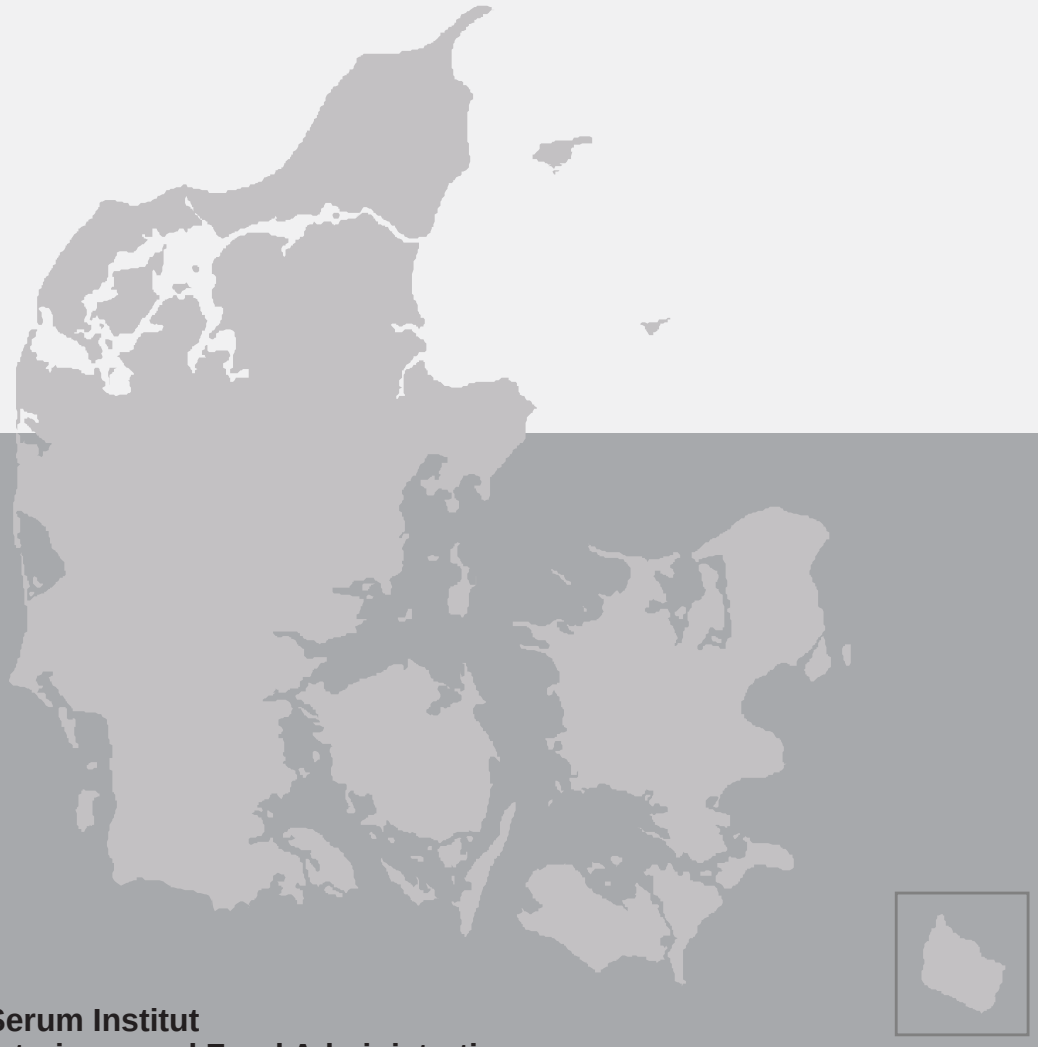
DANMAP 2003

DANMAP 2003 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark



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A similar report from Sweden is available at www.sva.se

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, food and humans. The programme is organised by Statens Serum Institute and the Danish Institute for Food and Veterinary Research (formerly Danish Veterinary Laboratory and the Danish Veterinary and Food Administration). The objectives of DANMAP are:

- To monitor the consumption of antimicrobial agents for food animals and humans
- To monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans
- To study associations between consumption and resistance
- To identify routes of transmission of antimicrobial resistance 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 antimicrobial resistance caused by use of antimicrobials in the respective reservoirs. Zoonotic bacteria are included because they can develop antimicrobial resistance in the animal reservoir and may subsequently lead to treatment failure in humans. Indicator bacteria are included due to their ubiquitous nature in animals, food and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in these reservoirs.

This report describes the annual consumption of antimicrobial agents and the occurrence of antimicrobial resistance in different reservoirs. Trends and comparisons 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*
AGP	Antimicrobial Growth Promoter
ATC	Anatomical Therapeutic Chemical *
CHR	Central Husbandry Register*
CI	Confidence Interval
CPR	Danish Civil Registry
DFVF	Danish Institute for Food and Veterinary Research
DMA	Danish Medicines Agency
DDD	Defined Daily Dose*
DVFA	Danish Veterinary and Food Administration
GAS	Group A *streptococcus*
GI	Gastro Intestinal
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

*** Explanatory notes**

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 sites housing production animals. It contains numerous information concerning ownership, farm size, animal species, age groups, number of animal and production type. The CHR 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 emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (http://www.whocc.no/atcddd/indexdatabase/).

Defined Animal Daily Dose (ADD). This is an estimated average daily dose, 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. In press). The ADD_{kg} is the ADD per kg animal. Consumption calculated in ADD_{kg} allows summation of consumption in 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 under test is not inhibited.

Sammendrag

Forbrug af antibiotika
DANMAP giver en samlet fremstilling af anvendelsen af antibiotika til dyr såvel som til mennesker. Oplysninger om forbrug af receptpligtig medicin til mennesker er blevet indsamlet af Lægemiddelstyrelsen siden begyndelsen af 1990'erne. Lægemiddelstyrelsen har bidraget med data til denne og tidligere DANMAP rapporter. I 2000 blev det landsdækkende register for receptpligtig veterinærmedicin, VetStat, taget i brug. I VetStat registreres oplysninger om forbruget af medicin til dyr på besætningsniveau, herunder forbruget til de enkelte husdyrarter og aldersgrupper, samt forbruget til behandling af kæledyr og heste i dyrlægepraksis.

Antibiotikaforbruget til dyr
Udfasningen af antibiotiske vækstfremmer i Danmark i 1997-1999 medførte en 50% reduktion i det totale antibiotikaforbrug, trods en stigning i forbruget af terapeutisk antibiotika på 68% fra 1998 til 2001. Forbruget til produktionsdyr var uændret i 2002, men steg yderligere 6,5 ton til 101,9 ton i 2003. Næsten 80% af det veterinære forbrug af antibiotika blev anvendt ved behandling af svin.
Stigningen i 2003 kan tilskrives et øget forbrug til behandling af svin, bl.a. omfattende 2,8 ton tetracyklin, 1,5 ton β-lactamase sensitive penicilliner, 1,1 ton penicillin med udvidet spektrum og 1,2 ton tiamulin, mens forbruget af makrolider faldt med 300 kg og forbruget af fluoroquinoloner faldt til 13 kg.
Det samlede forbrug af antibiotika til fravænningsgrise faldt marginalt, medens forbruget til slagtesvin og søer/smågrise steg. Stigningen i forbruget har tilsyneladende ingen sammenhæng med vækstfremmerophøret.

I løbet af 2003 er der sket en kraftig spredning af virussygdommen Post-weaning Multisystemic Wasting Syndrom (PMWS) i den danske svineproduktion. Stigningen i forbruget af tiamulin til søer, tetracyklin til slagtesvin og i mindre grad til fravænningsgrise, ses udelukkende i områder med forekomst af PMWS. Muligvis anvendes tetracyklin empirisk før en endelig diagnose er stillet, medens tiamulin i nogle tilfælde anvendes til at forhindre følgesygdomme hos pattegrise. Stigningen i penicillinforbruget skyldes primært behandling af lidelser i bevægeapperat og hud. Antibiotikaforbruget til behandlingen af mavetarmlidelser hos fravænningsgrise faldt med 2,6% i 2003 (målt i definerede daglige doser, ADD).

Det veterinære forbrug af fluorokinoloner faldt kraftigt fra 183 kg i 2001 til 53 kg i 2003, formentlig som følge af en lovændring i foråret 2002, der havde til formål at begrænse brugen af netop denne gruppe af antibiotika til produktionsdyr. Fluorokinoloner betragtes som særligt vigtige ved behandling af alvorlige infektioner hos mennesker. En anden gruppe af antibiotika der betragtes som særligt vigtig til behandling af infektioner hos mennesker er cephalosporinerne. Det veterinære forbruget af cephalosporiner steg fra 385 kg i 2002 til 461 kg i 2003, som følge af et øget forbrug til kvæg og svin. En relativ stor mængde af det samlede veterinære forbrug af cephalosporin og flourokinoloner blev imidlertid anvendt til kæledyr.

I 2003 blev der i Danmark anvendt 0,5 mg antibiotika/kg kyllingekød produceret og 52 mg antibiotika/kg oksekød produceret (inklusive malkekvæg), medens der i aquakultur blev anvendt 96 mg antibiotika/kg fisk produceret. Tilsvarende blev anvendt 43 mg antibiotika/kg svinekød produceret. I svineproduktionen er behandlingshyppigheden særlig høj i fravænningsgrise (ca. 4-11 uger), idet der anvendes 6,5 ADD/fravænningsgris, sammenlignet med 1,9 ADD/slagtesvin (ca. 11-25 uger).

Antibiotikaforbruget til mennesker
I 2003 blev der brugt 29,5 millioner definerede døgn doser (DDD) eller 15,0 DDD/1000 indbygger-dage antibiotika til systemisk behandling af mennesker i Danmark, hvilket er på niveau med forbruget i 2002. I alt blev der bruget 43,9 ton til systemisk behandling, hvilket er en stigning på 1,3% i forhold til 2002. Hovedparten af denne stigning skyldtes et øget forbrug af β-lactamase sensitive, og β–lactamase resistente penicilliner samt fluoroquinoloner.
Fra 1997 til 2002 har der været et støt stigende forbrug af antibiotika på hospitalerne fra 392 til 514 DDD/1000 sengedage. Denne stigning i antibiotikaforbrug skyldtes primært en 26% stigning i antallet af DDD ordineret fra hospitals-apotekerne (fra henholdsvis 2,3 millioner DDD i 1997 til 2,9 millioner i 2002), mens antallet af sengedage faldt med 6% i sammen periode. Siden 2000 er det totale antal af sengedage på danske hospitaler årligt faldet med 1% eller mindre.
Fra 2002 til 2003, har der været en moderat stigning i forbruget af antibiotika på hospitalerne, fra 514 til 519 DDD/1000 sengedage. Det ser derfor ud til, at den store stigning i anbitiotikaforbrug på hospitalerne der er

blevet observeret siden 1997, er stoppet. Den moderate stigning i forbruget fra 2002 til 2003, skyldes et fald i antallet af sengedage, hvorimod der ikke var nogen ændring i antallet af DDD. Resultatet fra 2003 skal dog tolkes med forsigtighed, idet det er baseret på et foreløbigt antal DDD og et estimeret antal sengedage.

