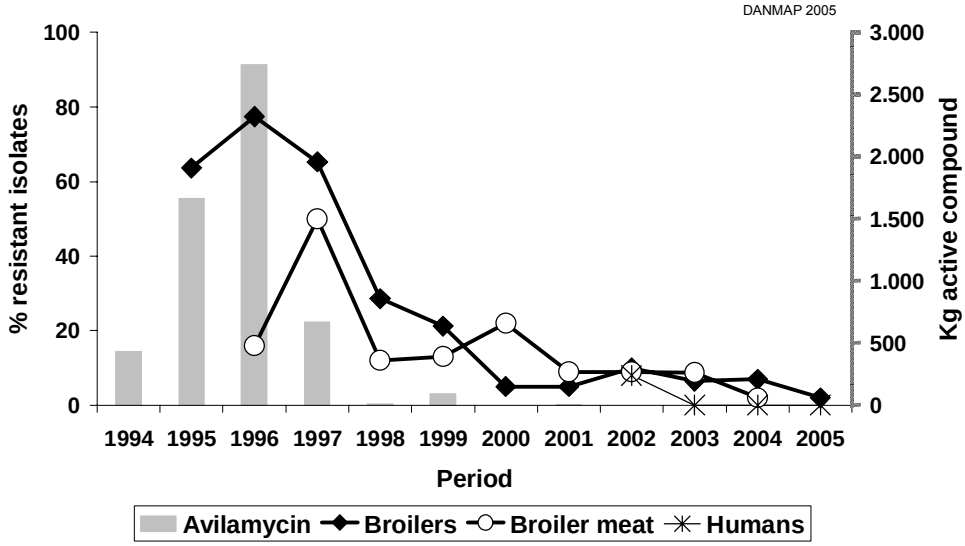

Figure 21.Trends in avilamycin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avilamycin in animals, Denmark*

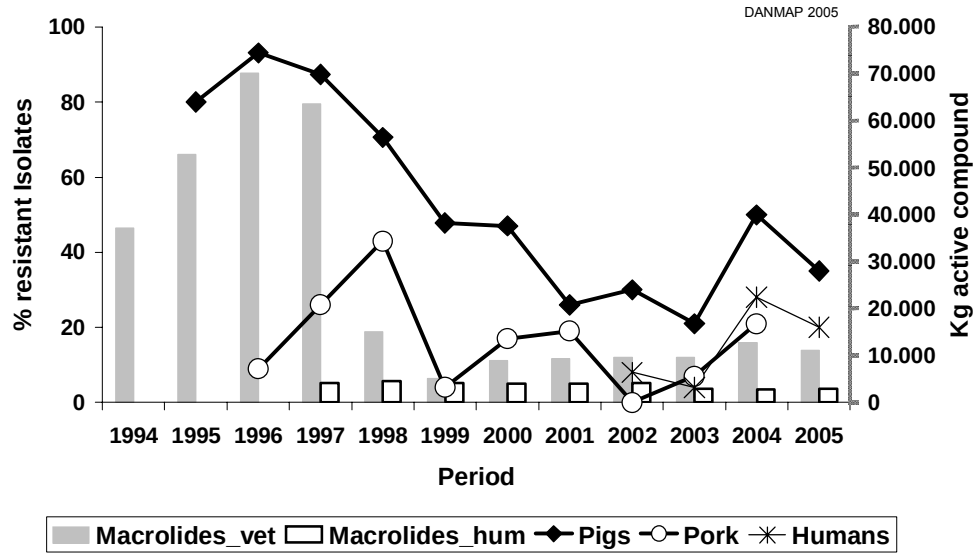

Figure 22.Trends in erythromycin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the total consumption of macrolides, both as growth promoters in animals and therapeutics in animals and humans, Denmark*

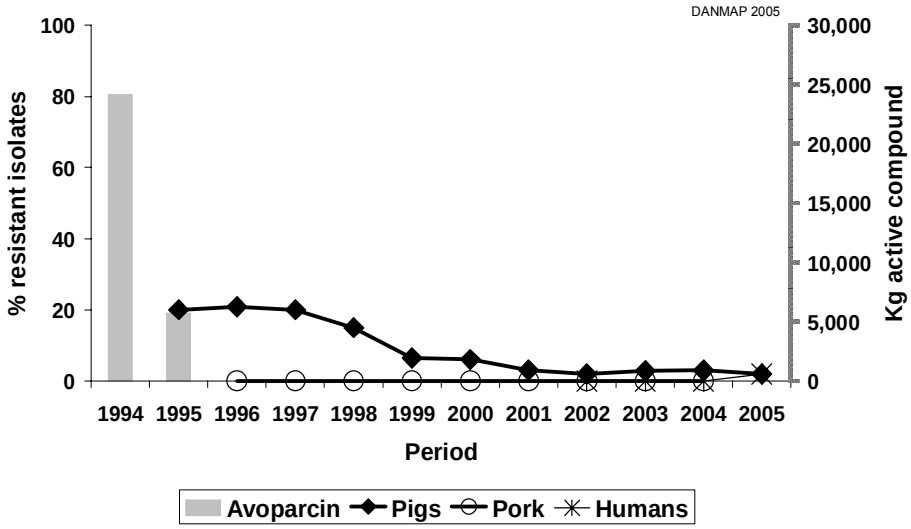

Figure 23. Trends in glycopeptide resistance among Enterococcus faecium *from pigs and pork and the consumption of the growth promoter avoparcin, Denmark*

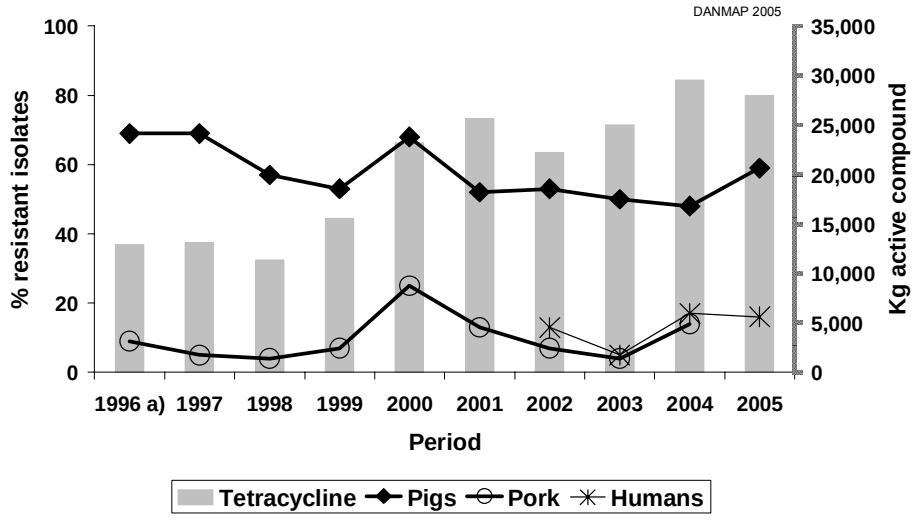

Figure 24. Trends in tetracycline resistance among Enterococcus faecium *from pigs, pork and healthy humans and the comsumption of tetracycline in pig production, Denmark*

Table 35. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from healthy humans (n=50), Denmark*

DANMAP 2005

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	16	[7.1-29.1]			84.0				2.0	14.0							
Chloramphenicol	0	[0.0-7.1]					32.0	66.0	2.0								
Florfenicol	0	[0.0-7.1]					100										
Penicillin	0	[0.0-7.1]				74.0	22.0	4.0									
Erythromycin	20	[10.0-33.7]		20.0	2.0	32.0	26.0	16.0			4.0						
Gentamicin	0	[0.0-7.1]										100					
Kanamycin	4	[0.49-13.7]										48.0	42.0	6.0			4.0
Streptomycin	6	[1.3-16.6]										94.0				4.0	2.0
Vancomycin	2	[0.05-10.7]				98.0					2.0						
Quinupristin/dalfopristin	54	[39.3-68.2]		26.0	8.0	12.0	54.0										
Avilamycin	0	[0.0-7.1]				42.0	58.0										
Salinomycin	0	[0.0-7.1]				98		2.0									
Linezolid	0	[0.0-7.1]			100												

Lines indicate breakpoints for resistance

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

Table 36. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from healthy humans (n=50) , Denmark*

DANMAP 2005

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	30	[17.9-44.6]			70.0						30.0						
Chloramphenicol	10	[3.3-21.8]				14.0	18.0	56.0	2.0	2.0	6.0	2.0					
Florfenicol	0	[0.0-7.1]					100										
Penicillin	0	[0.0-7.1]				52.0	46.0	2.0									
Erythromycin	12	[4.5-24.3]		22.0	26.0	28.0	12.0				12.0						
Gentamicin	2	[0.05-10.7]										98.0					2.0
Kanamycin	10	[3.3-21.8]										86.0	2.0		2.0		10.0
Streptomycin	10	[3.3-21.8]										82.0	4.0			4.0	10.0
Vancomycin	0	[0.0-7.1]				100											
Avilamycin	0	[0.0-7.1]				100											
Flavomycin	0	[0.0-7.1]				100											
Salinomycin	0	[0.0-7.1]				100											
Linezolid	0	[0.0-7.1]			54.0	46.0											

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 37. Comparison of resistance (%) among Enterococcus faecium *from food animals and healthy humans, Denmark*

DANMAP 2005

Compound	Pigs	Broilers	Healthy humans
	Danish %	Danish %	%
Tetracycline	59	7	16
Chloramphenicol	1	2	0
Florfenicol	0	0	0
Penicillin[a)]	18	26	0
Erythromycin	35	23	20
Gentamicin	0	0	0
Kanamycin	28	3	4
Streptomycin	29	5	6
Vancomycin	2	2	2
Quinupristin/dalfopristin	16	13	54
Avilamycin	0	2	0
Salinomycin	0	0	0
Linezolid	0	0	0
Number of isolates	105	131	50

a) For penicillin, 49 isolates from pigs and 38 isolates from broilers were tested

Table 38. Comparison of resistance (%) among Enterococcus faecalis *from food animals and healthy humans, Denmark*

DANMAP 2005

Compound	Pigs	Broilers	Healthy humans
	Danish %	Danish %	%
Tetracycline	82	39	30
Chloramphenicol	1	0	10
Florfenicol	0	0	0
Penicillin[a)]	0	0	0
Erythromycin	42	13	12
Gentamicin	7	0	2
Kanamycin	22	0	10
Streptomycin	30	2	10
Vancomycin	0	0	0
Avilamycin	0	0	0
Flavomycin	0	0	0
Salinomycin	0	0	0
Linezolid	0	0	0
Number of isolates	119	54	50

a) For penicillin, 46 isolates from pigs and 12 isolates from broilers were tested

Escherichia coli

Escherichia coli from food animals

Table 39 presents the MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates from animals at slaughter. A total of 369 isolates from broilers, cattle and pigs were collected and susceptibility tested in 2005. Figure 25 presents the trends in resistance to selected antimicrobial agents from 1996 to 2005.

From 2004 to 2005, significant decreases in resistance to tetracycline (P=0.002), chloramphenicol (P=0.02), ampicillin (P=0.002) and sulfonamide (P=0.002) were observed among indicator *E. coli* isolates from pigs. This coincided with a decrease in consumption of penicillins in weaners, whereas the decrease in occurrence of tetracycline and sulfonamide resistance coincided with increased consumption of tetracycline and sulfonamides.

From 2004 to 2005, a significant decrease in multi-resistance among *E. coli* isolates from pigs (resistant to ≥ 4 of 8 antimicrobial agents; ampicillin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, sulfonamides, tetracycline, trimethoprim) from 31.7% to 14% was observed (P=0.0002).
From 2004 to 2005, a decrease in concurrent resistance (co-resistance) to the three antimicrobial agents streptomycin, sulfonamide and tetracycline occurred (from 29.3% of the *E. coli* isolates in 2004 to16.2% in 2005), as well as a decrease in concurrent resistance to the combination ampicillin, streptomycin, sulfonamide and tetracycline (from 19.3% of the *E. coli* isolates in 2004 to 8.8% in 2005) (Figure 29).

No significant changes in resistance were observed in indicator *E. coli* isolates from broilers or cattle from 2004 to 2005.

Escherichia coli from food

Results from susceptibility testing of *E. coli* isolates from food were not available for analysis before the current version of this report was published.

Escherichia coli from healthy human volunteers

In 2005, stool samples from 110 healthy human volunteers were collected and 101 *E. coli* isolates were subsequently isolated. Table 40 presents the MIC distributions and occurrence of antimicrobial resistance of the 101 isolates. No significant changes in resistance were observed from 2004 and 2005. Resistance to sulfonamide, ampicillin and streptomycin was most common. Gentamicin resistance was detected in 2% of the isolates. This is the first isolation of gentamicin resistance in *E. coli* in our study of healthy human volunteers. Nalidixic acid resistance was observed in 5% of the isolates.

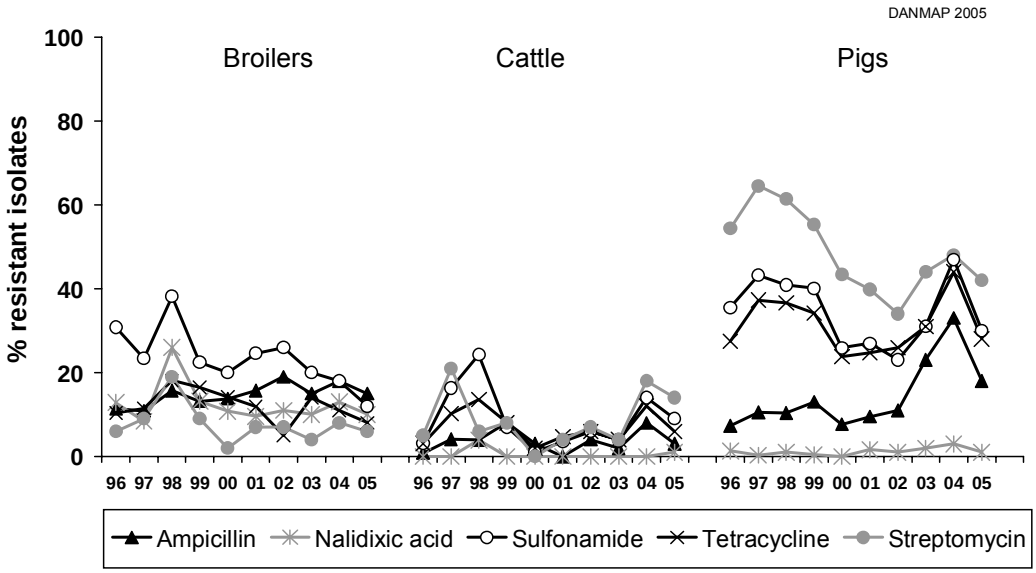

Figure 25. Trends in resistance to some selected antimicrobials among Escherichia coli *from food animals, Denmark*

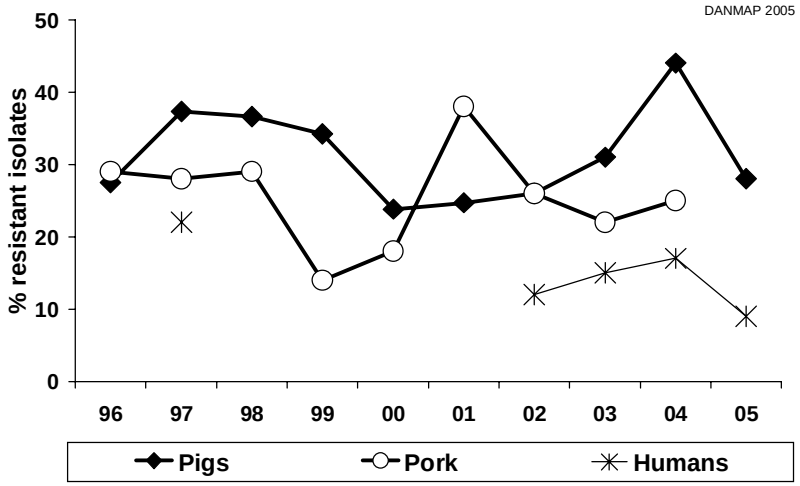

Figure 26. Trends in tetracycline resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

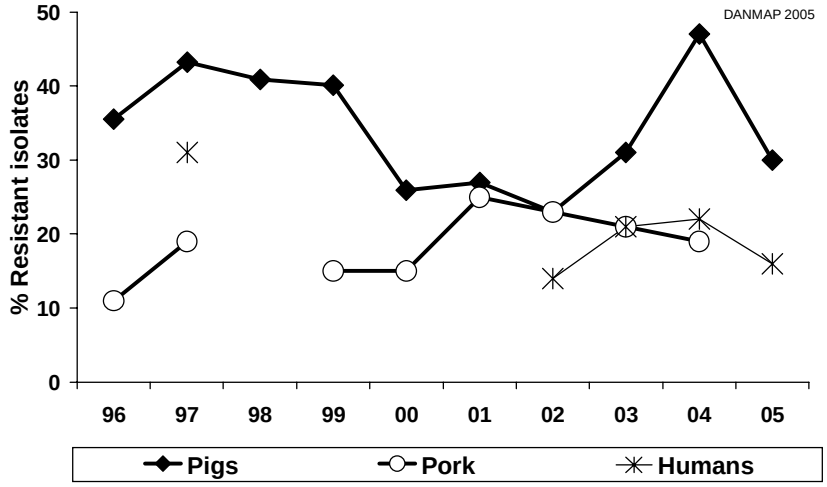

Figure 27. Trends in sulfonamide resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

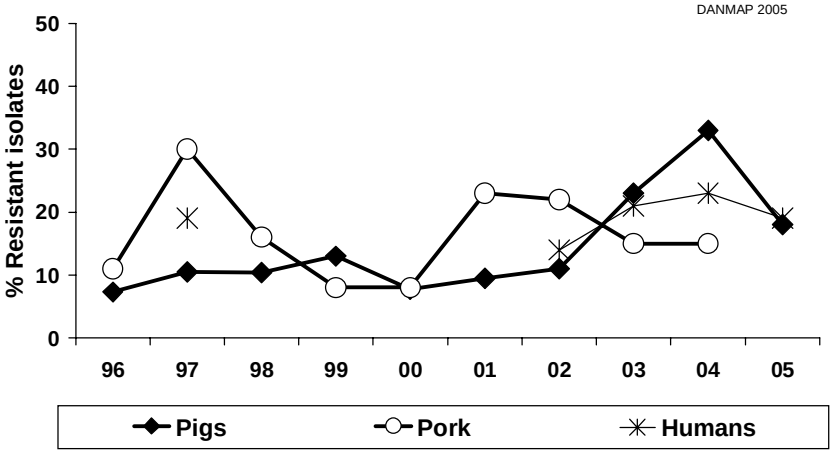

Figure 28. Trends in ampicillin resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

Comparison of Escherichia coli *from animals and healthy human volunteers*

The occurrence of resistance in food animals and healthy human volunteers is compared in Table 41.

Trends in resistance among *E. coli* isolates from pigs, pork and humans to sulfonamide, ampicillin and tetracycline are presented in Figures 26-28. The occurrences of 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. In 2005, *E. coli* isolates from pigs were significantly more resistant to tetracycline (P=0.0005), sulfonamide (P=0.02), spectinomycin (P<0.0001) and streptomycin (P=0.0002) than *E. coli* from healthy humans.

In general, resistance levels in *E. coli* from healthy human volunteers were similar to resistance levels in *E. coli* from broilers and cattle.

Table 39. Distribution of MICs and occurrence of resistance among Escherichia coli *from broilers (n=131), cattle (n=101) and pigs (n=136), Denmark*

DANMAP 2005

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Broilers	8	[3.7-13.6]							92.4					7.6					
	Cattle	6	[2.2-12.5]							93.1		1.0			5.9					
	Pigs	28	[20.6-36.3]							72.1					27.9					
Chloramphenicol	Broilers	0	[0.0-2.8]							6.9	73.3	19.9								
	Cattle	1	[0.03-5.4]							5.0	41.6	51.5	1.0			1.0				
	Pigs	2	[0.2-5.2]							3.7	49.3	44.9	0.7	1.5						
Florfenicol	Broilers	0	[0.0-2.8]							8.4	78.6	13.0								
	Cattle	0	[0.0-3.6]							2.0	42.6	52.5	3.0							
	Pigs	0	[0.0-2.7]							3.7	45.6	47.1	3.7							
Ampicillin	Broilers	15	[9.6-22.6]						19.1	48.9	16.0	0.8			15.3					
	Cattle	3	[0.6-8.4]						5.9	41.6	47.5	2.0		1.0	2.0					
	Pigs	18	[11.7-25.1]						8.8	43.4	28.7	1.5			17.7					
Amoxicillin/clavulanic acid[a)]	Broilers	0	[0.0-2.8]							49.6	35.1	15.3								
	Cattle	0	[0.0-3.6]							27.7	64.4	7.9								
	Pigs	0	[0.0-2.7]							36.8	41.2	21.3	0.7							
Cephalothin	Broilers	<1	[0.0-4.2]								48.9	35.9	14.5	0.8						
	Cattle	0	[0.0-3.6]								24.8	62.4	12.9							
	Pigs	2	[0.02-5.2]								30.2	51.5	16.9	0.7	0.7					
Cefpodoxime	Broilers	0	[0.0-2.8]			13.0	65.7	19.1	2.3											
	Cattle	0	[0.0-3.6]			5.9	44.6	46.5	3.0											
	Pigs	<1	[0.02-4.0]			4.4	39.0	50.0	5.9		0.7									
Ceftiofur	Broilers	0	[0.0-2.8]					98.5	1.5											
	Cattle	0	[0.0-3.6]					100												
	Pigs	0	[0.0-2.7]					99.3	0.7											
Sulfonamide	Broilers	12	[7.2-19.1]												84.7	2.3	0.8			12.2
	Cattle	9	[4.2-16.2]												90.1	1.0				8.9
	Pigs	30	[22.6-38.6]												69.1	0.7				30.2
Trimethoprim	Broilers	4	[1.3-8.7]								96.2				3.8					
	Cattle	1	[0.03-5.4]								99.0				1.0					
	Pigs	14	[8.6-21]								86.0				14.0					
Apramycin	Broilers	0	[0.0-2.8]								80.9	19.1								
	Cattle	0	[0.0-3.6]								98.0	2.0								
	Pigs	0	[0.0-2.7]								96.3	3.7								
Gentamicin	Broilers	0	[0.0-2.8]						99.2	0.8										
	Cattle	0	[0.0-3.6]						99.0	1.0										
	Pigs	0	[0.0-2.7]						100.0											
Neomycin	Broilers	2	[0.5-6.6]							98.5				0.8	0.8					
	Cattle	2	[0.24-7.0]							98.0					2.0					
	Pigs	6	[2.6-11.3]							92.7	1.5		0.7	2.2	2.9					
Spectinomycin	Broilers	2	[0.2-5.4]										61.8	35.9		1.5	0.8			
	Cattle	2	[0.24-7.0]										85.2	12.9		1.0	1.0			
	Pigs	29	[21.3-37.1]										57.4	10.3	3.7	10.3	18.4			
Streptomycin	Broilers	6	[2.7-11.7]								16.0	74.1	3.8	2.3	3.1	0.8				
	Cattle	14	[7.8-22.2]								62.4	22.8	1.0	4.0	5.0	5.0				
	Pigs	42	[33.5-50.7]								41.9	12.5	3.7	14.0	13.2	14.7				
Ciprofloxacin[b)]	Broilers	0	[0.0-2.8]	89.3	0.8	3.1	5.3	1.5												
	Cattle	0	[0.0-3.6]	99.0			1.0													
	Pigs	0	[0.0-2.7]	99.3			0.7													
Nalidixic acid	Broilers	10	[5.4-16.4]									91.1		0.8	3.1	6.1				
	Cattle	1	[0.03-5.4]									99.0				1.0				
	Pigs	<1	[0.02-4.0]									99.3				0.7				
Colistin	Broilers	0	[0.0-2.8]								100									
	Cattle	0	[0.0-3.6]								100									
	Pigs	0	[0.0-2.7]								100									

Vertical lines indicate breakpoints for resistance

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

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

b) The dotted line indicate the lower breakpoint used for *Salmonella* spp.

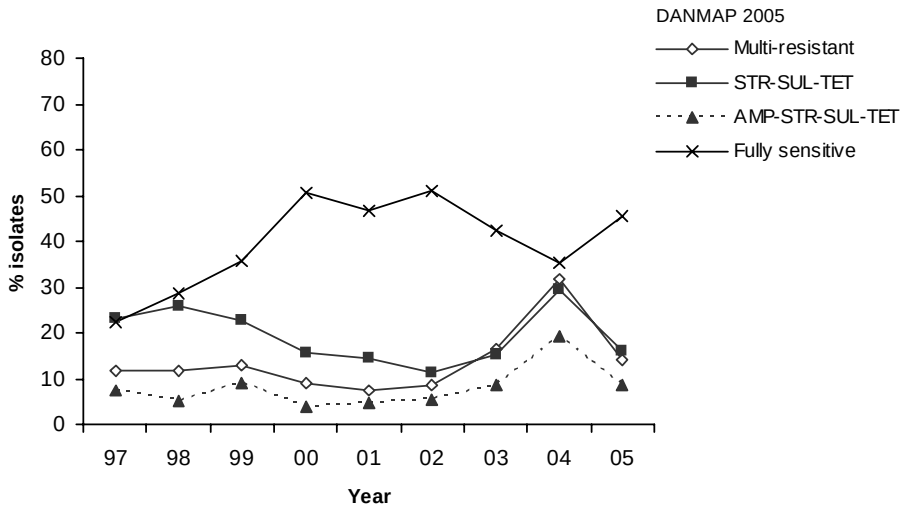

Figure 29. Trends in multi-resistance and selected resistance patterns in indicator E. coli *from pigs in Denmark*
Multi-resistance defined as isolates resistant to ≥4 of 8 antimicrobial agents (ampicillin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, sulfonamides, tetracycline or trimetoprim); STR-SUL-TET, at least resistant to streptomycin, sulfonamides and tetracyclines; AMP-STR-SUL-TET, at least resistant to ampicillin, streptomycin, sulfonamides and tetracycline; Fully sensitive, isolates which were sensitive to all 8 antimicrobial agents

Table 40. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy humans (n = 101), Denmark*
DANMAP 2005

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	9	[4.4-16.3]							91.1				1.0	7.9					
Chloramphenicol	3	[0.6-8.4]							1.0	40.6	55.4		1.0	2.0					
Florfenicol	0	[0.0-3.6]							5.9	54.5	38.6	1.0							
Ampicillin	19	[11.7-27.8]						3.0	31.7	44.5	2.0		1.0	17.8					
Amoxicillin/clavulanic acid[a)]	0	[0.0-3.6]							10.9	55.4	31.7	2.0							
Cephalothin	4	[1.1-9.8]								7.9	59.4	28.7	4.0						
Ceftiofur	0	[0.0-3.6]					98.0	2.0											
Sulfonamide	16	[9.3-24.5]												82.2		2.0	6.8	5.0	4.0
Trimethoprim	8	[3.5-15.0]								92.1				7.9					
Apramycin	<1	[0.0-5.4]								85.1	12.9	1.0		1.0					
Gentamicin	2	[0.2-7.0]						94.0	4.0		1.0		1.0						
Neomycin	0	[0.0-3.6]							96.0	4.0									
Spectinomycin	5	[1.6-11.2]										83.1	9.9	2.0	1.0	4.0			
Streptomycin	18	[11.0-26.7]								52.5	27.7	2.0	1.0	4.0	12.8				
Ciprofloxacin[b)]	<1	[0.0-5.4]	94.0	1.0			2.0	1.0	1.0		1.0								
Nalidixic acid	5	[1.6-11.2]									95.0			1.0		4.0			
Colistin	0	[0.0-3.6]								100									

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

Table 41. Occurrence of resistance (%) among Escherichia coli *from animals, food and healthy humans, Denmark*
DANMAP 2005

Compound	Broilers	Cattle	Pigs	Humans
	Danish %	Danish %	Danish %	%
Tetracycline	8	6	28	9
Chloramphenicol	0	0	2	3
Florfenicol	0	0	0	0
Ampicillin	16	3	18	19
Amoxicillin/clavulanic acid	<1	0	0	0
Cephalothin	2	0	2	4
Ceftiofur	0	0	0	0
Sulfonamide	12	9	30	16
Trimethoprim	4	1	14	8
Apramycin	0	0	0	<1
Gentamicin	0	0	0	2
Neomycin	2	2	6	0
Spectinomycin	2	2	29	5
Streptomycin	6	14	42	18
Ciprofloxacin	0	0	0	<1
Nalidixic acid	10	1	<1	5
Colistin	0	0	0	0
Number of isolates	132	101	136	101

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 indicator bacteria isolates originating from healthy animals sampled at slaughter.

Escherichia coli

The MIC distribution and the occurrence of resistance in *E. coli* isolates from cattle and pigs are presented in Table 42. Figure 31 presents trends in resistance to selected antimicrobial agents in *E. coli* isolates from pigs and cattle. From 2004 to 2005, significant decreases in resistance to neomycin (*P*=0.003) and nalidixic acid (*P*=0.01) were observed among *E. coli* isolates from diagnostic submissions from cattle. Furthermore, a significant decrease (*P*=0.02) in resistance to nalidixic acid was observed in *E. coli* isolates from diagnostic submissions from pigs from 2004 to 2005. Finally among the *E. coli* isolated from diagnostic submissions the first ESBL producing isolate (O149) from Danish productions animals from pigs was detected (see Report 3, page 94).

Staphylococci

Staphylococcus hyicus isolates originated from skin infections in pigs. The MIC distributions and the occurrence of resistance among *S. hyicus* from pigs are presented in Table 43. Trends in resistance to some selected antimicrobials from diagnostic submissions from pigs are presented in Figure 30. Resistance to penicillin among *S. hyicus* isolates from pigs decreased significantly from 86% in 2003 to 78% in 2004. Methicillin resistance was not observed. For all other antimicrobials in the test panel, the frequency of resistance remained unchanged from 2004 to 2005.

Table 42. Distribution of MICs and occurrence of resistance among Escherichia coli *from diagnostic submissions from cattle (n=46) and pigs (n=103), Denmark*

DANMAP 2005

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	Cattle	91	[79.2-97.6]							8.7					91.3					
	Pigs	72	[62.1-80.3]							27.2	1.0			5.8	66.0					
Chloramphenicol	Cattle	26	[14.3-41.1]								4.4	69.6				26.1				
	Pigs	25	[17.2-34.8]							1.0	65.1	6.8	1.9	5.8	1.9	17.5				
Florfenicol	Cattle	2	[0.1-11.5]								13.0	82.6	2.2			2.2				
	Pigs	0	[0.0-3.5]							6.8	71.8	14.6	6.8							
Ampicillin	Cattle	93	[82.1-98.6]							6.5					93.5					
	Pigs	37	[27.6-47.0]						7.8	47.6	4.9	1.9	1.0	1.0	35.9					
Amoxicillin/clavulanic acid[a)]	Cattle	2	[0.1-11.5]							4.4	8.7	63.0	21.7	2.2						
	Pigs	0	[0.0-3.5]							50.5	15.5	34.0								
Cephalothin	Cattle	2	[0.1-11.5]								17.4	58.7	21.7	2.2						
	Pigs	6	[2.2-12.3]								23.3	59.2	11.7	4.9	1.0					
Cefpodoxime[b)]	Cattle	0	[0.0-3.5]			41.3	43.5	15.2												
	Pigs	5	[1.3-11.6]			64.7	27.1	3.5	3.5		1.2									
Ceftiofur	Cattle	0	[0.0-3.5]					100												
	Pigs	1	[0.0-5.3]					99.0					1.0							
Sulfonamide	Cattle	85	[71.1-93.7]												15.2					84.8
	Pigs	75	[65.2-82.8]												24.3	1.0		2.9	6.8	65.1
Trimethoprim	Cattle	54	[39.0-69.1]								45.7				54.4					
	Pigs	31	[22.3-40.9]								68.9				31.1					
Apramycin	Cattle	7	[1.4-17.9]								87.0	6.5			6.5					
	Pigs	9	[4.1-15.9]								91.3				8.8					
Gentamicin	Cattle	9	[2.4-20.8]						84.8	6.5		4.4	2.2	2.2						
	Pigs	10	[4.8-17.1]						90.3			4.9	1.9	1.9	1.0					
Neomycin	Cattle	20	[9.4-33.9]							76.1	2.2	2.2		2.2	17.4					
	Pigs	38	[28.5-47.0]							61.2	1.0			6.8	31.1					
Spectinomycin	Cattle	39	[25.1-54.6]										47.8	6.5	6.5	17.4	21.7			
	Pigs	55	[45.2-65.1]										35.9	1.9	2.9	5.8	49.5			
Streptomycin	Cattle	76	[61.2-87.4]								6.5	8.7	8.7	23.9	15.2	37.0				
	Pigs	71	[61.1-79.4]								21.4	2.9	4.9	13.6	17.5	39.8				
Ciprofloxacin[c)]	Cattle	0	[0.0-3.5]	95.7		4.4														
	Pigs	0	[0.0-3.5]	86.4	2.9	10.7														
Nalidixic acid	Cattle	4	[0.5-14.8]									95.7			2.2	2.2				
	Pigs	13	[6.9-20.6]									87.3	15.5		1.0	11.6				
Colistin	Cattle	0	[0.0-3.5]								100	0.0								
	Pigs	0	[0.0-3.5]								99.0	1.0								

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

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

b) For cefpodoxime the number of tested isolates was n=46 for cattle and n=85 for pigs

c)The dotted line indicate the lower breakpoint used for *Salmonella* spp.