Ordinationen af antibiotika på danske hospitaler kan stadig beskrives som konservativ, men der er sket en stigning i ordinationen af bestemte grupper af antibiotika, herunder cephalosporiner, fluoroquinoloner og kombinationer af penicilliner inkl. β-lactamase inhibitorer og carbapenem. Brugen af disse 4 klasser af antibiotika udgjorde 53 % af stigningen i det totale forbrug fra 1997 til 2003. Dette langsomme, men vedholdende skift imod et øget forbrug af bredspektrede antibiotika på danske hospitaler er bekymrende, og bør overvåges nøje.

Resistens i zoonotiske bakterier

I såvel *Salmonella* Enteritidis isolater fra fjerkræ, som i *Salmonella* Typhimurium isolater fra fjerkræ, svin og kvæg forblev resistensniveauet uændret fra 2002 til 2003. Resistensforekomsten i *S.* Typhimurium isolater fra dyr var i lighed med sidste år, på niveau med resistensforekomsten i isolater fra mennesker.

I 2003 blev der observeret et yderligere fald i antallet af infektioner hos mennesker forårsaget af *Salmonella* og *Campylobacter.* Generelt er resistensniveauet i *S.* Enteritidis isolater fortsat lavt, men blandt isolater fra infektioner erhvervet i Danmark er andelen af nalidixinsyre resistente isolater steget fra 4% i 2002 til 15% i 2003. Forekomsten af resistens overfor nalidixinsyre i isolater af *Campylobacter jejuni, S.* Typhimurium og *S.* Enteritidis var dog signifikant højere ved infektioner erhvervet i udlandet end ved infektioner erhvervet i Danmark.
Ved behandling af mave-tarm infektioner er det vigtigt, at være opmærksom på, hvorvidt infektionen er associeret med udenlandsrejse. Dette skyldes den høje sandsynlighed for forekomst af resistens overfor nalidixinsyre og ciprofloxacin i isolater fra infektioner erhvervet i udlandet.

Resistens i indikatorbakterier

Forekomsten af resistens i enterokokker (*Enterococcus faecium og Enterococcus faecalis*) fra fjerkræ, kvæg og svin forblev uændret fra 2002 til 2003, med undtagelse af et fald i resistens overfor erythromycin blandt *E. faecium* fra svin. Dette er sammenfaldende med en reduktion i makrolid forbruget til svin. Den kraftige stigning i forbruget af tetracyklin og penicillin til svin, har tilsyneladende ikke haft nogen umiddelbar effekt på forekomsten af resistens i enterokokker fra svin.
I modsætning hertil steg forekomsten af resistens i *Eschericia coli* fra svin overfor en række forskellige antibiotika i 2003. Stigningen omfattede resistens overfor ampicillin, sulfonamid, trimethoprim, spectinomycin samt streptomycin, og faldt sammen med stigninger i forbruget af disse antibiotika til behandling af svin. Stigningen i forbruget af tetracyklin afspejles dog ikke umiddelbart i resistensforekomsten i *E. coli.*
Forekomsten af resistens i enterokokker fra mennesker er på nogenlunde samme niveau som forekomsten af resistens i enterokokker fra fødevarer. Dette syntes at stemme overens med antagelsen om, at resistensforekomst i bakterier i fødevare afspejles i resistensforekomst i bakterier hos mennesker.

Resistens i bakterier fra diagnostiske indsendelser fra dyr og mennesker

Forekomsten af resistens overfor penicillin i *Staphylococcus hyicus* isolater fra svin steg fra 54% i 2000 til 84% i 2003. Denne stigning falder sammen med en årlig stigning på 11-13% i forbruget af penicillin til svin i samme tidsrum. Forekomsten af resistens overfor øvrige antibiotika i testpanelet forblev uændret.
I 2003 blev der observeret 204 tilfælde af infektioner med methicillin-resistente *Staphylococcus aureus* (MRSA) hos mennesker i Danmark. Dette var mere end en fordobling i forhold til 2002. Der var ingen forskel på antallet af udenlands-erhvervede MRSA infektioner i forhold til 2002. Derfor må det øgede antal MRSA infektioner være erhvervet i Danmark. Mere end 50 % af MRSA infektioner var erhvervet uden for hospitalerne.
I 2003 blev der i *E. coli* isolater fra infektioner i mennesker observeret resistens overfor almindeligt anvendte antibiotika. Selvom denne resistens-forekomst stadig er lav, bør den overvåges nøje. Der blev i 2003 observeret et lavt niveau af fluoroquinolon-resistens, men et prisfald på fluoroquinoloner i 2003, samt den øgede forekomst af resistens overfor andre antibiotika, kan medføre et øget forbrug af fluroquinoloner og dermed en stigning i forekomsten af fluroquinolon-resistens.
Resistens overfor penicillin og makrolid var fortsat lav i isolater af *Streptococcus pneumoniae* og *Streptococcus pyogenes* i 2003.

Summary

Antimicrobial consumption
DANMAP presents use of antimicrobials in humans and animals. In humans, the use of prescription medicines has been monitored at the level of the individual patient since the early 1990s. In animals, data on all medicines prescribed by veterinarians for use in animals has been registered by the VetStat programme since 2001.

Antimicrobial consumption in animals
In Denmark, the use of antimicrobial growth promoters was terminated during 1997-1999, leading to a 50% reduction in the total consumption of antimicrobials, despite a 68% increase in use of therapeutic antimicrobials from 1998 to 2001. The total antimicrobial consumption in animal production was unchanged from 2001 to 2002. In 2003 the consumption increased by 6.5 tonnes to 101.9. Almost 80% of the veterinary consumption is used in the extensive Danish pig production.
The increase in 2003 was caused by increased consumption in pigs including 2.8 tonnes of tetracycline, 1.5 tonnes of β-lactamase sensitive penicillins, 1.1 tonnes of penicillins with extended spectrum, and 1.2 tonnes of tiamulin, while the consumption of other macrolides decreased by 300 kg and the use of fluoroquinolones decreased to a total of 13 kg. In pigs, the total consumption of antimicrobials for weaners was slightly reduced, whereas the consumption in finishers and sows/piglets decreased. The increase in use of antimicrobials does not seem to have any connection to the discontinued use of antimicrobial growth promoters in the late 1990s.

In 2003, the viral disease Post-weaning Multisystemic Wasting Syndrome (PMWS) spread in the Danish pig population. The increase in the use of tetracyclines in finishers is confined to geographic areas with out-breaks of PMWS. A large increase in usage of tetracycline in finishers and a minor increase in weaners were observed in these areas. Possibly, tetracycline is used emperically before a final diagnosis is established or to control secondary infections.

The veterinary consumption of fluoroquinolones was reduced from 183 kg in 2001 to 53 kg in 2003, most likely due to legal restrictions on the usage of fluoroquinolones in animal production being implemented in 2002. The consumption of cephalosporins increased from 385 kg in 2002 to 461 kg in 2003 due to increased use in pigs and cattle. However, a relatively large part of cephalosporins and flouroquinolones was used in companion animals.

The consumption of antimicrobials in pigs amounted to 43 mg/kg meat produced in 2003. The consumption in broilers was unchanged at 0.5 mg/kg meat produced, and the consumption in cattle was estimated at 52 mg/kg meat produced (including diary cattle). In aquaculture antimicrobial consumption amounted to 96 mg/kg fish produced. In pig production the treatment frequency was especially high in weaners (4-11 weeks of age), where 6.5 ADD/weaner was used. In comparison 1.9 ADD /finisher (11-25 weeks of age) was used.

Antimicrobial consumptions in humans
In 2003, the overall consumption of antimicrobials for systemic use in humans in Denmark amounted to 29.5 millions DDDs or 15.0 DDD/1,000 inhabitant-days, which is comparable to 2002. In 2003, 43.9 tonnes of antibacterials for systemic use were used in humans in Denmark, representing an increase of 1.3% compared to 2002. This increase was primarily due to an increased consumption of β-lactamase sensitive and β–lactamase resistant penicillins and fluoroquinolones.

From 1997 to 2002, the total consumption in hospitals showed a steady increase from 392 to 514 DDD/1,000 bed-days. This increase in consumption was mainly due to a 26% increase in the number of DDDs of antimicrobials registered by hospital pharmacies (from 2.3 to 2.9 million DDDs in 1997 and 2002 respectively) while there was a 6% decrease in the total number of hospital bed-days registered in Denmark in the same period. Since 2000, the total number of bed-days has decreased annually by 1% or less.

From 2002 to 2003, there was a moderate increase in total consumption of antimicrobials in hospitals from 514 to 519 DDD/1,000 bed-days. Thus, the increase in hospital consumption observed since 1997 has stopped. The moderate increase in consumption between 2002 and 2003, was primarily due to an estimated slight decrease in the number of bed-days for 2003, whereas there was no change in the number of registered DDDs. The results for 2003, however, must be interpreted with caution since they are based

on a provisional number of registered DDDs and an estimate of the number of bed-days.

The prescription of antimicrobials in Danish hospitals, although conservative, presented a steady increase in the use of specific classes including cephalosporins, fluoroquinolones, combinations of penicillins including β-lactamase inhibitors, and carbapenems. The use of these four classes represented 53% of the increase in total antimicrobial use between 1997 and 2003. This slow but steady shift towards use of „broad-spectrum" antimicrobials in Danish hospitals is of concern and requires close surveillance.

Resistance in zoonotic bacteria

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

The occurrence of resistance in *Salmonella* Enteritidis isolates from poultry, as well as in *Salmonella* Typhimurium isolates from poultry, cattle and pigs was unchanged from 2002 to 2003. In general a good correlation was observed between the frequency of resistance in *S.* Typhimurium isolates from humans and food animals.

In 2003, a further decline in the incidence of human salmonellosis and campylobacteriosis in Denmark was observed. Resistance in *S.* Enteritidis isolates remained low, although, for infections acquired domestically, the percentage of isolates resistant to nalidixic acid increased from 4% in 2002 to 15% in 2003. Resistance to nalidixic acid in isolates of *Campylobacter jejuni, S.* Enteritidis and *S.* Typhimurium from humans remains significantly higher in travel associated cases compared with those acquired within Denmark.
Inquiry about the patient's travel history and awareness of the high probability of resistance to quinolones are important in the management of patients with diarrhoea.

Resistance in indicator bacteria

In general, resistance in isolates of enterococci from broilers, cattle and pigs remained unchanged from 2002 to 2003, except for a decrease in resistance to erythromycin in *Enterococcus faecium* isolates from pigs. This decrease in occurrence of resistance coincides with a 12% reduction in macrolide consumption in pigs from 2002 to 2003. The substantial increase in consumption of tetracycline and penicillins in pigs, did not have any immediate effect on the occurrence of resistance in *E. faecium* isolates from pigs.

As opposed to previous years resistance to several antimicrobials in indicator *Eschericia coli* isolates from pigs increased significantly in 2003. Resistance to ampicillin, sulfonamide, trimethoprim, spectinomycin and streptomycin increased from 2002 to 2003, and was the most frequently observed resistance in indicator *E. coli* isolates from pigs in 2003. These observations coincide with increased consumption of penicillins, sulfonamides / trimethoprim and tiamulin in pigs in 2003. In contrast, the substantial increase in consumption of tetracycline in pigs in 2003, was not reflected in the occurrence of resistance to tetracycline among indicator *E. coli* in pigs in 2003.

The occurrence of resistance in enterococci from healthy humans was similar to resistance levels observed in isolates from food products.These observations may indicate exchange of bacteria between food products and humans.