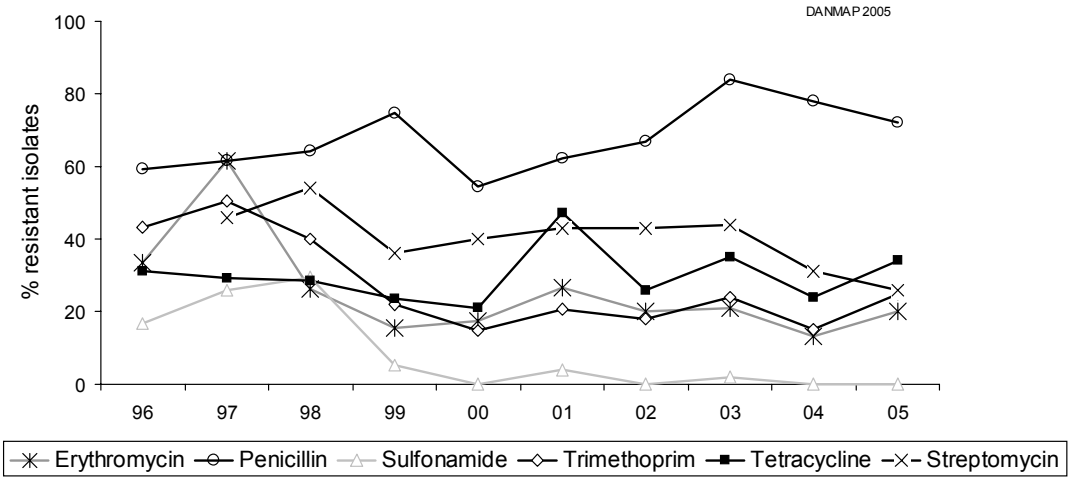

Figure 30. Trends in resistance to some selected antimicrobials among Staphylococcus hyicus *from diagnostic submissions from pigs, Denmark*

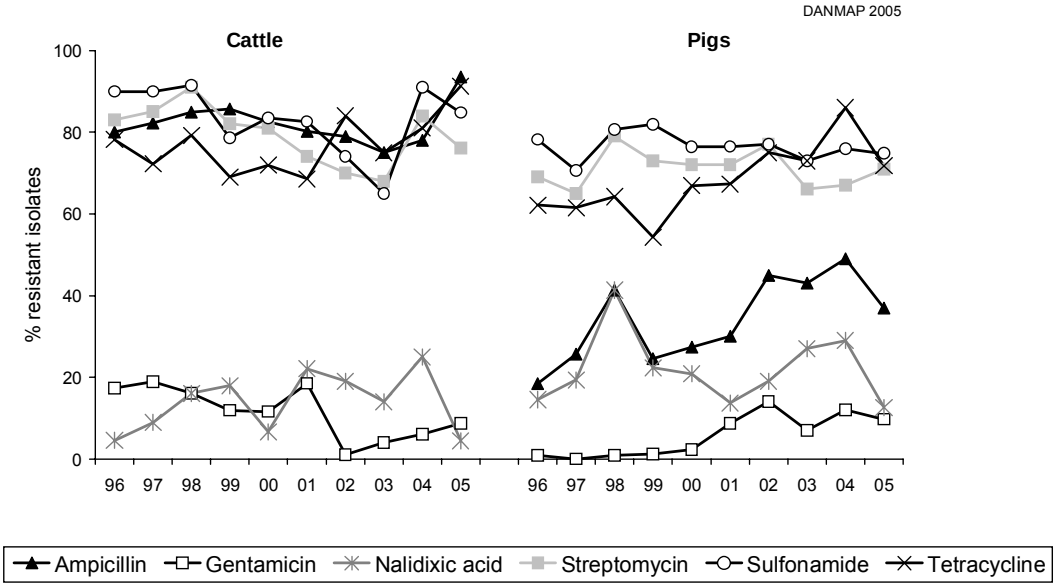

Figure 31. Trends in resistance to selected antimicrobials among Escherichia coli *from diagnostic submissions from animals, Denmark*

Table 43. Distribution of MICs and occurrence of resistance among Staphylococcus hyicus *from pigs (n=61), Denmark*

DANMAP 2005

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	34	[22.7-47.7]				65.6					6.6	18.0	9.8				
Chloramphenicol	2	[0.0-8.8]							88.5	9.8				1.6			
Florfenicol	2	[0.0-8.8]					3.3	91.8	3.3					1.6			
Penicillin	72	[59.2-82.9]	27.9		3.3	3.3	3.3	9.8	9.8	8.2	14.8	19.7					
Ceftiofur	0	[0.0-5.9]				42.6	57.4										
Sulfonamide	0	[0.0-5.9]								55.7	32.8	8.2	3.3				
Trimethoprim	25	[15.5-37.3]					57.4	18.0					24.6				
Erythromycin	20	[10.6-31.8]			72.1	8.2						19.7					
Spectinomycin	13	[5.8-24.2]										8.2	78.7			13.1	
Streptomycin	26	[15.8-39.1]						4.9	62.3	6.6			3.3	4.9	18.0		
Ciprofloxacin	3	[0.4-11.3]		82.0	14.8					3.3							
Tiamulin	31	[19.9-44.3]			3.3	52.5	6.6			4.9	1.6	4.9	26.2				

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

Bacteria from humans

Data on resistance levels in *Streptococcus pneumoniae* isolates, as well as *Salmonella* spp. and *Campylobacter* spp. isolates (see pages 38 and 43) 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, page 14.

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 1995-2005 are presented for each county in Figures 32, 33 and 34 showing resistance in blood and urine isolates in *E. coli* to selected antimicrobials.

Data on resistance in *E. coli* blood isolates from hospitals in all participating counties are presented in Figure 32. In *E. coli* blood isolates the generally high level of ampicillin resistance remained between 30 and 50% with an average of 40.3% (95% CI: 38.7-42.0). It was thus unchanged compared to 2004. In one county (Roskilde county), there was a significant decrease in ampicillin resistance in *E. coli* blood isolates from 48.1% in 2004 to 33.9% in 2005 (P=0.005), whereas in another county (West Zealand county) a significant increase in ampicillin resistance in *E. coli* blood isolates from 29.7% in 2004 to 41.7% in 2005 (P=0.04) was observed. There were no significant variations in ampicillin resistance in *E. coli* blood isolates in other counties. Gentamicin resistance in *E. coli* blood isolates was reported from 12 counties between 2003 and 2005. Overall, it significantly increased from 1.1% in 2003 to 2.4% in 2005 (P=0.0003). In one county (Copenhagen county), there was a significant increase in gentamicin resistance in *E. coli* blood isolates from 0.7% in 2004 to 3.7% in 2005 (P=0.004). In other counties there were no significant variations in gentamicin resistance in *E. coli* blood isolates. Cefuroxime resistance in *E. coli* blood isolates was reported from 11 counties between 2003 and 2005. It significantly increased from 2.1% in 2003 to 3.5% in 2005 (P=0.002) (excluding Rigshospitalet). In one county (Copenhagen county), there was a significant increase in cefuroxime resistance in *E. coli* blood isolates from 0.9% in 2004 to 4.6% in 2005 (P=0.001). Most of the cefuroxime resistant isolates reported in this county produced an extended beta-lactamase (ESBL) and were therefore resistant to third-generation cephalosporins. In other counties, there were no significant variations in cefuroxime resistance in *E. coli* blood isolates. Mecillinam resistance in *E. coli* blood isolates was reported from 10 counties in 2005, representing 70% of the Danish population. Overall, it was at an average 4.2% (95% CI: 3.5-5.0). Tetracycline resistance in *E. coli* blood isolates was reported from four counties as well as Rigshospitalet, representing around 30% of the Danish population. Overall, it was at an average 23.8% (95% CI: 21.3-26.4).

Data on resistance in *E. coli* urine isolates from primary health care in all participating counties are presented in Figure 33. Overall, ampicillin resistance in *E. coli* urine isolates from primary health care increased significantly from 39.3% in 2004 to 40.5% in 2005 (P=0.01). Sulfonamide resistance in *E. coli* urine isolates from primary health care also increased significantly from 36.6% in 2004 to 37.6% in 2005 (P=0.04). In one county (Ringkøbing county), there was a significant increase in sulfonamide resistance in *E. coli* urine isolates from primary health care from 32.9% in 2004 to 36.9% in 2005 (P=0.03). In other counties there were no significant variations in sulfonamide resistance in *E. coli* urine isolates. 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. A study performed in 1997-1999 showed that ampicillin and sulfonamide resistance in *E. coli* isolates from uncomplicated urinary tract infections in primary health care in Denmark was at only 20% and 22%, respectively, compared to 34% and 39%, respectively, in complicated urinary tract infections [Kerrn *et al.* 2002. J. Antimicrob. Chemother. 50: 513-516]. Data on ciprofloxacin resistance in *E. coli* urine isolates from primary health care in 2005 were available from 10 counties (see Figure 33), representing 68% of the Danish population. A significant increase in resistance to ciprofloxacin was observed: from 2.9% in 2004 to 4.3% in 2005 (P<0.0001). This increase was also highly significant (P<0.0001) when considering only the nine counties that reported ciprofloxacin resistance data in both 2004 and 2005. Between 2004 and 2005 and for the nine counties which reported resistance data, consumption

of fluoroquinolones in primary health care increased from 0.29 to 0.34 DDD per 1,000 inhabitant-days. Among the counties that test for ciprofloxacin resistance, three counties showed a significant increase in ciprofloxacin resistance in *E. coli* urine isolates from primary health care from 2004 to 2005. In Copenhagen and Frederiksberg municipalities (both having the status of counties) it increased from 2.7% in 2004 to 3.9% in 2005 (*P*=0.001), and in Funen county it increased from 2.0% in 2004 to 3.4% in 2005 (*P*=0.0002). In other counties there were no significant variations in ciprofloxacin resistance in *E. coli* urine isolates from primary health care. Data on nalidixic acid resistance in *E. coli* urine isolates from primary health care in 2005 were available from three counties, representing 26% of the Danish population. Overall, it was at an average 7.4% and unchanged compared to 2004 (95% CI: 6.7-8.2). The increase in ciprofloxacin resistance was concomitant to the increase in fluoroquinolone consumption in primary health care observed in Denmark since 2002 (see Table 12). Reasons for the increase in fluoroquinolone consumption are discussed in the section on antimicrobial consumption in humans (see page 31). All participating counties reported data on mecillinam resistance in *E. coli* urine isolates from primary health care. Overall, it was at an average 3.6% (95% CI: 3.4-3.9).

Data on resistance in *E. coli* urine isolates from hospitals in all participating counties are presented in Figure 34. Overall, ampicillin resistance in *E. coli* urine isolates from hospitals increased significantly from 37.6% in 2004 to 38.7% in 2005 (*P*=0.001). In one county (Viborg county), there was a significant increase in ampicillin resistance in *E. coli* urine isolates from hospitals from 36.6% in 2004 to 40.4% in 2005 (*P*=0.03). In other counties there were no significant variations in ampicillin resistance in hospital *E. coli* urine isolates. In *E. coli* urine isolates from hospitals, sulfonamide resistance remained unchanged compared to 2004 at an average of 32.7% (95% CI: 32.2-33.1). In one county (Copenhagen county), there was a significant decrease in sulfonamide resistance in hospital *E. coli* urine isolates from 37.1% in 2004 to 35.3% in 2005 (*P*=0.04), whereas in another county (Vejle county) there was a significant increase in sulfonamide resistance in hospital *E. coli* urine isolates from 29.0% in 2004 to 32.4% in 2005 (*P*=0.04). In other counties there were no significant variations in sulfonamide resistance in hospital *E. coli* urine isolates. Data on ciprofloxacin resistance in *E. coli* urine isolates from hospitals in 2005 were available from 9 counties (see Figure 34), representing 61% of the Danish population. A significant increase in resistance to ciprofloxacin was observed: from 3.0% in 2004 to 5.4% in 2005 (*P*<0.0001). This increase was also highly significant (*P*<0.0001) when considering only the 8 counties that reported ciprofloxacin resistance data in both 2004 and 2005. Among the counties that test for ciprofloxacin resistance, three counties showed a significant increase in ciprofloxacin resistance in hospitals, as compared to 2004 (Copenhagen and Frederiksberg Municipalities, P<0.0001; Rigshospitalet, P<0.05; Funen county, P=0.005). Data on nalidixic acid resistance in *E. coli* urine isolates from hospitals in 2005 were available from four counties, representing 31% of the Danish population. Overall, it was at an average 6.6%, remaining unchanged compared to 2004 (95% CI: 6.1-7.1). This increase was concomitant to the steady increase in consumption of fluoroquinolones reported from both hospitals and primary health care in recent years (see Tables 12 and 14). All participating counties reported data on mecillinam resistance in *E. coli* urine isolates from hospitals. Overall, it was at an average 4.4% (95% CI: 4.2-4.6).

Coagulase-negative staphylococci

In 2005, the average level of penicillin resistance in coagulase-negative staphylococci blood isolates from hospitals was unchanged compared to 2004, at an average 79% among counties (min. 66% - max. 85%), and has since 1996 been subject to small variations only. Resistance to erythromycin was unchanged compared to 2004 and averaged 36% among counties (min. 29% - max. 53%). In the thirteen counties reporting methicillin resistance data in 2004 and 2005, resistance increased from 45.7% in 2004 to 50.3% in 2005 (*P*<0.0001), although it varied among counties (min. 26% - max. 78%). 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 comparisons of resistance levels between counties.

Methicillin-resistant *Staphylococcus aureus*

In 2005, a total of 1,530 *S. aureus* bacteraemia (SAB) cases were reported from the 15 participating Danish counties/municipalities, covering 95% of the Danish population. This corresponded to an incidence of 29.7 per 100,000 inhabitants, which is similar to the incidence reported in 2004. Of these, 18 (1.2%) were methicillin resistant *S. aureus* (MRSA). This is similar to what was reported in 2004. The resistance profile for SAB compared to methicillin resistant *S. aureus* (MRSA) cases is shown in Table 44. A more detailed description of the SAB cases will be published in the yearly *S. aureus* bacteremia report, which can be downloaded at http://www.ssi.dk/sw3425.asp.

For MRSA, there has been an 8-fold increase in the number of cases (infections as well as colonisations) reported from all body sites since 2002 (Figure 35). In 2005, a total of 856 new persons (cases) were reported as MRSA-positive nationwide, which corresponded to an incidence of 15.8 per 100,000 inhabitants and a 51% increase from 2004 (number of cases: 568, incidence: 10.7 per 100,000 inhabitants). It is noteworthy that 13% of the MRSA isolates were resistant only to beta-lactams and susceptible to all other antimicrobials tested. Three MRSA isolates showed a reduced susceptibility to vancomycin (Table 44). MRSA isolates were generally more resistant to antibiotics compared to SAB isolates, which is in accordance to what has been reported in other countries. However, resistance frequencies are highly influenced by the distribution and the frequency of predominant MRSA strains and therefore can exhibit great variations, both geographically within Denmark and over time.

Large regional variations in the incidence of MRSA cases were observed. The highest incidence was reported in Vejle County with 87 cases per 100,000 inhabitants. This was due to a large hospital outbreak with a ST22, t022 strain (also known as EMRSA-15), which had been ongoing since the end of 2002. Due to a massive search-and-destroy intervention, the outbreak now seems under control as reflected by the significant decrease in the number of new MRSA cases in this county in the second half of 2005 and in 2006. A high MRSA incidence was also reported in the Greater Copenhagen area, i.e. Copenhagen Municipality, Frederiksberg Municipality and Copenhagen County, with an incidence of 25 cases per 100,000 inhabitants. The epidemiology in this region was more diverse consisting of several different clones and of community acquired-MRSA, as well as cases related to cross-transmission in hospitals and nursing homes.

Molecular typing showed that 92% of the MRSA isolated in Denmark in 2005 belonged to 7 clonal complexes: CC22 (34%), CC8 (26%), CC5 (13%), CC80 (9%), CC30 (7%) and CC45 (3%). In 2004, the same 7 clonal complexes accounted for 95% of MRSA isolates with an almost identical relative distribution. These clonal complexes also are among the most frequently isolated worldwide.

For each MRSA case, clinical and epidemiological data were retrospectively requested from hospitals and general practitioners. Based on this information, each case was classified as “screening“ or “infection“. MRSA infections were further classified according to the onset of the infection: acquisition outside Denmark (imported), hospital acquired (HA), community onset with health care associated risk (CO-HCA), community onset with a non-health care associated risk, e.g. a close family member being MRSA positive (CO-CR), and community onset but no identified risk factor (CO-NR).

As of June 1st 2006, discharge summaries had been obtained for 800 (93%) of the cases. The preliminary results for 2005 show that 444 (55%) of the MRSA cases had an infection at the time of diagnosis, whereas 349 (44%) corresponded to active screening. For 7 cases (1%), this information could not be retrieved. In 51 cases, the MRSA infection was imported from abroad. Among the remaining 387 MRSA infections, 103 were acquired in Danish hospitals (HA) and 284 were detected outside hospitals (onset in the Danish community, CO). Among the latter, 135 had a healthcare associated risk of acquisition (contact to hospitals or nursing homes within the last 12 months, CO-HCA) and the remaining 149 cases had either a non-health care associated risk, e.g. close family member to an MRSA case (CO-CR, 18 cases) or no identified risk factor (CO-NR, 131 cases) (Figure 36). The age distribution was significantly different between cases classified as CO-NR/CO-CR vs CO-HCA and HA with a median age of 34, 73 and 73 years, respectively (Figure 36).
Skin and soft tissue infection was the most frequent type of infection (68%), followed by urinary tract infection (9%), post operative infection (6%), lower respiratory tract infection (6%) and bacteraemia (4%). Due to the rapid increase of MRSA cases in Denmark, the National Board of Health is in the process of issuing a new national guideline for handling and preventing MRSA cases, which will also make MRSA infection, as well as colonisation, a notifiable disease.

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 2005, susceptibility testing was performed on 1,143 non-duplicate isolates from blood or cerebrospinal fluid samples. The percentage of *S. pneumoniae* isolates not susceptible (resistant plus intermediate isolates) to penicillin was 2.2% in 2003, 3.2% in 2004, and 4.2% in 2005 (Figure 38). This level of resistance is much lower than reported in many other European countries.

Macrolide resistance in *S. pneumoniae* isolates from blood and spinal fluid has been around 5% since 2000. The percentage of macrolide resistant *S. pneumoniae* was 5.2% in 2003, 4.6% in 2004, and 5.5% in 2005 (Figure 38).

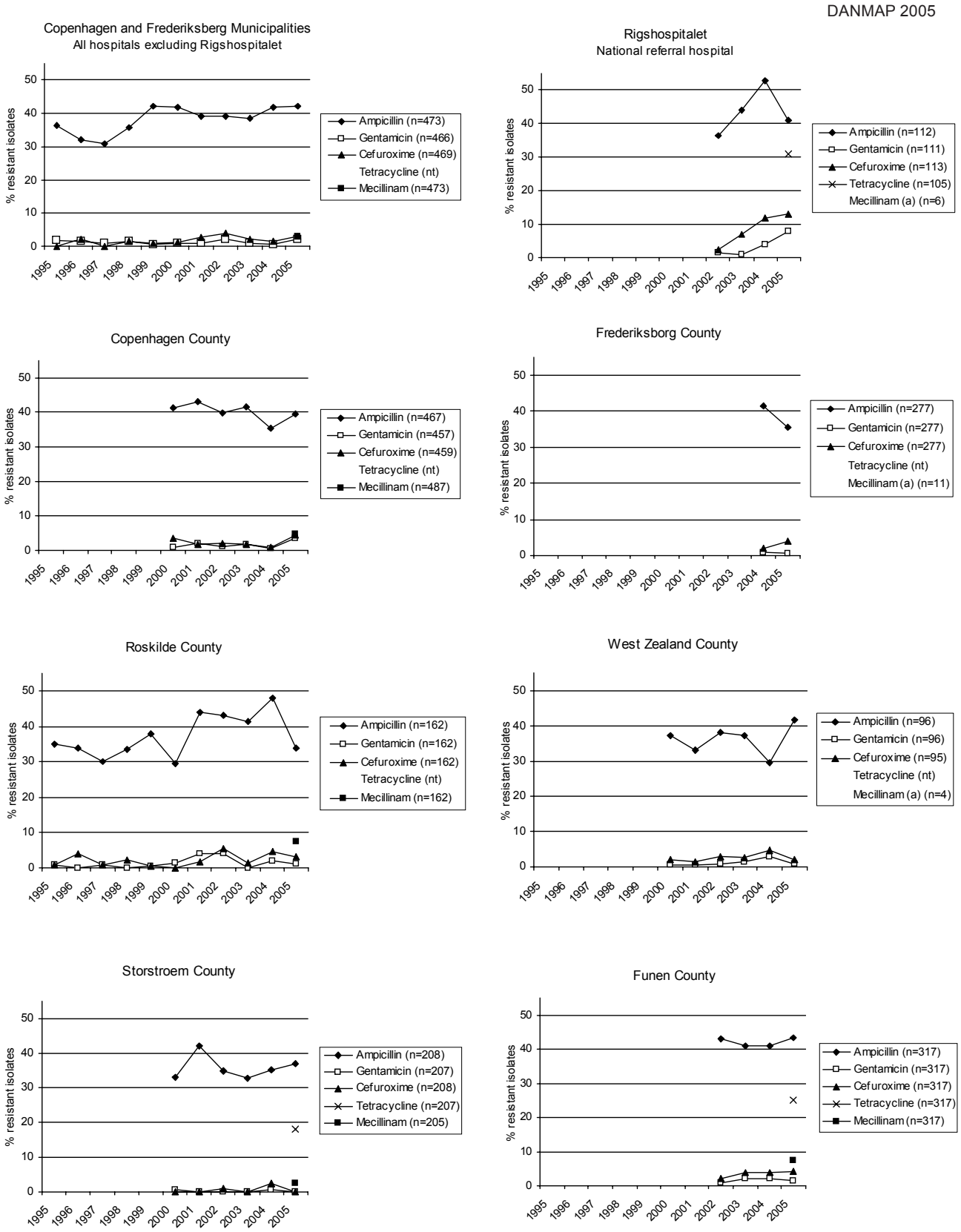

Figure 32. Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam and tetracycline in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.*

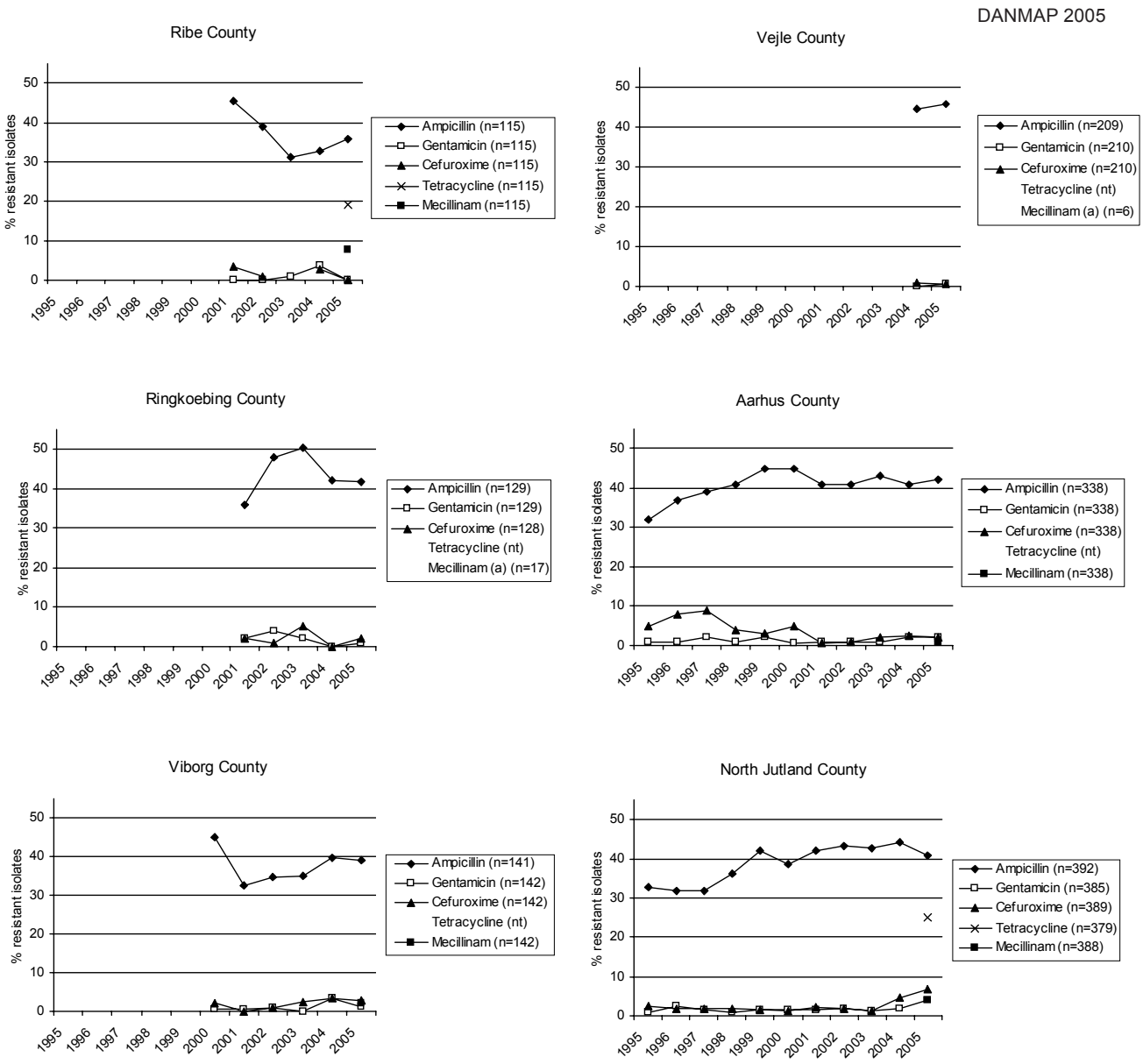

Figure 32. (Continued). Resistance (%) to ampicillin, gentamicin, cefuroxime, mecillinam and tetracycline in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.*

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

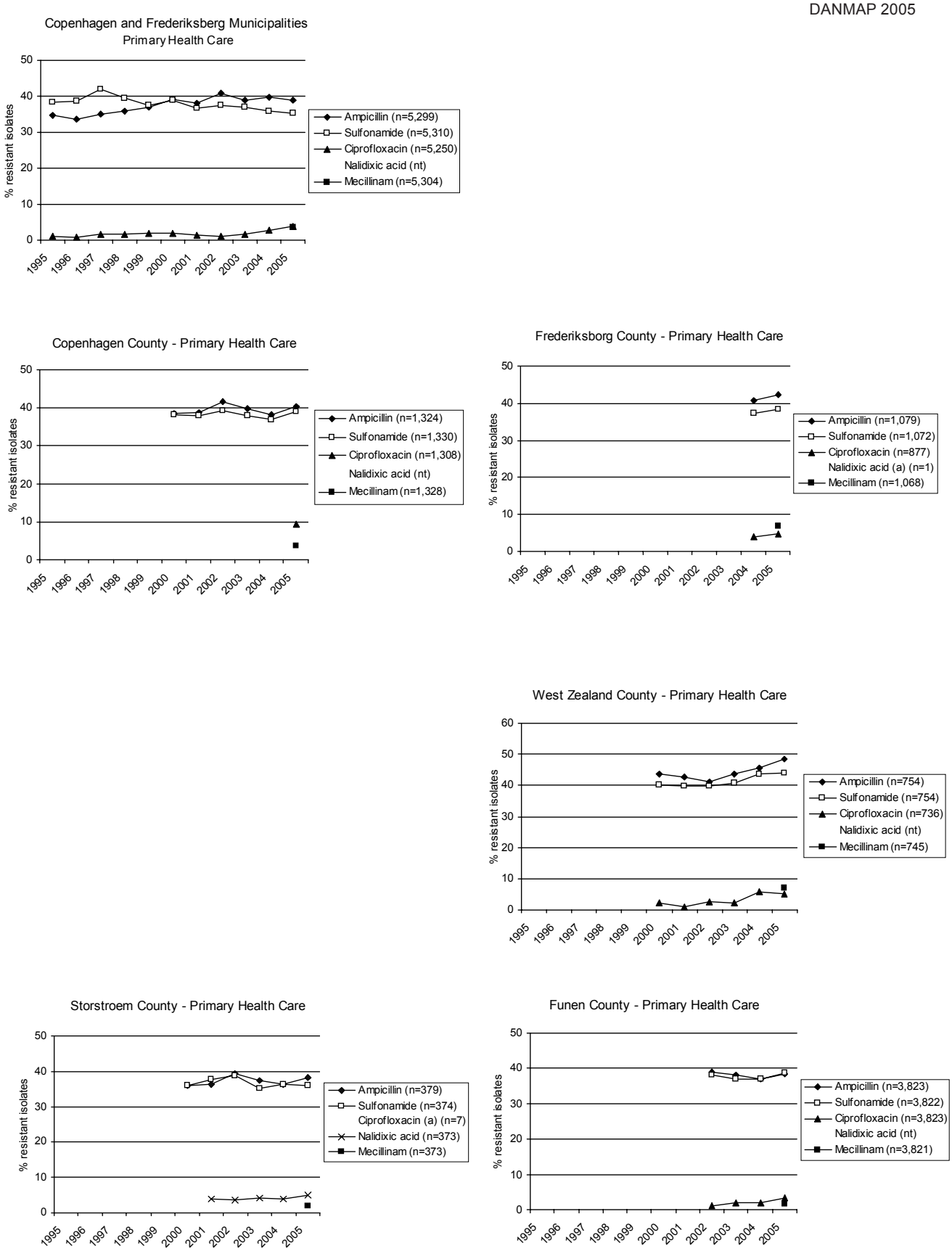

Figure 33. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in primary health care by county, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.*