Resistance in bacteria from diagnostic submissions

In isolates of *Staphylococcus hyicus* from pigs resistance to penicillin increased from 54% in 2000 to 84% in 2003. This increase coincided with a yearly increase of 11-13% (ADD_{kg}) in consumption of penicillins in pigs from 2001 to 2003. For all other antimicrobials in the test panel, the frequency of resistance in *Staphylococcus hyicus* remained unchanged in 2003.
In humans, the number of Methicillin-Resistant *Staphylococcus aureus* MRSA infections in Denmark more than doubled from 2002 to reach 204 cases in 2003. The number of MRSA infections imported from abroad did not change between 2002 and 2003. Thus, the increase in MRSA infections was due to an increase in infections acquired in Denmark. More than 50% of MRSA infections in 2003 were community onset.

Although at a low frequency, resistance to commonly used antimicrobials was observed in *E.coli* from infections in humans and calls for close surveillance. Resistance to fluoroquinolones remained low in 2003. A significant price-drop and high levels of resistance to other antimicrobials may increase fluoroquinolone consumption and subsequently levels of resistance. Resistance to penicillins and macrolides in *Streptococcus pneumoniae* and *Streptococcus pyogenes* remained low in 2003.

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 2003.

Table 1 shows the production of food animals (including animals for live export), meat, and the population of dairy cows. From 2002 to 2003, the production of broilers and cattle decreased by 4.8% and 6.4%, respectively, while the production of pigs increased by 1%.
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.

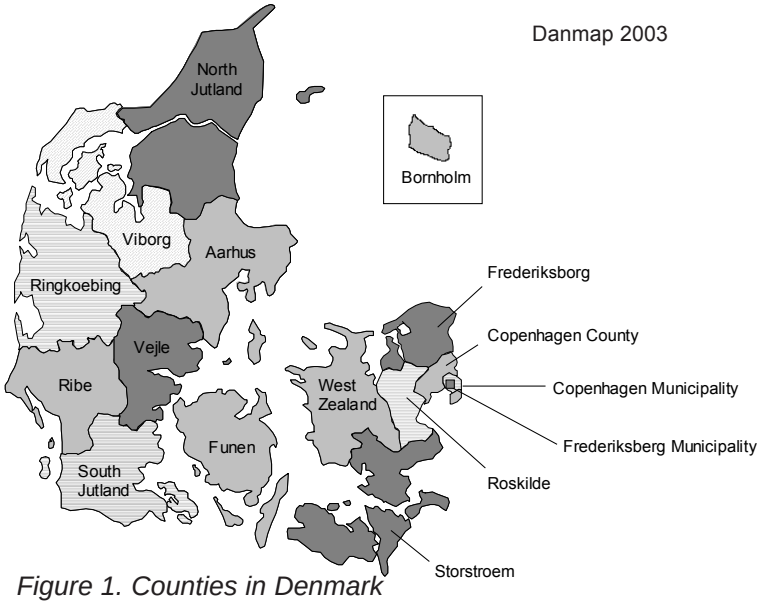

Figure 1. Counties in Denmark

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

Danmap 2003

Year	Broilers		Cattle (slaughtered)		Dairy cows		Pigs		Farmed fish	
									Fresh water	Salt water
	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	mill. kg	mill. kg	mill. kg
1990	94,560	116	789	219	753	4,542	16,425	1,260		
1992	107,188	137	862	236	712	4,405	18,442	1,442	35	7
1994	116,036	152	813	210	700	4,442	20,651	1,604	35	7
1996	107,895	149	789	198	701	4,494	20,424	1,592	32	8
1998	126,063	168	733	179	669	4,468	22,738	1,770	32	7
2000	133,987	181	691	171	636	4,520	22,414	1,748	32	7
2001	136,603	192	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	625	161	596	4,540	24,434	1,898	29	8

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

DANMAP 2003

County	No. inhabitants (1/1/2003)	No. inhabitants/km^2 (2003)	No. inhabitants/GP c) (2002)	No. bed-days d) (2002, provisional)	No. hospitals (2002)
Copenhagen Municipality a)	501,285	5,680	1,571	981,000 e)	4
Frederiksberg Municipality a)	91,435	10,426	1,604	-	1
Copenhagen County b)	618,016	1,175	1,559	610,000	3
Frederiksborg	372,276	276	1,626	335,000	4
Roskilde	236,151	265	1,571	222,000	2
West Zealand	300,729	101	1,542	266,000	4
Storstroem	261,188	77	1,602	271,000	5
Bornholm	44,060	74	1,335	39,000	1
Funen	473,471	136	1,589	510,000	6
South Jutland	253,013	64	1,454	210,000	4
Ribe	224,257	72	1,557	192,000	3
Vejle	353,284	118	1,621	334,000	6
Ringkoebing	275,044	57	1,554	242,000	5
Aarhus	649,177	142	1,553	672,000	8
Viborg	234,496	57	1,553	253,000	3
North Jutland	495,625	80	1,568	487,000	7
Denmark	5,383,507	125	1,559	5,624,000	66

a) Inner Copenhagen: both municipalities have county status
b) Outer Copenhagen
c) GP = general practitioner
d) Excluding psychiatry, private hospitals and one rehabilitation center
e) Public hospitals in Copenhagen and Frederiksberg municipalities (inner Copenhagen) represent one single administrative body and include the national referral hospital

Antimicrobial consumtion

Antimicrobial consumption in animals

Distribution of antibacterial agents

In Denmark, all antimicrobials, except for those approved by the EU as feed additives, are prescription only medicines, and must be purchased through a pharmacy or a feed mill. In 2003, 4% of the prescribed antimicrobials were purchased at the feed mills and 96% were purchased from pharmacies. Antimicrobial drugs for use by the veterinary practitioner are purchased at the pharmacies and comprise 14% of the usage. The other 86% are purchased and used by farmers according to directions given by a veterinary practitioner.

From 2001, the Danish register of veterinary medicines, VetStat, has collected detailed data close to the point of use, i.e. by pharmacies, veterinary practitioners, and by feed mills. Further details on the VetStat system are presented in Appendix 1**.**

Trend in consumption of therapeutics

Table 3 shows the trend from 1990 to 2003 in the consumption of antimicrobials used in production animals. For the years prior to the implementation of VetStat, the estimates are based on reports from the pharmaceutical industry of total annual sales, excluding antimicrobial drugs that are used exclusively in companion animals.

In the years 1996-2001, the yearly increase averaged nine tonnes.The usage seemed to reach a more stable level in 2001-2002, where the increase in animal production exceeded the increase in antimicrobial consumption (DANMAP 2002).

The quantity of antimicrobials used in production animals increased by 6% from 2002 to an estimated 101.9 tonnes active compound in 2003. The increase was mainly in tetracyclines, penicillins and to a lesser extent in the tiamulin/macrolide/lincosamide group. The increase was caused by a 10 % increase in antimicrobial usage in pigs, exceeding the 1% increase in the pig production (Table 3).

Antimicrobial consumption measured in kg active compound

Table 4 shows the total veterinary consumption of antimicrobial drugs in 2003 in kg active compound by animal species and age groups, including usage in companion animals.

The veterinary consumption of fluoroquinolones was reduced from 183 kg in 2001 to 53 kg in 2003, due to legal restrictions on the usage of fluoroquinolones in animal production being implemented in 2002. The consumption of cephalosporins increased from 385 kg in 2002 to 461 kg in 2003, including 250 kg used in companion animals. The increase occured in use of intramammaries in cattle (30 kg) and injectables in pigs (30 kg).

Please note that the amounts distributed through veterinary practice are not separated into animal species in Table 4. Of the total 14.4 tonnes of antimicrobials, used or re-sold by veterinary practitioners, an estimated 8-8.5 tonnes of antimicrobials were used in cattle, an estimated 2-3 tonnes were used in small animal practice, and 3-4 tonnes were used in other species. However, due to

Table 3. Trends in the estimated total consumption of antimicrobials for treatment of food animals, Denmark (kg active compound, rounded to nearest 50 or 100)

DANMAP 2003

ATC-group a)	Compound	1990	1992	1994	1996	1998	1999	2000	2001 b)	2002 b)	2003 b)
QJ01AA	Tetracyclines	9,300	22,000	36,500	12,900	12,100	16,200	24,000	28,400	24,400	27,100
QJ01CE	Penicillins, β–lactamase sensitive	5,000	6,700	9,400	7,200	14,300	14,700	15,100	16,400	17,400	18,900
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	6,600	7,300	8,800	9,900	11,000
QJ01EW	Sulfonamides + trimethoprim c)	3,800	7,900	9,500	4,800	7,700	6,800	7,000	9,500	10,500	10,500
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	1,000	950	900	850
QJ01F/QJ01XX	Macrolides, lincosamides, tiamulin	10,900	12,900	11,400	7,600	7,100	8,700	15,600	18,200	19,100	20,500
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	7,500	10,400	11,500	11,600	11,600
	Others c)	6,700	6,800	4,400	600	650	350	300	900	1,600	1,400
Total		53,400	73,200	89,900	48,000	57,300	61,900	80,700	94,600	95,400	101,900

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 2001-2002: VetStat. For comparability between VetStat data and previous data, see DANMAP 2000. Only veterinary drug are included. Veterinary drugs almost exclusively used in companion animals (tablets, capsules, ointment, eye/ear drops) are excluded.
a) Only the major contributing ATC groups are mentioned.
b) Data from VetStat 2001-2003. Data for 2001-2002 has been corrected, including delayed reporting from one pharmacy
c) Does not include consumption in aquaculture before 2001

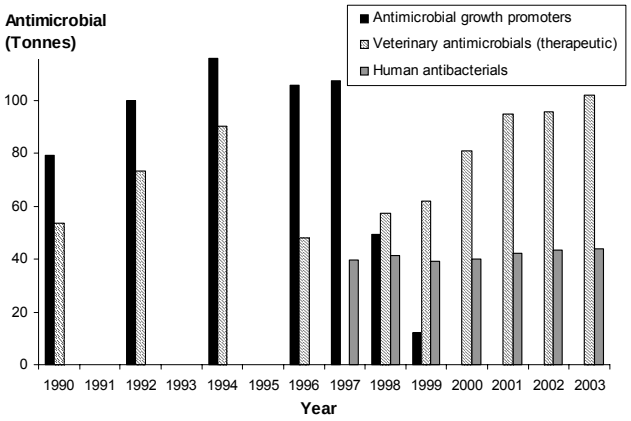

Figure 2. Antimicrobials use for therapy and growth promotion in animal production and in humans, Denmark

considerable underreporting from the veterinary practitioners in 2003, only 6.7 tonnes used in cattle and one tonne used in other production animal species were reported (See Appendix 1).

Analyses of trends in antimicrobial consumption in cattle is hampered because the major part of the antimicrobials used in cows is underreported from veterinary practice, lacking an estimated 20-25%.

The total veterinary antimicrobial consumption (incl. companion animals) increased by 5.6 % to 103.2 tonnes active compound in 2003. This was caused by a 10% increase in antimicrobial consumption in the pig production, from 73 tonnes in 2002 to 81 tonnes in 2003.