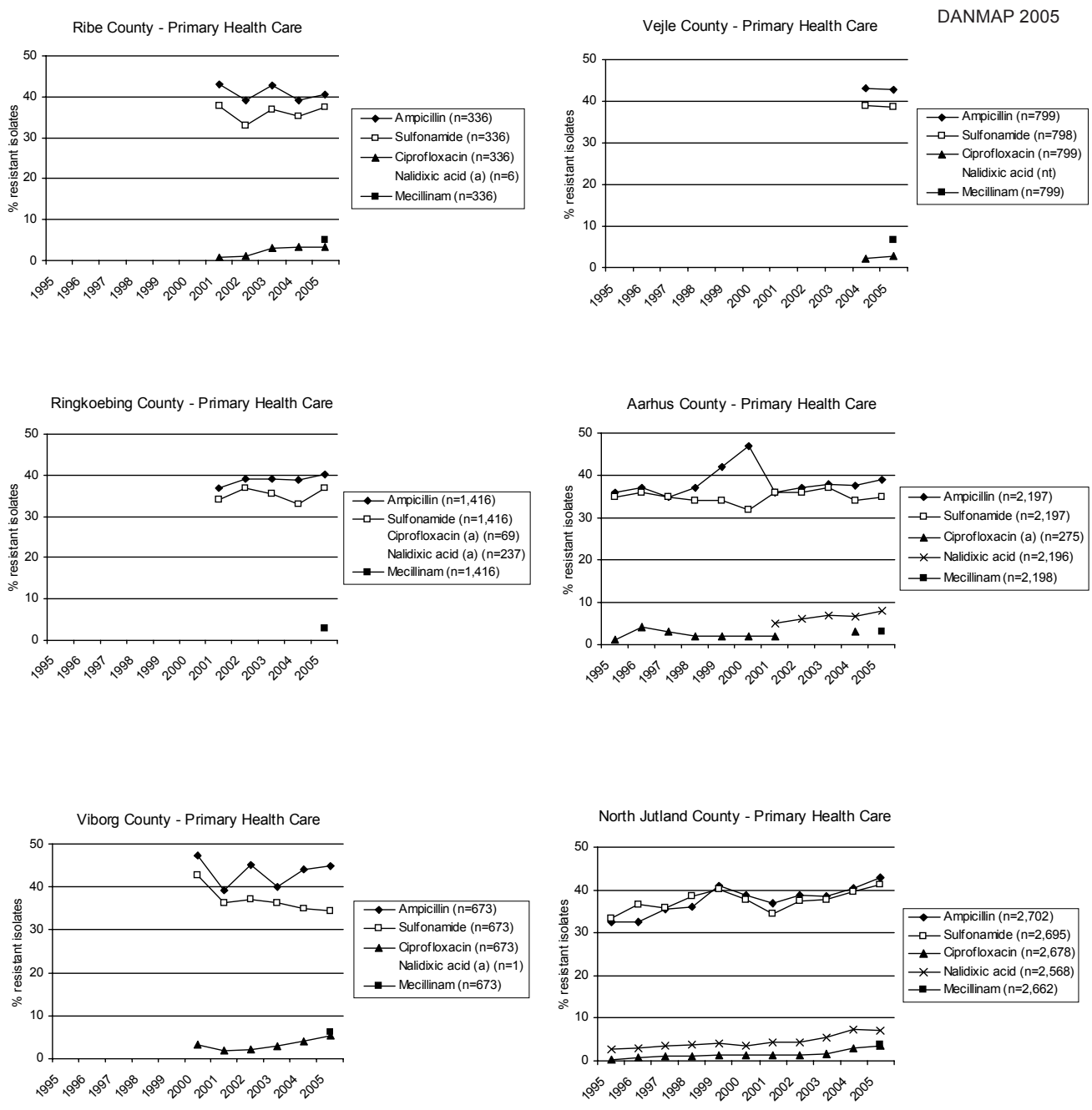

Figure 33. (Continued). Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in primary health care by county, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.*

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

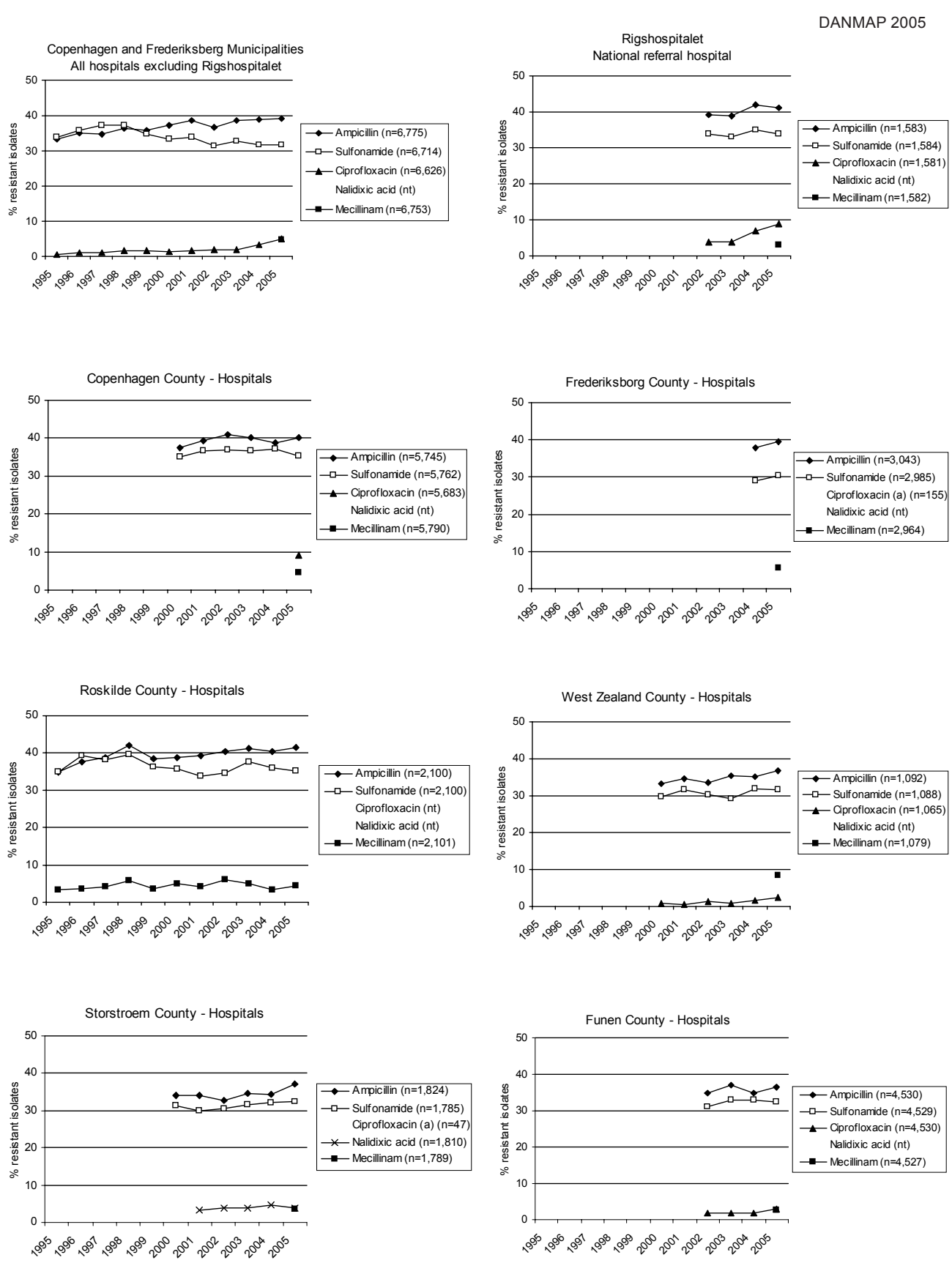

Figure 34. Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli *urine isolates from humans in hospitals by county, Denmark. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.*

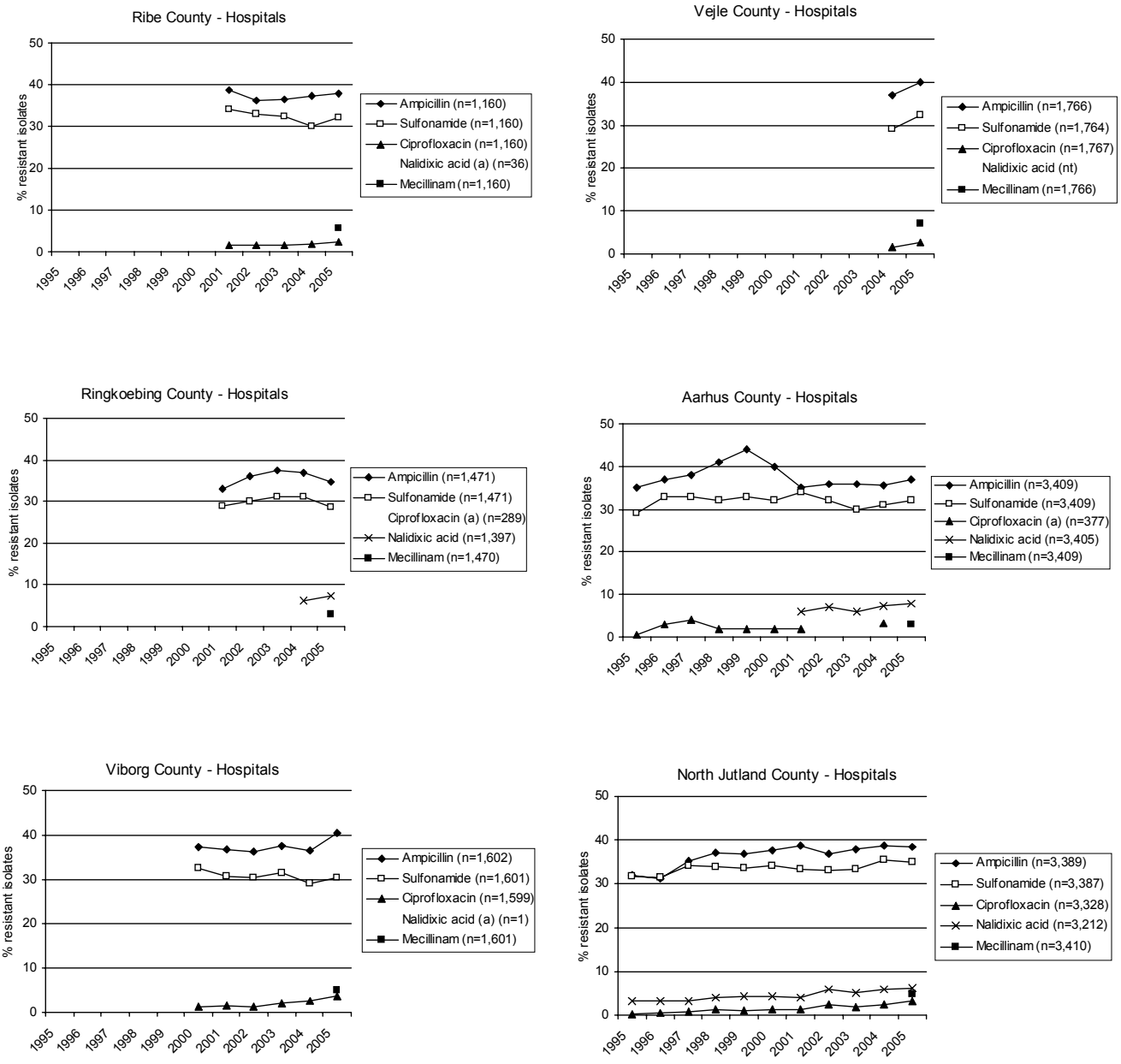

Figure 34. (Continued). Resistance (%) to ampicillin, sulfonamides, ciprofloxacin, nalidixic acid and mecillinam in Escherichia coli urine isolates from humans in hospitals by county, Denmark. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2005.

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

Table 44. Resistance frequencies among Staphylococcus aureus *bacteraemia and methicillin resistant Staphylococcus aureus (MRSA) isolates* DANMAP 2005

	Percent resistant	
	Among *S. aureus* bacteraemia isolates	Among MRSA, all body sites
Compound	(n=1530)[a)]	(n=856)
Penicillin	78%	100%
Cefoxitin	1%	100%
Erythromycin	4%	40%
Clindamycin	4%	36%
Tetracycline	3%	18%
Fluoroquinolones	2%	52%
Rifampin	1%	4%
Fusidic acid	9%	17%
Kanamycin	2%	25%
Streptomycin	1%	14%
Vancomycin	0%	VRSA (0%), 3 hVISA (0.4%)[b)]

a) Includes 18 bacteraemia MRSA
b) VRSA=*van*A positive MRSA, hVISA=heterogeneous vancomycin intermediate *S. aureus*

DANMAP 2005

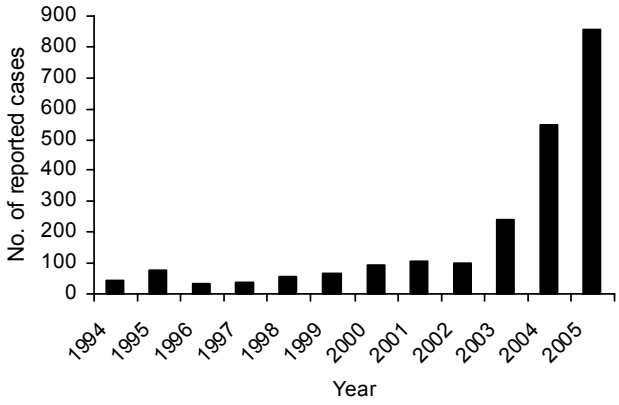

Figure 35. Number of reported cases of methicillin resistant Staphylococcus aureus *(MRSA), Denmark, 1994-2005. Only one MRSA isolate per patient/person was included*

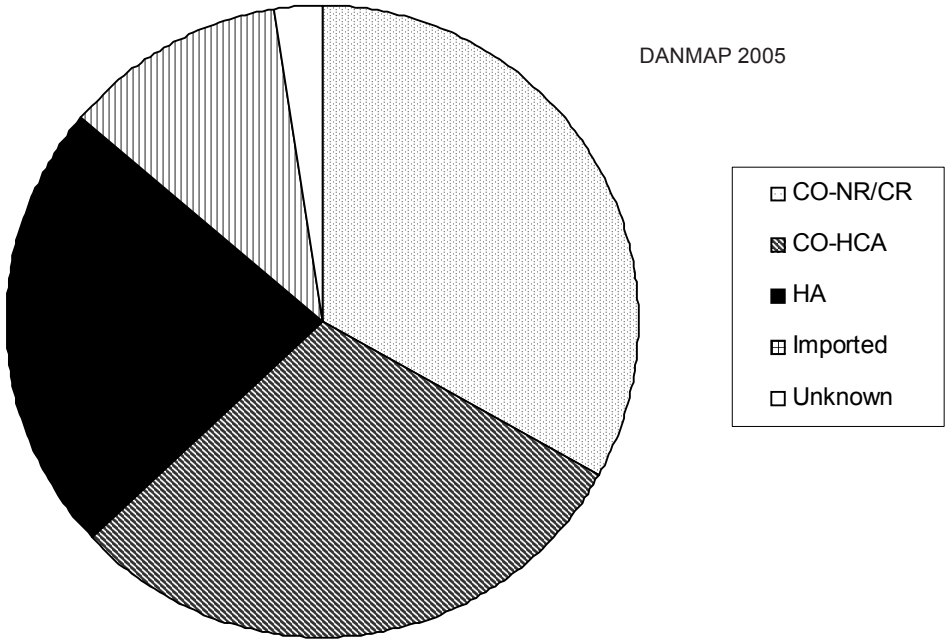

Figure 36. Distribution of methicillin resistant Staphylococcus aureus *(MRSA) infection cases according to origin, Denmark, 2005. CO-NR (community onset infection, no identified risk); CO-CR (community onset infection, community risk identified); CO-HCA (community onset infection, health care risk identified); HA (hospital acquired infection)*

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

In 2005, data were reported on 5,244 non-invasive GAS isolates from clinical samples in 12 counties. Resistance to macrolides (erythromycin) in GAS isolates was 2.0% compared to 2.2% in 2004. County-to-county variations ranged from 0.0% to 3.4%. In 2005, data on 116 invasive GAS isolates were reported to the national reference center at Statens Serum Institut. Resistance to macrolides in invasive isolates was 1.7% in 2005 compared to 1.6% in 2004. As in previous years, no resistance to penicillin in GAS isolates was reported in 2005.

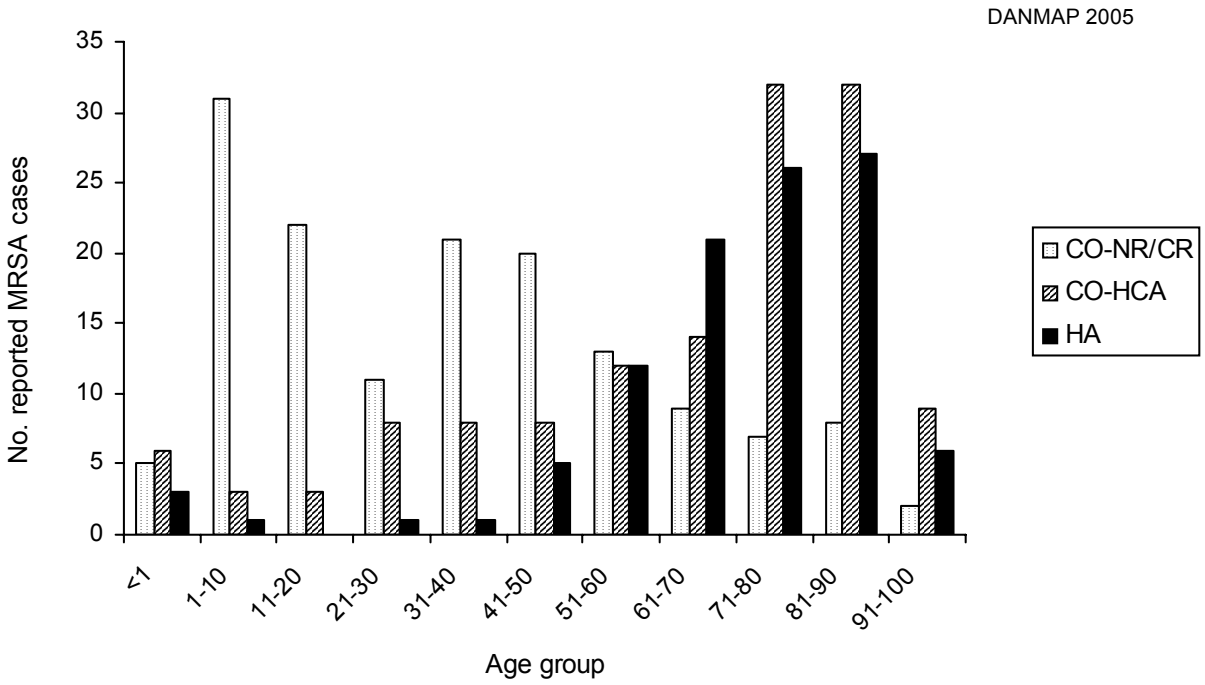

Figure 37. Age distribution of methicillin resistant Staphylococcus aureus *(MRSA) cases according to origin, Denmark, 2005. CO-NR (community onset infection, no identified risk); CO-CR (community onset infection, community risk identified); CO-HCA (community onset infection, health care risk identified); HA (hospital acquired infection)*

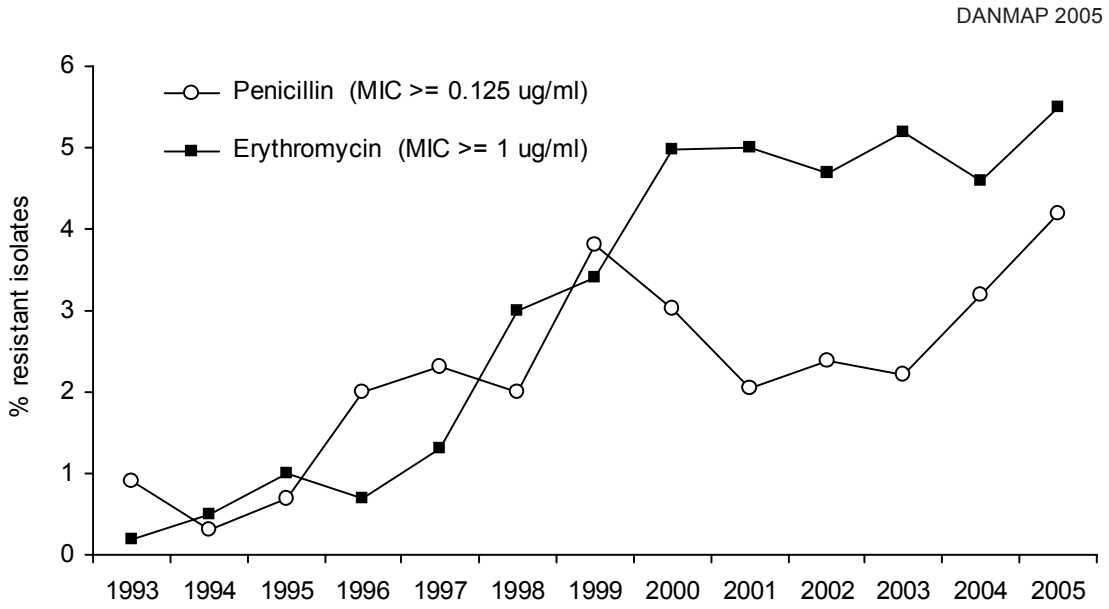

Figure 38. Resistance (%) to penicillin and macrolides in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

Appendix 1

Materials and Methods

Materials and methods

Demographics

Hospitals in Denmark

The reported number of hospitals in each county in Denmark corresponds to the number of geographically distinct public hospitals which do not specialise in psychiatric care (somatic hospitals) and report data to the Danish Medicines Agency and the National Board of Health. It is larger than the official number of hospitals in Denmark since reorganisation of the hospital sector has resulted in regrouping hospitals that are distant geographically under the same administration and therefore the same name.

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

Data on consumption of antimicrobials

Consumption of antimicrobial agents in animals

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

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

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

Data on all sales of veterinary prescription medicine from the pharmacies are sent electronically to VetStat. Veterinarians are required by law to report to VetStat the use of all prescription medicines in production animals. The amount of drugs reported by the veterinary practitioners is validated against pharmacy data on the total sales of therapeutic drugs for use in practice. Feed mills report all sales of medicated feed directly to VetStat.

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

Consumption of antimicrobial agents in humans

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

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

The present report includes data on the consumption of antibacterials for systemic use, or group J01 of the 2005 update of ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antimicrobials in primary health care is expressed as a number of DDD per 1,000 inhabitants and per day (DDD/1,000 inhabitant-days). Consumption in primary health care is also reported as a number of packages per 1,000 inhabitants. Consumption of antimicrobials in hospitals is expressed as a number of DDD per 1,000 occupied beds and per day (DDD/1,000 occupied bed-days). Since antimicrobial consumption expressed as DDD/1,000 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DDD/ 1,000 discharged patients. Data on the number of occupied bed-days (or patient-days) and number of discharges in each hospital were obtained from the National Board of Health (http://www.sundhedsdata.dk).

Collection of bacterial isolates

Isolates from animals

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

Table A1. Antibacterials used in humans and/or in animals in Denmark
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 in humans and animals are underlined [a)]

DANMAP 2005

ATC/ATCvet codes	Therapeutic group	Names of antibacterials in group
J01AA/*QJ01AA/QJ51AA*	Tetracyclines	Doxycycline, *chlortetracycline* , lymecycline, oxytetracycline, tetracycline, tigecycline b)
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, *cefoperazone, ceftiofur*
J01DE/*QJ51DA*	Fourth-generation cephalosporins	Cefepime, *cefquinome*
J01DF	Monobactams	Aztreonam
J01DH	Carbapenems	Meropenem, imipenem/cilastatin, ertapenem
J01EA	Trimethoprim and derivatives	Trimethoprim
J01EB/*QJ01EQ/QJ51R*	Short-acting sulfonamides	Sulfamethizole, *sulfadimidine, sulfathiazole*
J01EE/*QJ01EW*	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfamethoxazole/trimethoprim, *sulfadiazine/trimethoprim* , *sulfadoxine/trimethoprim*
J01FA/*QJ01FA*	Macrolides	Erythromycin, *spiramycin* , roxithromycin, clarithromycin, azithromycin, *tylosin* , *tilmicosin, acetylisovaleryltylosin, tulathromycin*
J01FF/*QJ01FF*	Lincosamides	Clindamycin, *lincomycin*
J01FG/*QJ01XX*	Streptogramins	*(Virginiamycin)* c)
J01G/A07AA/*QJ01G/QA07AA* d)	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* d)	Polypeptides (incl. polymyxins)	Colistin, *(bacitracin)*
J01XC	Steroid antibacterials	Fusidic acid
J01XD/P01AB/*QJ01XD* d)	Imidazole derivatives	Metronidazole
J01XE/*QJ01XE*	Nitrofurane derivatives	Nitrofurantoin
J01XX/*QJ01XX/QJ01FF*	Other antibacterials	*Spectinomycin* , methenamine, linezolid, daptomycin b)
QJ01XX9	Pleuromutilins	*Tiamulin, valnemulin*
QP51AH	Pyranes and hydropyranes (ionophores)	*(Monensin, salinomycin)*
Not in ATCvet	Oligosaccharides	*(Avilamycin)*
Not in ATCvet	Flavofosfolipols	*(Flavomycin)*

a) Antibiotics for intrammamary use in animals are included. Antibiotics only used topically in humans or in animals are not included
b) Tigecycline and daptomycin were not yet registered for use in Denmark
c) Pristinamycin and quinupristin/dalfopristin (for humans) are not used in Denmark
d) Although intestinal antiinfectives (A07AA) and nitroimidazole derivatives for protozoal diseases (P01AB) are used to treat human patients in Denmark, their consumption is not reported by DANMAP

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 taken at the slaughter plants is proportional to the number of animals slaughtered at each plant per year. Each sample represents one herd or flock. Samples are collected once a month (once weekly for broilers) in the period January-November. The broiler, cattle and pig slaughter plants included in the surveillance programme account for 95%, 90% and 95%, respectively, of the total number of animals slaughtered in Denmark per year. Accordingly, the bacterial isolates may be regarded as representing a stratified random sample of the respective populations, and the observed prevalence of resistant isolates provides an estimate of the true occurrence in the populations.

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 Swine Diseases, Danish Meat Association, Kjellerup. Accordingly, the programme achieves nationwide coverage for these pathogens.

Isolates from food

All food samples were collected at wholesale and retail outlets by the Regional Veterinary and Food Control Authorities (RFCA) during the course of routine inspection carried out by the authorities, or on request from the Danish Veterinary and Food Administration (DVFA) for the DANMAP surveillance programme. The collected material consisted of Danish and imported foods. The collection of food samples for isolation of indicator bacteria (enterococci and *E. coli*) was planned by the DFVF and coordinated by DVFA. The food samples were collected according to the guidelines for microbiological examination of foods from the DVFA [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 on-going surveillance comprising of approximately 200 stool samples per year was initiated in 2002. The subjects for participation in the surveillance were selected through the Danish Civil Registry system (CPR) which is a continuously updated register of all residents in Denmark. In total, 644 individuals were invited to participate in the study in 2005. 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 *Staphylocoocus 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 *Neisseria* and Streptococci 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 22-25 g material in a 1:10 dilution with buffered peptone water (BPW) and incubated 16-20 hours at 37°C. A plate with Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium was inoculated with 0.1 ml of BPW deposited on the agar as 3 drops and in addition for cattle samples 1.0 ml BPW was inoculated in 9 ml selinite cystein broth. After enrichment overnight at 41.5°C material from MSRV swarming zones and 0.01 ml broth were inoculated onto Brilliant Green Agar. Overnight incubation at 37°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 (samples from pigs and poultry) or by selective enrichment (samples from cattle). The selective agar (mCCD) was incubated in micro-aerophilic atmosphere for 1-5 days at 42°C. Selective enrichment was done by inoculation of Preston broth at a ratio of 1:10, followed by incubation in microaerophilic atmosphere for 24 h at 42°C. Ten µl of the enrichment culture was inoculated onto mCCD agar and incubated 1-5 days at 42°C. Campylobacter-like colonies were identified by their catalase activity and by their ability to hydrolyse hippurate and indoxyl acetate. For isolates from cattle and pigs, oxidase activity was also tested.

***Escherichia coli* from healthy animals (indicator *E. coli*).** The material was inoculated directly onto Drigalski agar and incubated at 37°C overnight. 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 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 sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified by the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose.

Enterococci from broilers 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

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

***Campylobacter* spp.** was isolated according to the guidelines for microbiological examination of foods from the Danish Veterinary and Food Administration. [NMKL No. 119, 2nd ed., 1990] At the DFVF, isolates were identified 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.

Indicator bacteria were isolated by the RVFCA. Subsequently, due to outsourcing, the isolates were sent to a private Danish laboratory (Eurofins), where species identity and MIC-determinations were performed. One isolate per species per food sample was tested for antimicrobial susceptibility:

Indicator *E. coli* was isolated by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulfphate-broth, which was incubated overnight at 44°C, and subsequently streaked onto violet red bile agar and incubated for 24h at 44°C. Presumptive *E. coli* were identified as *E. coli* using API 20E test (BioMérieux, France).

Enterococci was isolated by adding 5 g of the sample to 45 ml of azide dextrose broth, which was incubated overnight at 44°C, and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours the plates were examined for growth, and colonies typical of *E. faecium* and *E. faecalis*, respectively, were sub-cultured on blood agar. Species identification was performed by PCR according to Dutka-Malen S et al. *J. Clin.Microbiol*., 1995; **33**:24-27.

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

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

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

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

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

Susceptibility testing

Isolates of animal origin and *Salmonella* spp. and *Campylobacter* spp. isolated from food, were susceptibility tested at DFVF, whereas indicator *E. coli and Enterococcus* spp. isolated from food were susceptibility tested by a private Danish laboratory (Eurofins). *Salmonella* spp., *Campylobacter* spp., indicator *E. coli and Enterococcus* spp. isolates of human origin were susceptibility tested at SSI.

All antimicrobial susceptibility testing of *Salmonella* spp., *Campylobacter* spp., indicator *E. coli, Enterococcus* spp. and *Staphylococcus hyicus* was

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

DANMAP 2005

Antimicrobial agent	*E. coli / Salmonella*		*Staphylococcus hyicus*		Enterococci		*Campylobacter*	
	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range	Breakpoints µg/ml	Test range
Amoxicillin/clavulanic acid a)	32	2-32						
Ampicillin	32	1-32			16	2-64		
Apramycin	32	4-32						
Avilamycin					16	2-16		
Cefpodoxime b)	2	0.125-4						
Ceftiofur	8	0.5-8	8	0.125-16				
Cephalothin	32	4-32						
Chloramphenicol	32	2-64	32	2-64	32	2-64	32	2-32
Ciprofloxacin c)	0.125 / 4	0.03-4	4	0.125-8			4	0.03-4
Colistin	16	4-16						
Daptomycin b)					8	0.12-16		
Erythromycin			8	0.125-16	8	0.5-32	32	0.5-32
Flavomycin					16	4-32		
Florfenicol	32	2-64	32	1-64	32	4-32		
Gentamicin	8	1-32			1,024	128-2,048	16	0.125-16
Kanamycin					2,048	128-2,048		
Linezolid					8	1-8		
Nalidixic acid	32	8-64					64	2-64
Neomycin	16	2-32						
Penicillin			0.25	0.06-16				
Salinomycin					16	2-16		
Spectinomycin	128	16-128	128	8-256				
Streptomycin	32	4-64	32	2-128	2,048	128-2,048	16	2-16
Sulfonamide	512	64-1,024	512	8-512				
Quinupristin/dalfopristin d)					4	0.5-16		
Tetracycline	16	2-32	16	0.5-32	16	1-32	16	0.25-16
Tiamulin			32	0.25-32				
Tigecycline b)					0.5	0.015-2		
Trimethoprim	16	4-32	16	1-32				
Vancomycin					32	2-32		

a) Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1
b) Only food and animal isolates
c) >= 0.125 µg/ml was the ciprofloxacin breakpoint applied for *Salmonella* and indicator *E. coli* isolates
d) Trade name Synercid

performed with a commercially available MIC technique using dehydrated antimicrobials in microtitre wells (Sensititre, Trek Diagnostic Systems Ltd., UK). The wells were inoculated and incubated according to the CLSI guidelines (formerly NCCLS). The MIC was defined as the lowest concentration of antimicrobial with no visible growth. The breakpoints used are shown in Table A2.

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

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

Other human isolates

***Salmonella* spp.** Besides susceptibility testing using Sensititre, *Salmonella* spp. isolates were screened for mecillinam and fosfomycin resistance using a 33 mg mecillinam tablet and a 70+40 mg fosfomycin tablet, respectively, (Neo-Sensitabs®, A/S Rosco) on Mueller-Hinton agar (SSI Diagnostika). The breakpoints used are those defined by the CLSI.

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

***Streptococcus pneumoniae*.** The *Neisseria* and Streptococci Reference laboratory at SSI screens for

penicillin-resistant *S. pneumoniae* using a 1 mg oxacillin disk (Oxoid, Greve, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant *S. pneumoniae* using a 78 g erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika). Penicillin and erythromycin MIC's are determined using the E-test (AB Biodisk, Solna, Sweden) on Danish Blood Agar (Resistensplade, SSI Diagnostika). The breakpoints used are those defined by the CLSI.

Invasive *Streptococcus pyogenes.* The *Neisseria* and Streptococci Reference laboratory at SSI screens for penicillin-resistant *S. pyogenes* using a 1 mg oxacillin disk (Oxoid, Greve, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant *S. pyogenes* using a 78 mg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika). Erythromycin resistant *S. pyogenes* are tested with 15 mg erythromycin disk (Oxoid) and 15 mg clindamycin disk (Oxoid, Greve, Denmark) on Danish Blood Agar (Resistensplade, SSI Diagnostika). Erythromycin MIC's are determined using the E-test (AB Biodisk) 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 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 laboratories serving Ribe and Vejle counties used the above described method for testing isolates of *E. coli* and *Streptococcus pyogenes*. However, in Ribe county, March 2005, a shift to Müeller-Hinton II agar (SSI Diagnostika) was made when testing *E. coli*. When testing isolates of coagulase-negative staphylococci Ribe and Vejle counties 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 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).