The increase of 8 tonnes for treatment of pigs comprised an increase of 2.8 tonnes tetracycline, 1.5 tonnes β-lactamase sensitive penicillins, 1.1 tonnes of penicillins with extended spectrum, and 1.2 tonnes tiamulin, while the use of macrolides decreased by 300 kg. Table 5 shows the use of antimicrobials in pigs by route of administration. The increase in penicillins, aminoglycosides, amphenicols and sulfonamide/ trimethoprim was primarily in drugs for parenteral administration (injectables). The major increase

Table 4. Antimicrobials sold from pharmacies and feedmills by animal species and age group, Denmark. Usage is given in kg active compound.

DANMAP 2003

Therapeutic group	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macrol	Pen-β-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATC_{vet} groups a)	QJ01B	QJ01G	QJ01DA	QJ01MA	QJ01MB	QJ01FF	QJ01FA QJ01XX	QJ01CE	QJ01CA	QJ01E	QJ01AA	QJ01X b)	
Pigs													
Sows and piglets	25	2,719	55	12	0	711	2,196	7,426	3,170	3,955	2,949	23	23,240
Weaners	25	6,028	10	0.5	0	913	6,615	1,001	2,853	1,631	10,224	218	29,519
Finishers	22	908	7	0.5	0	1,255	6,876	4,091	1,800	205	11,369	5	26,538
Age not given	3	133	0.6	0	0	52	419	262	164	142	471	5	1,651
Cattle													
Cows and bulls	1	15	2	0	0	1	11	59	25	40	21	0	177
Calves<12 mo.	60	270	3	0.7	0	11	40	394	273	223	333	2	1,609
Heifers, Steers	1	3	0.1	0	0	0.3	0.8	7	6	2	8	0	28
Age not given	3	22	0.4	0.1	0	1	27	30	33	34	23	0.2	174
Poultry													
Broilers	0	3	0	3	0	0	0.5	0	21	1	4	0	32
Layers	0	0	0	2	0	0	0.8	0	19	0.7	0.4	0	23
Rearing flocks	0	1	0	1	0	0	11	0	13	33	6	0	65
Turkeys	0	0	0	0.3	0	0	0.5	0	64	0.8	0.1	0	66
Gamebirds, geese and ducks	0	2	0	0	0	0	20	0	16	18	13	0	69
Production category not given	0	0	0	0.2	0	0	2	0	5.6	4	2	0	13
Small ruminants	0	3	0	0	0	0.3	3	4	12	29	12	0	63
Mink	0	206	0	0.1	0	44	94	0.2	381	32	14	0	771
Aquaculture	48	0	0	0	834	0	0.1	0.2	36	2,589	2	0	3,510
Other production animals	0.9	8	0.5	0.2	0	1	2	24	28	15	5	0.1	85
Horses	0	6	0.2	0.1	0	0.3	0.9	21	2	110	2	0.2	144
Companion animals	0.3	9	71	6	0	40	14	18	71	77	22	10	338
Species not given													
- Farm identified c)	2	60	0.6	1	0	24	115	145	120	80	183	1	730
- For use in vet. practice	52	1,282	310	25	0	168	926	5,487	1,991	2,604	1,522	28	14,396
Total (pharmacy, feed mill)	244	11,678	461	53	834	3,222	17,373	18,970	11,102	11,827	27,184	293	103,241
Reported from vet. practice d)	37	382	163	8	15	37	523	3,641	1,036	935	912	3	7,692

Amcol=amphenicols, Amglc=aminoglycosides, Ceph=cephalosporins, FQ=fluoroquinolones, Quinol=other quinolones, Linco=lincosamides, Macro=macrolide+tiamulin, Pen-β-sens=β-lactamase sensitive penicillins, Pen-others=penicillins with extended spectrum, cloxacillin and amoxicillin/ clavulanic acid, SulfaTMP=sulfonamides+trimethoprim, Tet=tetracyclines.

a) Only the ATCgroup contributing most to the antimicrobial group is shown. Combination drugs are divided into active compounds.

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

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

d) Comprise reports from practitioners of usage in production animals in relation to consultations. Originates from pharmacy sales for use in practice. NB: incomplete data.

Table 5. Consumption of antibacterials for treatment of pigs in Denmark by administration route[a]. Usage is given in kg active compound

DANMAP 2003

ATC_{vet} -code b)	Therapeutic group	Peroral		Parenteral		Others c)	
		2002	2003	2002	2003	2002	2003
QJ01A	Tetracyclines	19,668	22,645	2,081	2,369	0.1	0.1
QJ01B	Amphenicols	0	0	3	76	0	0
QJ01CE	Penicillin, β-lactamase sensitive	0	0	11,269	12,779	1	1
QJ01CA/CR	Penicillins, others	4,353	5,064	2,500	2,922	0.1	0.1
QJ01DA	Cephalosporins	0	0	43	73	0	0
QJ01E/QP51	Sulfonamides, trimethoprim	2,297	2,347	3,034	3,581	0.8	4
QJ01FA/QJ01X	Macrolides, tiamulin	13,937	15,595	402	511	0	0
QJ01FF	Lincosamides	1,375	1,409	1,307	1,522	0	0
QJ01G/QA07A	Aminoglycosides	7,167	7,196	2,336	2,591	2	1
QJ01MA	Fluoroquinolones	2	0	39	13	0	0
QJ01XX d)	Other antibacterials	180	0	0	250	0	0
		48,979	54,256	23,015	26,686	4	6

a) Based on VetStat data from pharmacies and feedmills.
b) For each compound, only the ATC code for the more important antimicrobial group is shown
c) For intramammary, intra-uterine, or topical administration

occurred in drugs for oral use, i.e., tetracyclines, tiamulin and penicillins with extended spectrum. The use of fluoroquinolones decreased to 13 kg, which were almost entirely used in sow herds. The use of amphenicols in pigs increased from close to none (3 kg in 2002) to 76 kg in 2003.

In poultry, the consumption of therapeutic prescription antimicrobials (including sulfaclozin) amounted to a total of 400 kg active compound. The consumption decreased by 50% compared to 2002, reaching the 2001 level. The increase in 2002 was due to unusual disease outbreaks in the turkey production. In the Danish poultry production, amoxicillin remains to be the most commonly used antimicrobial, comprising 65% (262 kg) of therapeutic antimicrobial consumption in 2003. In poultry, fluoroquinolones were the second most used antimicrobial, although the consumption decreased from 14 kg in 2002 to 11 kg in 2003.

Almost 80% of the veterinary consumption of therapeutic antimicrobials is used in the extensive Danish pig production. Relative to production figures (Table 3), the consumption of antimicrobials in pig production amounted to 43 mg/kg. In comparison, the consumption in poultry was low, amounting to 0.4% of the total consumption in animals. The consumption in broilers was unchanged at a very low level**,** 0.5 mg/kg meat produced in 2003. The consumption in cattle relative to production is estimated at 52 mg/kg meat produced (the waste majority used in dairy cows).

The antimicrobial consumption in aquaculture was high in 2002 due to an unusually warm summer. Although decreasing in 2003, the usage in aquaculture was high compared to the production, amounting to 3.4% of the total antimicrobial consumption in animals or 96 mg/kg fish produced.

Antimicrobial consumption measured in Defined Animal Daily Doses (ADD)

In VetStat, the target animal species is known, enabling the presentation of usage statistics in Defined Animal Daily Doses (ADD) on species and age group level.

Table 6 shows the total usage of antimicrobials calculated as Defined Animal Daily Dose (ADD). The total antimicrobial usage in ADD_{kg} (doses for treatment of one kg body weight) increased by 7% (not including topical treatment and aquaculture). The antimicrobial consumption in aquaculture declined, whereas the consumption in horses, goats, sheep and more exotic production animals (e.g., deer, rodents, ostrich) increased slightly, as estimated from pharmacy and feed mill figures.

Tables 7-9 shows the usage of antimicrobials in ADD for swine, cattle, and poultry by ATC group and age group. Figure 1 shows the development from 2001 to 2003 in the different antimicrobials (in ADD) used in pigs.

In 2003, the number of pigs slaughtered increased by 1% to 23.1 millions. Furthermore, 1.6 million pigs were exported at 10-11 weeks of age.
For each pig produced**,** 6.5 ADD/pig were used in the weaners (4 to 11 weeks of age) and 1.9 ADD/pig were used in the finishers (11 to 25 weeks of age). The usage in sow herds (sows/gilts and piglets) was 7.5 ADD/sow-year in 2003.

In the pig production, antimicrobial usage in ADD_{kg} increased by 8% from 2002 to 2003. The change in

Table 6. Usage of antimicrobials given as Defined Animal Daily Doses (ADDs), ADD_{kg} [a)] and kg antimicrobial, based on sales from pharmacies and feed mills to specified species and age group, Denmark

DANMAP 2003

			Pharmacies and feed mills				Veterinary Practice b)
		Defined animal	kg antimicrobial	ADDkg (1000s) a)		ADD (1000s) a)	ADD (1000s)
Species	Agegroup	Bodyweight (kg)	2003	2002	2003	2003	2003
Pigs	Sows and piglets	200	23,229	1,506,860	1,694,770	8,474	57
	Weaners	15	29,518	2,387,940	2,407,983	160,532	592
	Finishers	50	26,536	1,964,750	2,232,773	44,655	465
	Age not given	50	1,649	110,569	140,322	2,806	1
Cattle	Cows and bulls	600	178	24,223	30,872	51	1848
	Intramammamaries c)	600	381	692,806	684,762	1,141	-
	Calves<12mo.	100	1,603	102,656	115,464	1,155	476
	Heifers, Steers >12mo.	300	28	3,570	2,238	7	65
	Age not given	600	172	4,157	11,925	20	0
Small ruminants	>12 mo.	50	17	694	1,047	21	4
	<12 mo.	20	1	125	87	4	5
	Age not given	50	32	1,264	1,359	27	0
Poultry	Broilers	1	32	3,193	1,955	1,955	2,216
	Layers	1	23	700	1,617	1,617	338
	Rearing flocks	1	65	1,447	1,889	1,889	141
	Game birds	1	57	1,631	1,793	1,793	1
	Ducks, Geese	1	11	31	404	404	0
	Turkeys	20	66	15,510	4,641	232	336
	Productiontype not given	1	12	1,025	496	496	0
Horses	Age not given	500	112	4,888	5,134	10	nc
Mink (ADD)	Age not given	1	770	40,546	39,400	39,400	4,289
Aquaculture	Age not given	1	3560 d)	nc	nc	nc	nc
Other prod. animals	(intramammaries)	unknown	81	nc	nc	1324	0
	Age not given	1		3,872	4,518	nc	36
Companion animals	(intramammaries)	unknown	310	nc	nc	1229	-
	Age not given	1		14,789	14,854	nc	not reported
ADD not defined e)		-	101	-	-	-	-
Species not given f)	(kg active compound)	-	14,695	-	-	-	-
Total			103,241	6,887,247	7,400,302	-	-

nc: not calculated (species not given or ADD not defined)
a) The total comsumption is presented both as ADDkg and converted into ADD (ADD= ADDkg/bodyweight). In cases when convertion is not possible, the consumption is shown as only one of either
b) Data are incomplete (see Appendix 1)
c) Intrammamaries used in practice, ie. species not given. Almost entirely used in cows.
d) Quinolones and sulfonamide/trimethoprim used in aquaculture-practice are included with the data from pharmacies.
e) Includes drugs where ADD is not given for the recipient species (mostly topical drugs).
f) Species not given at the pharmacy, i.e., mainly for use in practice (14,015 kg; Intramammaries excluded).

Table 7. Usage of antmicrobials in pigs given as defined Animal Daily Doses (ADDs 1,000) in 2002 and 2003, Denmark.

DANMAP 2003

		Pharmacies and feed mills								Veterinary practice a)		
Age group		Sows/piglets		Weaners		Finishers		Age not given		Sows/piglets	Weaners	Finishers
Animal standard weight		200 kg)		15 kg		50 kg		50 kg		200 kg	15 kg	50 kg
		2002	2003	2002	2003	2002	2003	2002	2003		2003	
ATCvet code	Therapeutic group	ADD (1,000s) b)										
QJ01A	Tetracyclines	880	914	32,133	32,367	8,722	11,119	461	545	9	162	108
QJ01B	Amphenicols	0.2	6	3	84	-	22	1	3	0	0.1	0.2
QJ01CE	Penicillins, β–lact. sen. b)	1,786	2,012	2,514	2,894	4,587	5,117	239	313	13	49	102
QJ01CA/CR	Penicillins, other	1,025	1,117	9,956	12,712	2,146	2,418	162	224	8	70	61
QJ01DA	Cephalosporins	60	99	146	254	36	56	3	4	6	1	5
QJ01E/QP51	Sulfonam./trimeth.	944	1,083	3,965	4,145	203	173	101	129	5	9	15
QJ01FA	Macrolides	814	729	46,405	41,201	11,593	12,242	609	695	3	41	72
QJ01FF	Lincosamides	561	579	17,468	19,791	3,814	4,407	187	233	3	70	36
QJ01G/A07AA	Aminoglycosides	254	238	23,939	22,207	224	193	78	98	3	54	10
QA07AA10	Colistin (local GI)	17	23	2,108	2,909	14	18	6	18	0.2	11	1
QJ01MA	Fluoroquinolones	49	21	182	11	67	5	2	0	3	7	1
QJ01R	Combinations	642	703	2,150	2,210	349	422	34	51	4	17	8
QJ01X	Other antibacterials c)	495	945	18,226	19,749	7,539	8,462	330	492	2	102	47
QJ51	Intramammaries	7	4	1	1	1	0.5	0	0.2	0	0	0
QG01AA	Gynecologic (local)	0.1	0.1	0	0	0	0	0	0	0	0	0
Total		7,534	8,474	159,196	160,532	39,295	44,655	2,211	2,806	57	592	465

a) Data from veterinary practice is shown separately, because reporting from veterinary practice is incomplete, lacking an estimated 50-70%
b) β-lactamase sensitive penicillins
c) QJ01X is mainly tiamulin and a small part is valnemulin

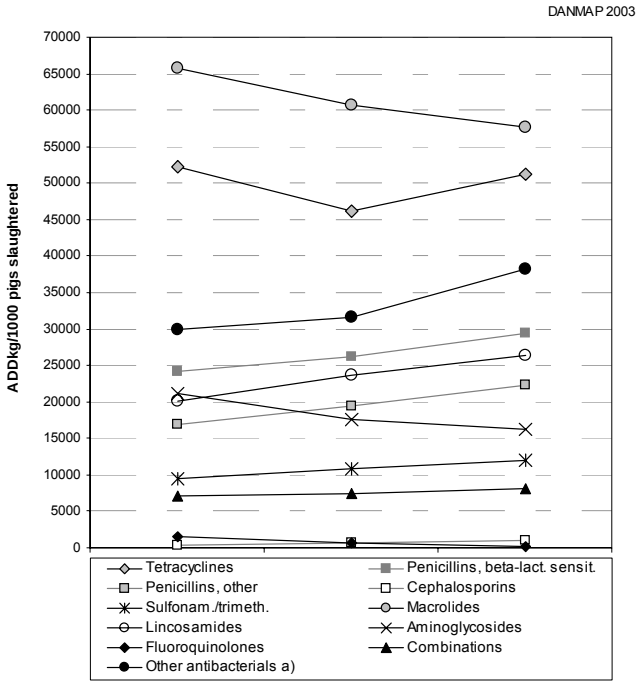

Figure 3. Antimicrobial drugs (ADD_{kg}) used in pigs, trends in 2001-2003, Denmark

Antibacterial groups refer to ATCvet groups (see Table 7). Amphenicols, colistin, intramammaries and gynecologicals are not included in the figure
Data from veterinary practice are not included (amounts to <2%)
a) QJ01X is mainly tiamulin and a small part is valnemulin

total consumption was primarily due to an increasing use of tetracycline, penicillins and tiamulin (Table 7and Figure 3).

In finishers, the increase occurred in prescription for gastrointestinal (GI) disease, respiratory diseases and disease of the skin or locomotor system. The increase in prescriptions for GI use was primarily in tetracyclines, and may be connected with the emergence of Post-weaning Multisystemic Wasting Syndrome (PMWS), rather than primary GI disease (discussed below). The consumption of penicillins increased in sow herds and in the finishers, primarily for treatment of diseases of the skin or locomotor system, respiratory tract, reproductive system and udder. The consumption of tiamulin increased in all age groups, particularly in sows.

In weaners, a major decline (2.6%) was seen in treatment of GI, related to a decline in use of macrolides (28%) - and a minor increase (8%) in use of tiamulin. Prescription for treatment of respiratory disease increased by 34%, causing an increase in use of penicillins and less of tetracycline, macrolide and tiamulin. In conclusion, the increase in use of antimicrobials does not seem to have any connection with the cessation of use of antimicrobial growth promoters in the late 1990s (Figure 2). On the contrary, the use of antimicrobials for GI disease in weaners is declining.

The viral disease PMWS is currently spreading in the Danish pig population. The number of diagnosed cases increased from 68 cases in 2002 to 276 cases in 2003. The increase in use of tetracyclines is confined to geographic areas with outbreaks of PMWS, but it has not yet been investigated whether these events are related. In these areas a large increase in tetracycline usage in finishers and a minor increase in weaners was observed. Possibly, tetracycline is used empirically before a final diagnosis of the syndrome, or due to secondary infections.

Increasing macrolide resistance in *Brachyspira hyodysenteriae* (causing swine dysenteria) may have contributed to the decrease in use of macrolides. The cause of the increase in tiamulin use is not known. The use of tiamulin increased by 100% in the sow herds (Table 7), causing 54% of the total increase in tiamulin. This was also restricted to areas with PMWS and may be related to the practice of using tiamulin for prevention of secondary disease in piglets in relation to PMWS.

Table 8 shows the usage of antimicrobials in cattle in ADDs. The antimicrobial usage in cattle was distributed on approximately 85% in cows, 13% in calves and 2% in heifers/steers (in ADD_{kg}). In cows, the major therapeutic group was intramammaries, representing 54% of the doses applied. Due to a 50% increase in cephalosporin-intramammaries from 2001 to 2003, these are now the most common type (360 x 10^3 ADD), closely followed by narrow spectrum penicillin-intramammaries (350 x 10^3 ADD). As in previous years, β-lactamase sensitive penicillins are used in almost half of the systemic treatments of cows, whereas tetracycline was used in 17% of the systemic treatments. In calves, the antimicrobial usage increased by 12% compared to the 2002 level, due to a 63% increase in broad spectrum penicillins (QJ01CA and QJ01CR) and a 13% increase in tetracycline. Tetracycline and penicillins represented 65% of the total antimicrobial usage in calves. Increases were also seen in use of amphenicols, cephalosporins, and sulfonamide/trimethoprim, whereas the use of fluoroquinolones decreased by 67%.

The therapeutic antimicrobial usage in poultry is shown in Table 9. The decline in treatment of turkeys amounted to 58% measured in ADDs. The antimicrobial use in broilers decreased by 21%, exceeding the 5% decrease in broiler meat production. In broilers, a decline was seen in all antimicrobial groups, except for aminoglycosides. Usage of fluoroquinolones in broilers declined by 24% to 3 ADD_{kg}/1000 broilers produced. In 2003,

Table 8. Usage of antimicrobials in cattle given as Defined Animal Daily Doses (ADD), Denmark

DANMAP 2003

		Pharmacies and feed mills								Veterinary Practice a)		
Age group		Cows, bulls		Calves <12 months		Heifers, steers		Age not given		Cows, bulls	Calves	Heifer/steer
Animal standard weight		600 kg		100 kg		300 kg		600 kg		600 kg	100 kg	300 kg
		2002	2003	2002	2003	2002	2003	2002	2003		2003	
ATCvet code	Therapeutic group	ADD (1,000s) b)										
QJ01A	Tetracyclines	3	3	348	394	6	3	2	3	128	141	12
QJ01B	Amphenicols	0.1	0.1	20	30	0.2	0.2	0.2	0.2	1	10	0
QJ01CE	Penicillin, β-lact. sen. b)	5	7	210	184	2	1	0.5	3	390	54	20
QJ01CA/CR	Penicillins, others.	3	3	121	185	1	1	0.9	4	72	104	4
QJ01DA	Cephalosporin	0.5	0.9	15	24	0.4	0.4	0.1	0.2	59	16	5
QJ01E	Sulfonamid./trimeth.	1	3	74	89	0.3	0.3	0.4	2	53	53	2
QJ01FA	Macrolides	3	3	30	39	0.5	0.3	0.0	2	74	13	7
QJ01FF	Lincosamiders	0.6	0.8	21	16	0.1	0.1	0.3	0.3	1	7	0
QJ01G/QA07AA	Aminoglycosides	0.5	1	51	66	0.1	0	0.7	2	2	26	0
QA07AA10	Colistin (local GI)	0	0.1	3	5	0	0	0	0.1	0	1	0
QJ01MA	Fluoroquinolones	0.1	0	10	2	0	0	0.1	0.1	1	2	0
QJ01R	Combinations	0.7	1	122	117	0.6	0.5	0.4	1.0	14	48	2
QJ01X	Other antibiotics	0.5	0.3	0.6	3.0	0.5	0	0.9	2	0	0	0
QJ51	Intramammaries	22	29	0.8	0.9	0.1	0.1	0	0	1,002	0	13
QG01AA	Gynecologic (local)	0.3	0.1	0	0	0	0	0	0	53	0	2
Total		40	51	1027	1155	12	7	7	20	1,848	476	65

a) Data from veterinary practice is shown seperately, because the use in cattle practice in underreported by an estimated 20-25%
b) β-lactamase sensitive penicillins

Table 9a. Usage of antimicrobials in domestic fowl [a)] given as Defined Animal Daily Doses (ADD), Denmark

Danmap 2003

Age group/production type		Broilers		Layers		Rearing		Production type not given	
Animal standard weight		1 kg		1 kg		1 kg		1 kg	
Year		2002	2003	2002	2003	2002	2003	2002	2003
ATCvet code	Therapeutic group	ADD (1000s) a)							
QA07AA	Aminoglycosides		133	0	0	0	67	0	0
QJ01A	Tetracyclines	155	76	4	7	136	110	63	10
QJ01CA	Amoxicillin	4883	3521	521	1,725	1,858	1,025	717	379
QJ01E/QP51	Sulfonamides	145	23	65	11	355	568	56	65
QJ01FA	Macrolides	36	6	13	11	204	150	29	19
QJ01FF	Lincosamides	0	0	0	0	0	0	0	0
QJ01MA	Fluoroquinolones	542	410	490	200	160	111	160	20
QJ01X	Other antibacterials	3	3	0	0	3	0	0	3
Total		5,763	4,171	1,094	1,954	2,716	2,030	0 1,025	496

Includes data from all sources (pharmacies, feedmills and veterinary practice)
a) *Gallinus domesticus* (hens and chickens)

Table 9b. Usage of antimicrobials in other poultry given as Defined Animal Daily Doses (ADD), Denmark

Danmap 2003

Age group/production type		Turkeys		Ducks, geese		Game birds	
Animal standard weight		20 kg		1 kg		1 kg	
Year		2002	2003	2002	2003	2002	2003
ATCvet code	Therapeutic group	ADD (1000s) a)					
QA07AA	Aminoglycosides	0	0	0	0	0	100
QJ01A	Tetracyclines	0	0	6	154	99	95
QJ01CA	Amoxicillin	1,342	545	50	250	896	838
QJ01E/QP51	Sulfonamides	0	3	0	0	319	306
QJ01FA	Macrolides	0	0	25	0	246	260
QJ01FF	Lincosamides	0	0	0	0	50	0
QJ01MA	Fluoroquinolones	0.5	20	0	0	0	1
QJ01X	Other antibacterials	0	0	0	0	10	10
Total		1,342	568	0 81	404	0 1,634	1,609

Includes data from all sources (pharmacies, feedmills and veterinary practice)

flouroquinolones were prescribed for 26 broiler flocks or in 1.3% of the produced flocks[1]. Penicillin remains to be the most important therapeutic antimicrobial used in broilers, comprising 85 % of the total use (in ADDs).
In 2003, total antimicrobial use apparently increased by 80% in layers, due to an increase in amoxicillin, while the use of fluoroquinolones decreased by 65%. The antimicrobial usage in rearing hens decreased by 25% due to a decrease in usage of all therapeutic groups except for sulfaclozin.