Fluoroquinolone breakpoint

The current Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) breakpoint for resistance to the fluoroquinolone ciprofloxacin is ≥4 mg/ml. Because of compelling evidence that the treatment efficacy of fluoroquinolones is reduced in humans infected with strains of *Salmonella enterica* with decreased susceptibility to fluoroquinolones (MIC values ≥0.125 mg/ml), it has been recommended that for *Salmonella* a breakpoint of ≥0.125 mg/ml for fluoroquinolones should be used [Aarestrup *et al.* 2003. Antimicrob. Agents Chemother. 47: 827-9]. Since 2004, a breakpoint of ≥0.125 mg/ml is used in DANMAP for both *Salmonella* spp. and indicator *E. coli.*

Gentamicin and apramycin breakpoints

Since 2004, the *E. coli* and *Salmonella* spp. breakpoints for resistance to gentamicin and apramycin has been changed from ≥16 to ≥8 mg/ml and from ≥16 to ≥32 mg/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

Table A3. Results of performance testing (Correct result/number of tests performed) among laboratories participating in DANMAP 2005, Denmark

DANMAP 2005

Antimicrobial agent	*E. coli*		*Salmonella* spp.		*Enterococcus* spp.		Campylobacter	
	S + I [a]	R[b]	S + I [a]	R[b]	S + I [a]	R[b]	S + I [a]	R[b]
Penicillin	-	-	-	-	-	-	-	-
Ampicillin	8/8	12/12	6/6	9/9	15/15	-	-	-
Amoxicillin/clavulanic acid	20/20	-	12/12	3/3	-	-	-	-
Cephalothin	16/16	4/4	9/9	6/6	-	-	-	-
Ceftiofur	16/16	4/4	9/9	6/6	-	-	-	-
Erythromycin	-	-	-	-	6/6	9/9	27/27	3/3
Tetracycline	8/8	12/12	9/9	6/6	-	15/15	29/30	-
Tigecycline	-	-	-	-	15/15	-	-	-
Chloramfenicol	20/20	-	9/9	6/6	15/15	-	30/30	-
Vancomycin	-	-	-	-	15/15	-	-	-
Daptomycin	-	-	-	-	15/15	-	-	-
Linezolid	-	-	-	-	15/15	-	-	-
Quinopristin/dalfopristin	-	-	-	-	1/6[c]	9/9	-	-
Nalidixic acid	16/16	4/4	9/9	6/6	-	-	27/27	3/3
Ciprofloxacin	20/20	-	9/9	6/6	15/15	-	27/27	3/3
Neomycin	12/12	8/8	12/12	3/3	-	-	-	-
Streptomycin	-	20/20	3/3	12/12	9/9	6/6	27/27	3/3
Apramycin	12/12	8/8	12/12	3/3	-	-	-	-
Kanamycin	-	-	-	-	9/9	6/6	-	-
Gentamicin	8/8	12/12	9/9	6/6	15/15	-	30/30	-
Spectinomycin	16/16	4/4	9/9	6/6	-	-	-	-
Colistin	20/20	-	15/15	-	-	-	-	-
Sulfamethoxazole	8/8	12/12	6/6	9/9	-	-	-	-
Trimethoprim	12/12	8/8	12/12	3/3	-	-	-	-
Florfenicol	20/20	-	12/12	3/3	15/15	-	-	-
Avilamycin	-	-	-	-	15/15	-	-	-
Flavomycin	-	-	-	-	9/9	6/6	-	-
Salinomycin	-	-	-	-	15/15	-	-	-
Total	232/232	108/108	162/162	93/93	199/204	51/51	197/198	12/12
	100%	100%	100%	100%	97.5%	100%	99.5%	100%

a) S + I: susceptible and intermediate
b) R: resistant
c) See text for further comments

Poultry at DFVF received 5 *E. coli* strains, 5 *Salmonella* spp., 5 *Enterococcus* spp. and 10 *Campylobacter* spp. All antimicrobial susceptibility testing of *Salmonella* spp., *Campylobacter* spp., indicator *E. coli,* and *Enterococcus* spp. was performed with a commercially available MIC technique using dehydrated antimicrobials in microtitre wells (Sensititre, Trek Diagnostic Systems Ltd., UK). All four laboratories inoculated and incubated according to the CLSI guidelines (formerly NCCLS). The *E. coli* strains were tested in all four laboratories, whereas the other three species were tested in three laboratories. A total of 1060 antibiotic-bacterium susceptibility tests were performed and the overall results were 0.99% failures. Some unexpected results were seen for quinupristin/dalfopristin. Two *E. faecium* strains, which were PCR negative for *vat*(D) and *vat*(E) (encoding resistance to streptogramin A), were selected for the performance test. In all three laboratories, *E. faecium* strain No.14 was found resistant (MIC = 4 or 8 µg/ml) and in two of three laboratories, the other *E. faecium* strain (No. 15) was found resistant (MIC = 4 µg/ml) to quinupristin/dalfopristin. These results may indicate a breakpoint-related problem (breakpoint might be too low) or an unknown resistance mechanism. The detailed results are shown in Table A3.

Data handling

Data on animal isolates

The results from the primary examination of samples from slaughterhouses and primary production for the bacteria of interest – positive as well as negative findings – and of the susceptibility testing were stored in an Oracle Database 8i Enterprise Edition®. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant breakpoint. Each isolate was identified by the bacterial species, including subtype as applicable and by the date of sampling and the species of animal. Information on the farm of origin was also recorded. All handling and evaluation of results was carried out using SAS®Software, 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 database administrated by the Danish Veterinary and Food Administration. For each bacterial isolate information is available on the type of food sample, bacterial species, date of examination of the sample, the RFCA that collected and processed the sample, and an identification number, which makes it possible to obtain further information about the isolate from the Authority. Furthermore, information about the country of origin was recorded whenever possible.

Data on human isolates

***Salmonella* spp. and *Campylobacter* spp.** Data on *Salmonella* spp. and *Campylobacter* spp. infections were exported from the Danish Registry of Enteric Pathogens (Microsoft®Access) maintained by the Unit of Gastrointestinal Infections 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*.** For MRSA, data on the characteristics of the MRSA isolates and the clinical/ epidemiological information were exported from the Danish MRSA registry (Microsoft® Excel) maintained by the Staphylococcus Laboratory at SSI. In this database, patients are only registered the first time they are diagnosed with MRSA, regardless of whether it was colonisation or infection. MRSA cases were classified as active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital (HA-MRSA) or infection diagnosed outside hospitals (community onset). Cases of community onset MRSA infection were further classified according to risk factors in the discharge summary as: community onset – no risk identified (CO-NR), community onset - community risk identified (CO-CR), community onset - health care risk identified within the last 12 months (CO-HCA).

Streptococcus pneumoniae. Data on susceptibility testing of *Streptococcus pneumoniae* isolates are stored as MIC's in a Microsoft® Access database at the *Neisseria* and Streptococci Reference laboratory at 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 last 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 Fishers exact test (2-tailed) was applied when appropriate. *P*-values were reported to the first significant figure except *P*-values smaller than 0.0001, these were reported as $P<0.0001$.

Appendix 2

DANMAP publications

DANMAP publications

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.

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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.

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2004

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Summary research reports

Report 3

Occurrence of *E. coli* and *S.* Typhimurium carrying Extended-Spectrum Beta-Lactamases (ESBLs) among food production animals and food products in Denmark

Escherichia coli and *Salmonella* Typhimurium are common commensals of the intestinal tract of production animals, but can also be important pathogens to both animals and humans. *Enterobacteriaceae* resistant to oxyiminocephalosporins due to the production of extended-spectrum beta-lactamases (ESBLs) have emerged worldwide and a number of different ESBL genes such as the bla_{SHV}, bla_{TEM}, bla_{CTX} and bla_{CMY} have been identified. These genes have in several cases been shown to encode ESBL resistance in bacteria of both animal and human origin.

The first ESBL-producing bacterium from food animals in Denmark was found in August 2003. This was a bla_{CMY-2}-containing *Salmonella* Heidelberg isolate obtained from the intestine of a boar imported from Canada [Aarestrup *et al*. 2004. Antimicrob. Agents Chemother. 48: 1916–7].
In October of the same year the Danish Institute for Food and Veterinary Research (DFVF) received three *Salmonella* Virchow isolates found in quails imported from France, which were found to contain $bla_{CTX-M-9}$ [Aarestrup *et al*. 2005. Emerg. Infect. Dis. 11:1984–5].

In 2004, the first ESBL resistant *E. coli* originating from a food product sold in Denmark was detected at DFVF as part of the DANMAP surveillance programme. The *E. coli* isolate was obtained from a sample of sliced beef (Goulash) imported from Germany and was also resistant to quinolones [nalidixic acid (MIC >128 mg/L) and ciprofloxacin (MIC >4 mg/L)], sulfonamides (MIC >1024 mg/L), tetracycline (MIC >32 mg/L) and trimethoprim (MIC >32 mg/L). Examination of the genetic background to the ESBL phenotype revealed the presence of the $bla_{TEM-52b}$ gene [Jensen *et al*. 2006. J. Antimicrob. Chemother. 57: 793–4]. A sampling of poultry meat sold in retail stores revealed the first bla_{CMY-2} from a food product sold in Denmark. bla_{CMY-2} was found in *E. coli* isolated from a poussin imported from the UK in 2005. The bla_{CMY-2} gene was present on a large plasmid capable of horizontal gene transfer to both *E. coli* and *S.* Typhimurium recipients [Agersø *et al.* 2006. ICEID].
In Denmark, ESBL-producing isolates of *E. coli* or *S.* Typhimurium have not previously been isolated from animals in primary food production. However, in July 2005, two *E. coli* isolates from infections in pigs showing resistance to cephalosporins were identified during the routine diagnostic testing. These isolates were from two different farms, where one was from a case of diarrhoea (sero-typed as O149) and the other one from septicaemia (sero-typed as O20). Both carried the $bla_{CTX-M-1}$ gene located on large transferable plasmids. *E. coli* carrying the $bla_{CTX-M-1}$ gene have also been reported from both animals and humans in other countries. Thus, there are several international reservoirs from where the resistance could have spread to these two Danish farms, but the precise origin of the $bla_{CTX-M-1}$ genes found here has not been determined [Aarestrup *et al*. 2006. J. Antimicrob. Chemother. Published online].

In conclusion, ESBL resistance among bacteria originating from production animals as well as animal products in Denmark are still rare. Until now, most of the ESBL were originated from imported animals or imported meat products. Therefore, the global spread of ESBL-mediated resistance is one of the emerging problems currently faced by Denmark and studies determining the way in which the spread occurs are urgently needed.

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Correlation between apramycin and gentamicin use and gentamicin resistance in *Escherichia coli* from pigs

Gentamicin is classified as a critically important drug in human medicine. Therefore, spread of gentamicin resistant *Escherichia coli* strains to humans is of great concern.

In Denmark, data on veterinary antimicrobial consumption (VetStat) and data on occurrence of antimicrobial resistance (DANMAP) makes it possible to analyse the correlation between antimicrobial consumption and occurrence of resistance at farm and animal species level, and resistance on subsequent levels in the in the animal-human food chain. Recently, the effect of apramycin and gentamicin use on occurrence of resistance in porcine isolates of *E. coli* was studied [Jensen *et al.* 2006, J. Antimicrob**.** Chemother. Published online].

After the marketing of apramycin in Denmark in 1998, consumption in pigs increased rapidly (Figure 1). As a consequence, the use of extemporaneously prepared gentamicin products decreased during 1999-2004, and less than 3 kg gentamicin was used annually in approved products.

Before the approval of apramycin in Denmark, apramycin resistance in porcine *E. coli* was rare. During 1999 to 2004, 1096 isolates of *E. coli* O149 from diagnostic submission were isolated from pigs and tested for apramycin and gentamicin resistance. In 2000, apramycin resistance in *E. coli* from pigs was found in 2.9% of clinical isolates and continued to increase until 2002, reaching 15.3% (Figure 1). The annual number of isolates resistant to gentamicin (not apramycin) has remained at a low level from 1998-2004.

On the national level, logistic regression showed a significant effect of time since approval of apramycin and amount of apramycin consumed yearly on apramycin/gentamicin cross-resistance among *E. coli* O149. At farm level, a significant correlation between apramycin use (+/-) within one year prior to sampling and apramycin/ gentamicin cross-resistant *E. coli* O149 in submissions was found.

In 2002, the first gentamicin resistant indicator *E. coli* strain was isolated in the DANMAP programme. In 2003 and 2004, three and seven resistant indicator apramycin/gentamicin cross-resistant *E. coli* were isolated, respectively. At farm level, the finding of resistant indicator *E. coli* at slaughter was significantly related to use of apramycin in weaners, while neither apramycin nor gentamicin had been prescribed for finishers within the past year before isolation.

The gene *aac(3)-IV* is the only identified gene causing enzymatic cross-resistance between gentamicin and apramycin. In our study, the *aac(3)-IV* gene was detected in all tested cross resistant isolates including 25 clinical isolates of *E. coli* and nine indicator *E. coli*.

The findings plead for a prudent use of antimicrobials in pigs, considering the human health risk associated with apramycin/gentamicin cross-resistance. The results in DANMAP 2003-2005 suggest a decreasing trend in occurrence of apramycin/ gentamicin resistant clinical *E. coli*, most likely as a result of the decreasing consumption since 2001.

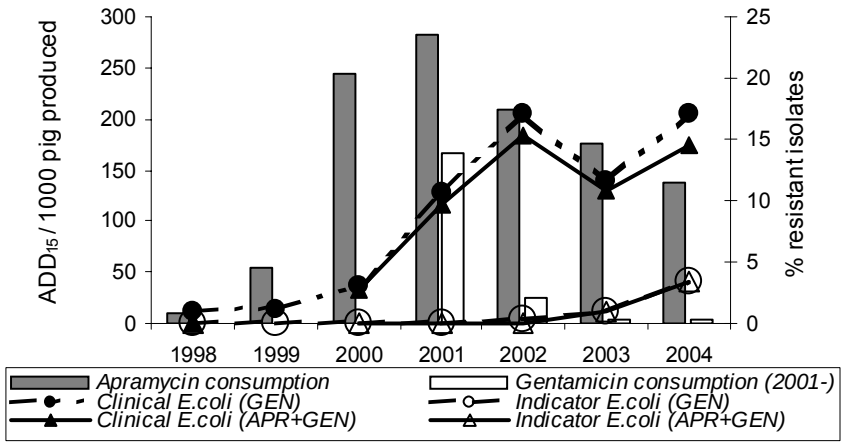

Figure 1: Consumption of apramycin and gentamicin in pigs and occurrence of resistance in porcine isolates of E. coli, *Denmark 1999-2004.*
Consumption of gentamicin in pigs before 2001 was unknown. GEN= gentamicin resistant. APR+GEN=apramycin/gentamycin cross-resistant. ADD15 = Approved Average Daily Dose for 15 kg pig

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Report 5

Antibiotic Consumption and Attitudes Towards Antibiotics in Danish Households

Several studies have suggested that there is considerable use of antibiotics without seeking professional medical advice in some European countries. However, information on self-medication with these drugs is limited. The first European wide study was conducted in 2003 in 19 European countries to address this gap. The survey was coordinated by the University Medical Center in Groningen, the Netherlands, and funded by a grant of Directorate General SANCO of the European Commission (Agreement SPC2002333) [Grigoryan *et al.* 2006. Emerg. Infect. Dis. 12: 452-459].

In Denmark, the survey was carried out by the National Center for Antimicrobials and Infection Control at the Statens Serum Institut in collaboration with the Institute of Public Health at the University of Southern Denmark. In June 2003, a questionnaire was sent to 3000 randomly chosen persons aged 18 years or above, half in urban and half in rural areas of Greater Copenhagen and the counties of Frederiksborg, Funen and Aarhus. The selection was made by the Danish Civil Registration System (Det Centrale Personregister) and the study was approved by the Danish Data Protection Agency (Datatilsynet). The questionnaire was returned by 1959 persons (65% response rate following a reminder). After exclusion of returned, but uncompleted questionnaires, due to reasons such as sickness or death, 1881 questionnaires were available for analysis.