[1)] Assuming that all houses on the farm receiving fluoroquinolons are treated and 6.5 rotations/year

Feed additives

The distribution system for antimicrobial growth promoters and coccidiostats is well defined and different from that used for prescription-only medicines. The distribution system is under control of the Danish Plant Directorate and the feed mills report directly to VetStat. The data were provided by the Danish Plant Directorate before 2001 and obtained from VetStat in 2001-2003.

Antimicrobial growth promoters

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

In 2001 to 2003, VetStat showed the use of very small quantities of flavomycin and avilamycin, both among the four AGPs remaining approved by the EU. AGPs sold in 2003, included only small amounts of flavomycin used in pigs (Table 10). These additives have been used in a few farms, exporting pigs for slaughter in other countries.

Coccidiostats

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

Table 11 shows usage of coccidiostats in poultry production. Almost all of the coccidiostats used

Table 10. Consumption of antimicrobial growth promoters (kg active compound), Denmark[a)] DANMAP 2003

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

a) Data from the Danish Plant directorate until 2002 and on VetStat data from 2001
b) n = not monitored, assumed to be zero
c) Sold to an exporting feed mill company and a farm near the border to Poland/Germany (pigs treated are presumed exported for slaughter)

Table 11. Usage of coccidiostats in poultry (kg active compound), Denmark[a)] DANMAP 2003

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

a) Data from the Danish Plant directorate until 2002 and on VetStat data from 2001

belonged to the ionophore group of compounds.
From 2002 to 2003, the consumption of coccidostats decreased by 2,900 kg (21%), while the broiler production declined by 5%.
In broiler production, the use of ionophores may to some degree replace the use of other AGPs in the prevention of necrotic enteritis. In Denmark, the use of ionophores increased yearly from 1994 to 1999, followed by a decrease in the years 2000 to 2003. In 2003, both the use of ionophores and the total use of coccidiostats were lower than before the ban of growth promoters.

Antimicrobial residues

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

In the 2003 monitoring programme, no positive results were found among 3,808 targeted samples of pigs/sows, 531 targeted samples of cattle, 6 sheep, 5 horses, 454 targeted samples of poultry, 54 aquaculture trouts, 210 milk samples, 141 eggs, 28 samples of farmed game nor in 26 samples of honey. The monitoring of other residues in eggs showed very low amounts (less than 4 µg/kg) of coccidiostats in 18 of 141 egg samples.

In 2003, three bovine samples, 52 porcine samples and 95 milk samples taken based on suspicion revealed antimicrobial residues in 2 bovines, 11 porcines and 1 milk sample.

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.fdir.dk/Foedevare/Foedevarekontrol/Indberetninger_EU/forside.htm)

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

Antimicrobial Consumption in humans

Overall

In 2003, the overall consumption of antibacterials for systemic use (ATC group J01, 2004 definition) in humans in Denmark amounted to 29.5 millions DDDs or 15.0 DDD/1,000 inhabitant-days, which is comparable to 2002. To allow comparison with consumption in animals, total human consumption is presented in kg (Table 12). In 2003, 43.9 tonnes of antibacterials for systemic use were used in humans in Denmark, representing an increase of 1.3% compared to 2002.

Primary health care sector

Table 13 presents the consumption of antibacterials for systemic use in primary health care from 1997 to 2003. In 2003, β-lactamase sensitive penicillins (mostly phenoxymethylpenicillin) represented 37.5% of the total antimicrobial consumption followed by penicillins with extended spectrum (18.5%) (mostly amoxicillin, pivampicillin and pivmecillinam), and macrolides (15.5%). These proportions were similar as for recent years. Detailed data on the consumption of the various macrolides is presented in Figure 24, page 49.

Between 2002 and 2003, the total consumption of antibacterials for systemic use in primary health care increased by 2%. From 1997 to 2003, the overall increase has been 10.5%. Figure 4 shows the changes from 1997 to 2003 for important classes of antibacterials. In 2000-2002, the increased consumption was primarily due to β-lactamase sensitive and β-lactamase resistant penicillins, penicillins with extended spectrum and macrolides. In 2003, 81.5% of the total increase in consumption from 2002 was due to increased consumption of β-lactamase sensitive, and β–lactamase resistant penicillins and fluoroquinolones, whereas macrolide consumption decreased slightly.

The consumption of β-lactamase resistant penicillins, mostly dicloxacillin, expressed in DDD/1,000 inhabitant-days continued to increase in 2003, and has more than doubled since 1997 (Figure 5). Since the number of prescriptions for this antibacterial has shown a parallel

Table 12. Consumption of antibacterials for systemic use in humans (kg active compound), Denmark. These data include data from both primary health care and hospitals and have been re-calculated from original data expressed as a number of DDDs. They must only be used for comparison with consumption in food animals. 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 13 and 14).

DANMAP 2003

ATC group a)	Therapeutic group	Year							
		1997	1998	1999	2000	2001	2002	2003	(lowest cal.limit - highest cal.limit) b)
J01AA	Tetracyclines	1,518	1,485	1,381	1,490	1,475	1,501	1,542	
J01B	Amphenicols	1	1	0	1	0	0	0	
J01CA	Penicillins with extended spectrum	5,513	5,467	5,181	5,135	5,371	5,340	5,283	
J01CE	β-lactamase sensitive penicillins	18,813	19,947	18,790	19,782	20,715	21,256	21,622	
J01CF	β-lactamase resistant penicillins	1,913	2,115	2,416	2,654	3,225	3,736	4,074	
J01CR	Combinations of penicillins, including β-lactamase inhibitors	48	55	51	51	144	245	331	
J01D	Cephalosporins and related substances	660	657	685	727	785	853	863	(404 - 1,320)
J01EA	Trimethoprim and derivatives	245	256	258	263	280	293	307	
J01EB	Short-acting sulfonamides	3,498	3,493	3,289	3,148	3,111	3,091	3,063	
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	337	322	279	285	283	282	269	
J01FA	Macrolides	4,227	4,536	4,147	4,040	4,089	4,150	3,876	(2,851 - 4,902)
J01FF	Lincosamides	28	38	33	33	42	46	52	(42 - 63)
J01G	Aminoglycosides	32	31	32	32	28	31	28	
J01MA	Fluoroquinolones	320	343	321	290	335	381	502	(343 - 660)
J01MB	Other quinolones	15	17	16	0	0	0	0	
J01XA	Glycopeptides	25	27	32	37	36	42	43	
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70	59	59	58	
J01XD	Imidazoles	128	127	140	154	168	179	191	
J01XE	Nitrofuran derivatives	137	141	141	149	152	163	166	
J01XX05	Methenamine	2,233	2,132	1,956	1,792	1,637	1,662	1,589	(1,272 - 1,907)
J01XX08	Linezolid	0	0	0	0	0	3	4	
J01	Antibacterials for systemic use (total) c)	39,764	41,262	39,227	40,133	41,933	43,314	43,864	(41,893 - 45,834)

cal.limit = calculated limit

a) From the 2004 edition of the ATC classification system

b) When two different DDDs of an antimicrobial existed for different presentations, i.e. oral and parenteral, e.g. for cefuroxime, erythromycin, clindamycin, ciprofloxacin and methenamine, an average DDD was used. For 2003, extremes values, i.e. estimates using the lowest and the highest DDD, are given in parentheses

c) Does not include polymyxins

increase, it is unlikely that the increased consumption is due to changes in treatment regimens, i.e. increased doses or longer duration of treatment. The consumption of fusidic acid for topical use has similarly increased (ATC code D06AX01, data not shown). Possible explanations for these changes could therefore be either an increased focus on staphylococcal infections, or an increased incidence of these infections in primary health care.

The increase in fluoroquinolone consumption (primarily ciprofloxacin, J01MA02) seen in 2003 is most likely explained by a markedly reduced price per DDD due to the opening of the market to generic ciprofloxacin (Figure 6). This change calls for a closer monitoring of fluoroquinolone consumption and its effect on fluoroquinolone resistance. Preliminary data show that fluoroquinolone resistance might already have increased in 2003 in *Escherichia coli* urine isolates from primary health care (see figure 26, page 52).

Hospitals

The consumption of antibacterials for systemic use in hospitals from 1997 to 2003 is shown in Table 14. From 1997 to 2002, total consumption in hospitals showed a steady increase from 392 to 514 DDD/1,000 bed-days. This increase in consumption was mainly due to a 26% increase in the number of DDDs of antimicrobials registered by hospital pharmacies (from 2.3 to 2.9 million DDDs in 1997 and 2002 respectively) while there was a 6% decrease in the total number of hospital bed-days registered in Denmark in the same period. Since 2000, the total number of bed-days has decreased annually by 1% or less.

From 2002 to 2003, there was a moderate increase in total consumption in hospitals from 514 to 519 DDD/ 1,000 bed-days. Thus, the increase in hospital consumption observed since 1997 has stopped. The moderate increase in consumption between 2002 and 2003, was primarily due an estimated slight decrease in the number of bed-days for 2003, whereas there was no change in the number of registered DDDs. The results for 2003, however, must be interpreted with caution since they are based on a provisional number of registered DDDs and an estimate of the number of bed-days.

In 2003, penicillins still represented 57% of hospital antimicrobial use in Denmark. Between 2002 and 2003, consumption of penicillins with extended spectrum (J01CA) decreased by 2.1%, however, total consumption of penicillins (J01C) increased due to the consumption of other penicillins. In 2003, cephalosporins (mainly cefuroxime), fluoroquinolones (mainly ciprofloxacin) and carbapenems represented 12.8%, 7.2% and 1.2% of total hospital use, respectively. The prescription of antimicrobials in Danish hospitals, although conservative, presented a steady increase in the use of specific classes including cephalosporins, fluoroquinolones, combinations of penicillins including β-lactamase inhibitors, and carbapenems (Table 14). The use of these four classes represented 53% of the increase in total antimicrobial use between 1997 and 2003. This slow but steady shift towards use of „broad-spectrum“ antimicrobials in Danish hospitals is of concern and requires close surveillance.

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

DANMAP 2003

ATC group a)	Therapeutic group	Year						
		1997	1998	1999	2000	2001	2002	2003
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	0.99	1.04	1.07
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	2.47	2.51	2.52
J01CE	β-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	4.91	5.00	5.07
J01CF	β-lactamase resistant penicillins	0.34	0.40	0.48	0.52	0.65	0.77	0.85
J01CR	Combinations of penicillins, incl. β-lactamase inhibitors	0.02	0.03	0.02	0.02	0.03	0.04	0.05
J01DA	Cephalosporins and related substances	0.02	0.03	0.02	0.02	0.03	0.03	0.02
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	0.35	0.36	0.38
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	0.36	0.36	0.36
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.08	0.04	0.03	0.03	0.04	0.03	0.03
J01FA	Macrolides	2.03	2.28	2.17	2.02	2.10	2.15	2.13
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.22	0.23	0.20	0.15	0.17	0.18	0.25
J01XB	Polymyxins	0.03	0.02	0.03	0.03	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
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	0.39	0.41	0.42
J01XX05	Methenamine	0.46	0.43	0.40	0.36	0.33	0.34	0.32
J01XX08	Linezolid	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	13.24	13.52

a) From the 2004 edition of the Anatomical Therapeutic Chemical (ATC) classification system

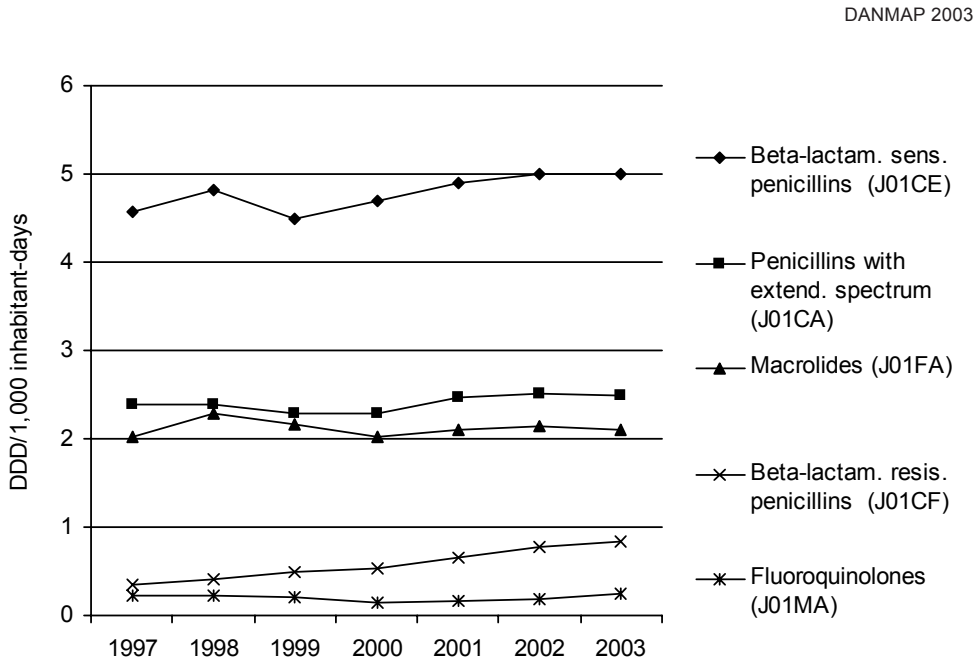

Figure 4. Consumption of selected antibacterials for systemic use in primary health care, Denmark

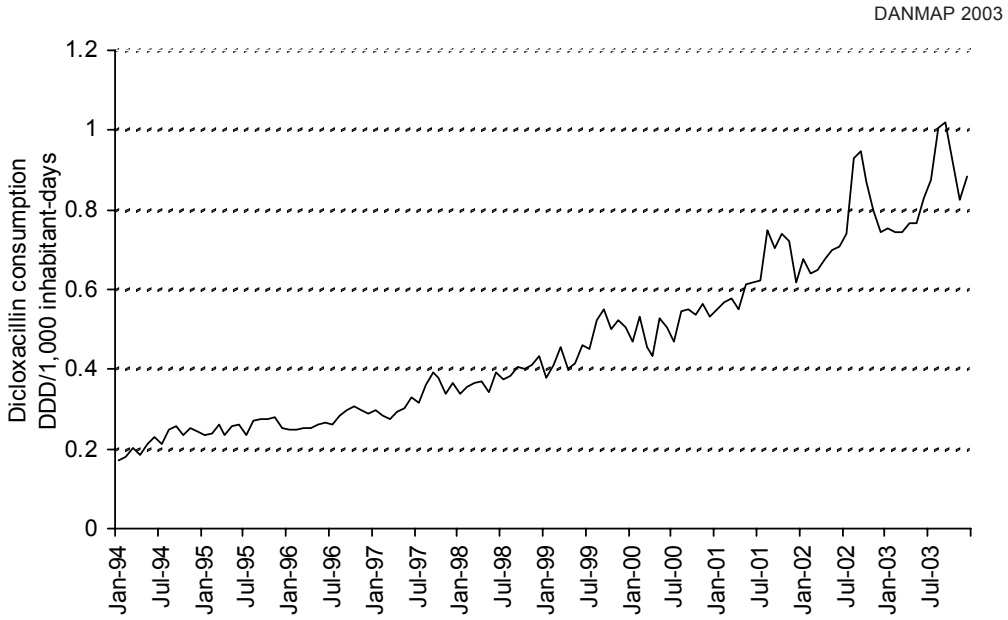

Figure 5. Monthly consumption of dicloxacillin (J01CF01) in primary health care, Denmark

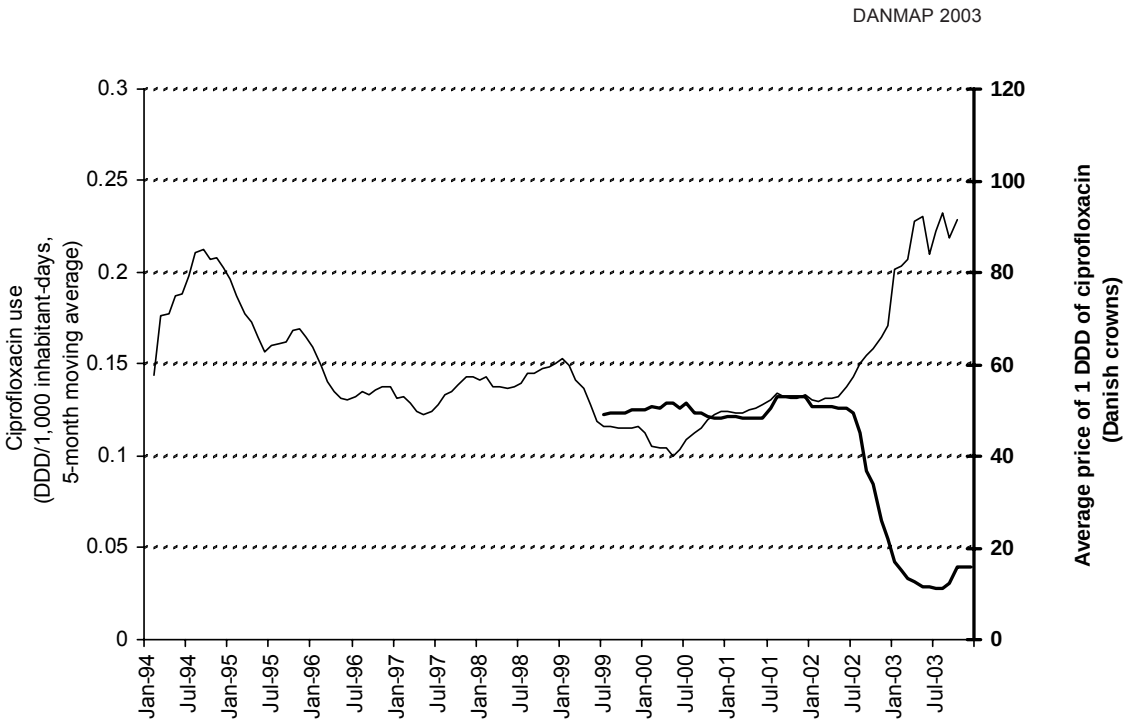

Figure 6. Comparison between the monthly average price and the monthly consumption of ciprofloxacin (J01MA02) in primary health care, Denmark. Line in bold refers to average price.

Table 14. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 occupied bed-days), Denmark. Data represented more than 98% of the total DDDs used in Danish hospitals in 2003. Psychiatric hospitals, private hospitals and one rehabilitation center were excluded.

DANMAP 2003

ATC group a)	Therapeutic group	Year						
		1997	1998	1999	2000	2001	2002 b)	2003 c)
J01AA	Tetracyclines	3.3	3.1	2.7	2.9	2.8	3.2	2.9
J01CA	Penicillins with extended spectrum	108.3	109.2	108.1	114.0	115.0	113.2	112.6
J01CE	β-lactamase sensitive penicillins	77.2	85.7	91.7	99.7	105.9	113.5	114.9
J01CF	β-lactamase resistant penicillins	42.4	44.3	46.8	53.1	59.9	62.3	63.4
J01CR	Combinations of penicillins, including β-lactamase inhibitors	0.3	0.4	0.4	0.9	1.7	3.1	4.8
J01DA	Cephalosporins	45.3	46.4	49.9	54.4	59.0	64.3	66.3
J01DF	Monobactams	0.6	0.1	0.1	0.2	0.1	0.0	0.0
J01DH	Carbapenems	3.6	2.4	3.2	3.9	4.2	5.8	6.3
J01EA	Trimethoprim and derivatives	4.0	4.2	3.6	3.7	4.3	4.1	4.2
J01EB	Short-acting sulfonamides	12.4	12.7	12.3	12.2	12.4	12.3	11.2
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	4.4	12.9	13.6	14.0	13.5	14.6	14.8
J01FA	Macrolides	31.0	34.0	32.1	32.4	32.5	31.9	29.0
J01FF	Lincosamides	1.3	1.8	1.4	1.6	1.7	1.9	1.8
J01GB	Aminoglycosides	19.5	19.4	20.2	21.3	18.5	17.6	16.6
J01MA	Fluoroquinolones	14.2	15.1	18.4	23.0	28.0	34.8	37.5
J01XA	Glycopeptides	2.1	2.3	2.8	3.3	3.2	3.8	4.0
J01XB	Polymyxins	0.4	0.2	0.3	0.4	0.3	0.3	0.3
J01XC	Steroid antibacterials (fusidic acid)	2.4	2.4	2.6	2.3	2.0	1.9	2.1
J01XD	Imidazoles	13.9	14.0	15.8	17.8	19.7	21.2	22.7
J01XE	Nitrofuran derivatives	3.6	3.3	2.9	2.8	2.9	2.8	2.6
J01XX05	Methenamine	1.7	1.8	1.5	1.4	1.3	1.2	0.8
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.5	0.4
J01	Antibacterials for systemic use (total)	391.9	415.9	430.4	465.4	488.9	514.2	519.2

a) From the 2004 edition of the ATC classification system
b) Provisional data
c) Estimated using a provisional number of DDDs and an estimate of the number of bed-days in 2003 based on past trends

Resistance in zoonotic bacteria

Salmonella

Table 15 shows the *Salmonella* serotype distribution of isolates from animals, food and humans in 2003. The phage type distributions of *Salmonella* Typhimurium and *Salmonella* Enteritidis are presented in Tables 16 and 17.