In Denmark, the large majority of the antibiotics were obtained with a medical prescription and less than 1% of the respondents actually self-medicated themselves with antibiotics. These results contrasted greatly with those observed in certain European countries where antibiotics can still be obtained from pharmacies without a prescription [Grigoryan *et al.* 2006. Emerg. Infect. Dis. 12: 452-459]. Eight percent of the Danish respondents had left-over antibiotics at home and 13% declared that they would take antibiotics without a prescription if this was possible. In Denmark, it is nearly impossible to obtain antibiotics at a pharmacy without a prescription. The population at risk of self medication is therefore represented by persons having left-over antibiotics at home and intend to use them for self-medication. In this study, this corresponded to only a very small fraction of the Danish respondents (Figure 1, overlap between the stripped disk and the checkered disk).

A more detailed analysis of the data pertaining to Danish households was recently performed [Muscat *et al.* 2006. Scand. J. Infect. Dis. published online]. Danish respondents who had been prescribed antibiotics during the previous 12 months more often intended to use antibiotics for self-medication (21%) than those who did not (12%) ($p<0.001$). Additionally, respondents who had reached high or intermediate education levels more often intended to self-medicate with antibiotics (18%) compared to those who attained a lower education level (10%) ($p<0.001$).

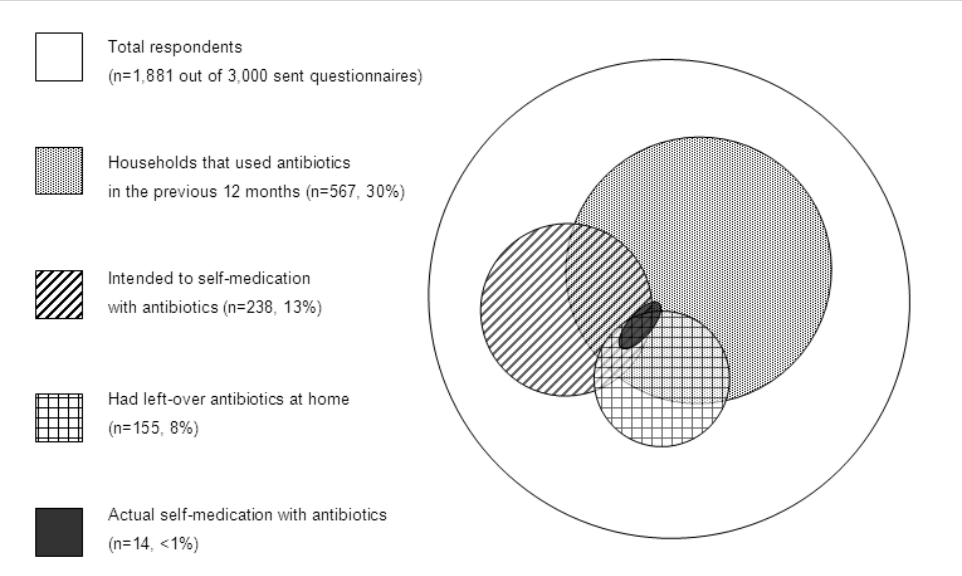

Figure 1. Antibiotic consumption and attitudes towards antibiotics in Danish households, 2003.

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Report 6

In vivo transfer of the *vanA* resistance gene from an *Enterococcus faecium* strain of animal origin to a human *E. faecium* in the intestine of human volunteers

In the beginning of the 1990s, the presence of vancomycin-resistant enterococci (VRE) was reported in the environment and in production animals. It was later shown that the growth promoter avoparcin, a glycopeptide very similar to vancomycin, selected for VRE in production animals [Bates *et al.* 1993. Lancet 342: 490–491; Klare *et al.* 1995. Microb. Drug Resist.1: 265–272]. Although transient colonization by vancomycin-resistant enterococci of animal origin has been identified in the intestine of humans, little is known about whether transfer of the *vanA* gene could occur in the human intestine.
The present study was performed to determine whether resistance genes from an *E. faecium* isolate of animal origin could be transferred to a human *E. faecium* isolate in the intestines of human volunteers without any selective antimicrobial pressure [Lester *et al.* 2006. Antimicrob. Agents Chemother. 50: 596-9].

Six volunteers each ingested 250 ml of a 10^9-CFU suspension of a vancomycin-susceptible *E. faecium* recipient of human origin. Three hours later, they each ingested 250 ml of a 10^7-CFU suspension of a vancomycin-resistant *Enterococcus faecium* of chicken origin. All suspensions were prepared in whole milk. Stool samples were collected from the volunteers 48 hours prior to ingestion of the *E. faecium* suspensions, on a daily basis for the following 7 days (days 1-7), and at days 14 and 35. All stool samples were homogenized in 0.9% saline and plated on antibiotic-supplemented Enterococcosel agar plates. The study protocol was approved by the scientific ethics committee for Copenhagen and Frederiksberg municipalities [(KF)01-153/03)].

Transconjugants were recovered from three of the six volunteers. In one volunteer, not only vancomycin resistance was transferred, but also quinupristin/dalfopristin resistance.
This study shows that transfer of the *vanA* gene from an *E. faecium* isolate of animal origin to an *E. faecium* isolate of human origin can occur in the human intestine.
At present, several years after the EU ban on avoparcin, VRE can still be found in poultry and in healthy humans in Denmark [Hammerum *et al.* 2004. J. Antimicrob. Chemother. 53: 547–549; Heuer *et al*. 2002. Microb. Drug Resist. 8: 133–138]. Before the ban, VRE were found in large amounts in poultry and pigs, and intestinal colonization by VRE after eating chicken or pork was probably frequent among humans. Our study suggests that transfer of the *vanA* gene probably did take place in the intestines of humans before the EU ban took place.

Our study suggests that transient intestinal colonization by enterococci carrying mobile elements with resistance genes represents a risk for spread of resistance genes to other enterococci that are part of the human indigenous flora and could be responsible for infections in certain groups of patients, e.g. immuno-compromised patients.

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Report 7

Identification of Tn*5397*-like and Tn*916*-like transposons and diversity of the tetracycline resistance gene *tet*(M) in enterococci from humans, pigs and poultry

In enterococci and other species, the tetracycline resistance gene *tet*(M) has been found associated with conjugative transposons related to the Tn*916*/Tn*1545* family [Rice. 1998. Antimicrob Agents Chemother 42: 1871-7]. Other conjugative transposons like Tn*5397* from *Clostridium difficile* have been found to harbour *tet*(M) as well [Roberts *et al.* 2001. Microbiology. 147: 1243-51]. Tn*5397* is related to Tn*916*; the central regions that are involved in conjugation of these two elements are very similar. However, Tn*5397* can be distinguished from Tn*916* in at least two important characteristics: first, Tn*5397* contains a group II intron inserted into a gene almost identical to *orf14* from Tn*916,* and second, the DNA sequences at the ends of Tn*5397* are completely different from those of Tn*916* [Roberts *et al.* 2001. Microbiology. 147: 1243-51]. Instead of having the *int* and *xis* genes that have been shown to be required for integration and excision of Tn*916*, Tn*5397* contains the gene *tndX*, which encodes a putative protein not related to Int or Xis but belonging to the large resolvase subgroup of site-specific recombinases [Roberts *et al.* 2001. Microbiology. 147: 1243-51; Wang *et al.* 2000. J Bacteriol 182: 3775-83].
This study was conducted to characterize and determine the occurrence of mobile elements associated with *tet*(M) in *E. faecium* and *E. faecalis* isolated from humans, pigs and broilers in Denmark, and to see if there was a correlation between the presence of certain mobile elements with the diversity of *tet*(M).

A total of 76 isolates were screened for Tn*916*/Tn*1545*-like and Tn*5397*-like transposons by PCR. *tet*(M) was sequenced in fifteen of the isolates and compared to *tet*(M) sequences submitted to GenBank (phylogenetic analysis and signs of recombination). Plasmids were extracted; filter mating experiments performed and Tn*5397*-like transposons were further characterized in selected isolates.
One *E. faecium* isolate from broilers with a Tn*5397*-like element was partially sequenced. The sequence including *tet*(M) and *tndX* gene (5070bp) had 97% identity to the corresponding sequence of *C. difficile* (GenBank no. AF333235, position 14631-19700). Tn*5397* from this isolate was transferred to *E. faecium* (7×10^{-8} transconjugant/donor) but not to *E. faecalis* JH2-2 (detection limit $>1.1 \times 10^{-8}$ transconjugant/donor).
In eight of thirteen isolates of *E. faecium* from broilers, *tet*(M) was present on Tn*5397*-like transposons, whereas *tet*(M) was predominantly associated with Tn*916*/Tn*1545*-like transposons in *E. faecium* from pigs and humans, as well as in *E. faecalis* from humans, pigs and broilers (50/63).
Phylogenetic analysis divided the *tet*(M) genes into three major subgroups. Subgroup I consisted of *tet*(M) from *Clostridium difficile* and *E. faecium* associated with Tn*5397*-like elements and also two *E. faecium* isolates from pig and broiler, respectively, which had *tet*(M) present on 80 kb plasmids branched separately in subgroup I. Subgroup II contained *tet*(M) located on Tn*916*/Tn*1545* family transposons and subgroup III consisted of *tet*(M) associated with composite elements containing several resistance genes. We found evidence of recombination both within and between these groups. Moreover, we identified an *E. faecium* isolate with both Tn*916*/Tn*1545*-like and Tn*5397*-like elements [Agersø *et al.* 2006. J. Antimicrob. Chemother. 57:832-9].

This study showed, that enterococci contain diverse *tet*(M) genes present on different mobile elements, which suggest that enterococci may play an important role in the evolution and horizontal spread of mobile elements carrying *tet*(M). This is the first report of Tn*5397*-like elements in enterococci [Agersø *et al.* 2006. J. Antimicrob. Chemother. 57:832-9].

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Detection of *sul1*, *sul2* and *sul3* in sulfonamide resistant *Escherichia coli* isolates obtained from healthy humans, pork and pigs in Denmark

In 2003, antibiotic treatment of pigs accounted for almost 80% of the total amount of antibiotics administered to food animals in Denmark. The combination of sulfonamide and trimethroprim was the second most used antimicrobial therapy for sows and piglets in 2003. In Denmark, sulfonamide or trimethroprim are used for treatment of the majority of uncomplicated urinary tract infections in humans. Potential transfer of sulfonamide resistant *Escherichia coli* from animals, directly or via handling of raw meat, to humans is therefore undesirable. Sulfonamide resistance is often encoded by *sul1* and *sul2* in *Enterobacteriaceae*. Recently a third gene, *sul3*, was found to encode for sulfonamide resistance.

Occurrence of sulfonamide resistance was investigated in 998 *Escherichia coli* isolates, obtained from pig faeces collected at slaughter, Danish pork collected at retail outlets and faeces from healthy persons in Denmark collected as a part of DANMAP in 2002 and 2003 [Hammerum *et al.* 2006. Int. J. Food Microbiol.106: 235-7]. Overall, 18% (n = 35), 20% (n = 38) and 26% (n = 161) of the *E. coli* isolates obtained from humans, pork and pigs, respectively, were resistant to sulfonamide. All sulfonamide resistant *E. coli* isolates were investigated for the presence of *sul1*, *sul2, sul3* and *intI1* genes by PCR. The *sul1* gene was detected in 40% (n=14), 29% (n=11) and 55% (n=88) of the sulfonamide resistant isolates from humans, pork and pigs, respectively. The *sul2* gene was detected in 80% (n=28), 76% (n=29) and 50% (n=81) of the sulfonamide resistant isolates from humans, pork and pigs, respectively. None of the human sulfonamide resistant isolates were PCR-positive for *sul3,* whereas *sul3* was present in 5% of the pork isolates and 11% of the pig isolates. Of the 113 *sul1* positive isolates, 97 carried the integron-associated integrase gene *intI1*. All 20 *sul3* positive isolates were positive for *intI1,* and in 12 of these isolates *sul3* was the only sulfonamide resistance gene detected.

In the present study, none of the healthy humans with sulfonamide resistant *E. coli* had received sulfonamide treatment, but two of them had received another antibiotic therapy within one month of sampling. A recent study has demonstrated a close similarity between certain food borne and human isolates of extraintestinal pathogenic *E. coli,* which suggests a possible acquisition through food products of *E. coli* causing urinary tract infections [Johnson *et al.* 2005. J. Infect. Dis.191: 1040-9]. The presence of sulfonamide resistant *E. coli* in faeces from pigs, in pork and in the gut flora of healthy humans is therefore of concern. The origin of *sul1* and *sul2* found in isolates from healthy humans is speculative, but their spread from pigs to humans via the food chain is possible.

Table 1. Prevalences of sul1, sul2 *and* sul3 *genes in sulfonamide resistant* Escherichia coli *isolates obtained from healthy humans, pork and pigs*

Origin	No. of isolates tested	No. (%) of PCR-positive isolates:			No. (%) of strains with more than one sulfonamide resistance gene
		sul1	*sul2*	*sul3*	
Humans	35	14 (40%)	28 (80%)	0	7 (20%)
Pork	38	11 (29%)	29 (76%)	2 (5%)	4 (11%)
Pigs	161	88 (55%)	81 (50%)	18 (11%)	25 (16%)

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Report 9

Determination of the genetic background for resistance to olaquindox, a Gram-negative growth promoter

Concern has often been raised, that the use of antimicrobial compounds as growth promoting animal food additives would lead to increased resistance to therapeutic antimicrobials. Among the previously used antimicrobial growth promoters only two, olaquindox and carbadox, had an effect on Gram-negative bacteria. Olaquindox (OQX) was used extensively as growth promoter in pig feed but was banned in 1998 due to carcinogenic effects. Until recently a genetically encoded resistance to this drug had not been identified. Hansen *et (al.)* isolated and identified a conjugative plasmid (pOLA52) encoding resistance to OQX [Hansen *et* al. 2004. Antimicrob. Agents Chemother.38: 3332-7, Sørensen *et al.* 2003. Antimicrob. Agents Chemother. 37: 798-9]. The resistance determinant was identified as a multidrug efflux pump of the Resistance Nodulation cell Division (RND) type family. Genes encoding multi drug efflux pumps of the RND family have previously been found on the chromosome of most if not all analyzed Gram-negative bacteria. Here they contribute to the increased resistance to antimicrobials, rendering organisms such as *Pseudomonas aeruginosa*, with several pumps, less sensitive to drug treatment [Poole 2001, J. Mol. Microbiol. Biotechnol. 3: 255-64].

The resistance mechanism is a three-component system consisting of a membrane fusion protein OqxA and a cytoplasmic membrane efflux pump OqxB in combination with an outer membrane channel protein (TolC in *E. coli*). This tripartite system spans the entire double membrane in Gram-negative bacteria where it effectively removes chemicals from the cell cytoplasm. Tauch *et al.* recently published the finding of another conjugative plasmid (pB4) bearing a different RND pump, MexCD, isolated from activated sludge [Tauch *et al,* 2003, Mol Genet Genomics, 268: 570-84]. These reports on plasmid encoded multidrug efflux pumps could indicate an emerging resistance problem.

Efflux pumps are known to interact with a wide range of substrates and the OqxAB pump has been shown to increase resistance of *E. coli* strains not only to olaquindox, but also to several antimicrobials including therapeutic antibiotics such as chloramphenicol and quinolones, quaternary ammonium compounds and detergents such as Sodium Dodecyl Sulphate (SDS) [unpublished data].

A survey of *E. coli* isolates from the DANMAP surveillance from 1995 to 1998, the year of termination of usage of olaquindox, supplemented with a limited number of *E. coli* from Sweden from the same period found that 10 of 556 (1.8%) of the isolates were resistant to olaquindox defined by having a MIC $\geq$ 64 µg/ml to olaquindox [Hansen *et al.* 2005, Microb. Drug Res. 11: 378-2]. In nine of these ten strains, the *oqxA* gene was detected. Sequencing of an internal fragment of *oqxA* from the *oqxA*-positive strains showed no variation, indicating highly conserved *oqxA* genes. All of the *oqxA*-positive strains contained plasmids with origins of replication similar to that of pOLA52. Southern hybridization verified that the *oqxAB* operon was situated on plasmids of different sizes in most resistant strains. Furthermore, horizontal transfer of olaquindox resistance from three olaquindox-resistant isolates confirmed that the *oqxAB* harboring plasmids were conjugative. Also, pOLA52 could be transferred to strains of *Salmonella* and *Klebsiella pneumoniae* [unpublished data].

The prevalence of *oqxAB* in human *E. coli* isolates is currently being investigated. Preliminary studies show that the *oqxAB* resistance determinant is present in all olaquindox resistant *E. coli* isolates of human origin. In addition, we recently have found *oqxAB* in two olaquindox resistant isolates of *Salmonella Dublin*. It remains to be investigated whether the resistance is plasmid borne and could have been transmitted through the food chain.

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