Table 15. Distribution (%) of Salmonella *serotypes isolated from animals, foods and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2003

Serotypes	Poultry a)	Broiler meat b)	Cattle a)	Beef c)	Pigs a)	Pork	Humans
	%	%	%	%	%	%	%
Agona	2	4			1		2
Bovismor-bificans					<1		1
Derby	3	2			16	11	2
Dublin			52	29			2
Enteritidis	22	37			<1		43
Hadar	3	5					1
Infantis	16	<1	2		7	1	2
Newport							2
Stanley							2
Typhimurium	21	2	44	41	64	61	26
Virchow		<1					2
Others including non-typable	33	48	2	30	12	27	16
Number of isolates	63	129	64	17	859	83	1,710

a) Only one isolate per serotype per farm
b) All but one isolate originate from imported meat
c) All but three isolates originate from imported meat

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.
Tables 18a, 18b and 19 show the MIC distribution and the occurrence of resistance in *S.* Enteritidis from poultry and in *S.* Typhimurium from poultry, cattle, pigs and pork in 2003.

Only 14 isolates of *S*. Enteritidis from poultry were available in 2003, making changes in the proportions of resistant isolates difficult to demonstrate (Table 18a). Resistance was observed only to nalidixic acid (14%). From 2002 to 2003 the consumption of fluoroquinolones in poultry was reduced by almost 50%.

Among *S*. Typhimurium isolates from poultry, cattle and pigs (Table 19), resistance to tetracycline, ampicillin, sulfonamide and streptomycin was most frequently observed, however the proportions of resistant and sensitive isolates were unchanged from 2002 to 2003. The substantial increase in consumption of tetracycline and penicillins in 2003 in pigs did not have any

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

DANMAP 2003

Phage type	Poultry	Cattle	Pigs	Pork	Humans a)
	%	%	%	%	%
1					1
3			1		<1
12	23	25	29	6	8
17	8	11	7		2
41	8				<1
66	8		5	2	<1
104/104b	15	18	6	4	14
110	8		1	2	<1
120		21	10	18	11
135	8		1		<1
170	8	11	14		10
193			6	6	5
U302			1	24 b)	13
Others including non-typable	14	14	18	38	35
Number of isolates	13	28	546	51	442

a) Not all isolates selected for susceptibility testing were phage typed
b) All phage type U302 isolates originated from imported pork

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

DANMAP 2003

Phage type	Poultry	Broiler meat a)	Humans b)
	%	%	%
1	29	17	9
4	29	52	32
6	7	10	5
6a			2
8	29		24
14b			5
21/21b		10	13
Others including non-typable	6	11	10
Number of isolates	14	48	466

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

Table 18a. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from poultry (broilers and layers) (n=14), Denmark*

DANMAP 2003

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs 0.03 0.06 0.125 0.25 0.5 1 2 4 8 16 32 64 128 256 512 1024
Tetracycline	0	[0.0-23.2]	100
Chloramphenicol	0	[0.0-23.2]	92.9 7.1
Florfenicol	0	[0.0-23.2]	100
Ampicillin	0	[0.0-23.2]	100
Amoxycillin/clavulanic acid a)	0	[0.0-23.2]	100
Cephalothin	0	[0.0-23.2]	92.9 7.1
Ceftiofur	0	[0.0-23.2]	78.6 21.4
Sulfonamide	0	[0.0-23.2]	92.9 7.1
Trimethoprim	0	[0.0-23.2]	100
Apramycin	0	[0.0-23.2]	100
Gentamicin	0	[0.0-23.2]	100
Neomycin	0	[0.0-23.2]	100
Spectinomycin	0	[0.0-23.2]	50.0 50.0
Streptomycin	0	[0.0-23.2]	92.9 7.1
Ciprofloxacin	14	[1.8-42.8]	85.7 7.1 7.1
Nalidixic acid	14	[1.8-42.8]	78.6 7.1 14.3
Colistin	0	[0.0-23.2]	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

immediate effect on the occurrence of resistance in *S*. Typhimurium isolates from pigs. However, since 1999 resistance to tetracycline in *S.* Typhimurium isolates from pigs has increased steadily.

Salmonella from food

In 2003, 146 Salmonella isolates were obtained from broiler meat and beef sampled at wholesale and retail outlets. Among 129 *Salmonella* isolates from broiler meat, all but one isolate originated from imported meat. Results of susceptibility testing of *S*. Enteritidis isolates from broiler meat are presented in table 20. Among 17 *Salmonella* isolates from beef, all but three isolates originated from imported meat. Due to the low number of *S*. Typhimurium isolates in this group, no results of susceptibility testing are reported. Resistance to nalidixic acid in *S*. Enteritidis isolates from imported broiler meat increased from 2002 to 2003.

Salmonella in humans

In 2003, 1,710 cases of human salmonellosis occurring in Denmark were reported to the Statens Serum Institut. This represents a decrease in incidence from 38.5 cases per 100,000 inhabitants in 2002 to 32 cases per 100,000 inhabitants in 2003. This steady decline is apparent since 1997 (EPI-NEWS 2004, no. 9: http://www.ssi.dk/sw8593.asp).The proportion of *Salmonella* infections reported as acquired abroad was 16%. 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 to those acquired domestically should be interpreted with caution.

The distribution of *Salmonella* serotypes is presented in table 15. Susceptibility testing was performed in 412 (56%) *S*. Enteritidis isolates. Resistance was generally low, in domestically acquired isolates as well as in those acquired abroad (Table 20). However, in domestically acquired isolates resistance to nalidixic acid increased significantly from 4% in 2002 to 15% in 2003. In isolates from cases acquired from abroad nalidixic acid resistance was 24%, a percentage comparable to that in 2002.

Almost all (>99%) of *S*. Typhimurium isolates were tested for antimicrobial susceptibility. The proportion of DT104 and related phage types (DT104b, DTU302) among the *S*. Typhimurium isolates was 27% in 2003. Resistance in *S*. Typhimurium isolates to trimethoprim, ampicillin, sulfonamides and aminoglycosides was significantly higher in cases where infection was acquired from abroad compared to those acquired in Denmark (Table 21). Among *S*. Typhimurium isolates

from cases with infection acquired in Denmark, a significant increase in resistance to tetracycline and streptomycin was observed in 2003. However, a significant decrease in resistance to trimethoprim occurred. In isolates from infections acquired abroad, no changes were observed in 2003.

Farm to table

The occurrence of resistance among *S*. Typhimurium isolates from food animals, pork and domestically acquired human cases is presented in Table 21, and in Figure 7 trends over time in resistance to *S*.

Table 19. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from poultry (broilers and layers) (n=13), cattle (n=28) and pigs (n=547), Denmark*

DANMAP 2003

Compound	Animal species	% Resistant [95% Confidence interval]	Distribution (%) of MICs																
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Poultry	31 [9.1-61.4]							69.2				7.7	23.1					
	Cattle	39 [21.5-59.4]							60.7				7.1	32.1					
	Pigs	36 [32.0-40.2]							63.1	0.9		1.3	4.6	30.2					
Chloramphenicol	Poultry	8 [0.2-36.0]							7.7	61.5	15.4	7.7			7.7				
	Cattle	7 [0.9-23.5]							10.7	46.4	35.7				7.1				
	Pigs	9 [6.7-11.7]							6.2	55.9	27.1	1.8	0.2	0.9	7.9				
Florfenicol	Poultry	8 [0.2-36.0]							15.4	69.2	7.7		7.7						
	Cattle	7 [0.9-25.3]							10.7	64.3	17.9		7.1						
	Pigs	5 [3.0-6.7]							8.0	70.7	15.9	0.7	3.8	0.5	0.2				
Ampicillin	Poultry	39 [13.9-68.4]						53.8	7.7					38.5					
	Cattle	25 [10.7-44.9]						50.0	25.0					25.0					
	Pigs	19 [15.8-22.6]						68.2	11.7	1.1				19.0					
Amoxicillin/clavulanic acid a)	Poultry	0 [0.0-24.7]							61.5	7.7	23.1	7.7							
	Cattle	0 [0.0-12.3]							75.0	3.6	10.7	10.7							
	Pigs	0 [0.0-0.7]							81.0	1.6	10.8	6.6							
Cephalothin	Poultry	0 [0.0-24.7]							61.5	15.4	23.1								
	Cattle	0 [0.0-12.3]							46.4	32.1	21.4								
	Pigs	<1 [0.1-1.6]							50.6	34.4	11.3	2.7	0.5						
Ceftiofur	Poultry	0 [0.0-24.7]					92.3	7.7											
	Cattle	0 [0.0-12.3]					50.0	50.0											
	Pigs	0 [0.0-0.7]					54.7	42.0	3.3										
Sulfonamide	Poultry	31 [9.1-61.4]												69.2					30.8
	Cattle	39 [21.5-59.4]												53.6	7.1				39.3
	Pigs	36 [32.0-40.2]												61.8	2.0	0.2		0.2	35.8
Trimethoprim	Poultry	8 [0.2-36.0]								92.3				7.7					
	Cattle	0 [0.0-12.3]								100									
	Pigs	9 [6.9-11.9]								90.9				9.1					
Apramycin	Poultry	0 [0.0-24.7]								100									
	Cattle	0 [0.0-12.3]								100									
	Pigs	2 [1.0-3.6]								97.4	0.5				2.0				
Gentamicin	Poultry	0 [0.0-24.7]						100											
	Cattle	0 [0.0-12.3]						100											
	Pigs	2 [0.8-3.1]						96.9	0.7		0.7	0.4	0.5	0.7					
Neomycin	Poultry	0 [0.0-24.7]							100										
	Cattle	4 [0.1-18.3]							96.4					3.6					
	Pigs	8 [5.4-10.0]							91.8	0.5	0.2		0.5	6.9					
Spectinomycin	Poultry	8 [0.2-36.0]										15.4	76.9			7.7			
	Cattle	11 [2.3-28.2]											89.3			10.7			
	Pigs	14 [11.1-17.1]										0.7	80.4	4.9	1.6	12.2			
Streptomycin	Poultry	23 [5.0-53.8]								15.4	46.2	15.4		7.7	15.4				
	Cattle	36 [18.6-55.9]								7.1	42.9	14.3	3.6	7.1	25.0				
	Pigs	34 [29.9-38.0]								3.5	53.7	9.0	1.8	5.5	26.5				
Ciprofloxacin	Poultry	0 [0.0-24.7]	92.3	7.7															
	Cattle	4 [0.1-18.3]	85.7	10.7	3.6														
	Pigs	<1 [0.04-1.3]	94.3	5.3			0.4												
Nalidixic acid	Poultry	0 [0.0-24.7]									100								
	Cattle	4 [0.1-18.3]									96.4					3.6			
	Pigs	<1 [0.04-1.3]									99.3	0.4				0.4			
Colistin	Poultry	0 [0.0-24.7]								100									
	Cattle	0 [0.0-12.3]								100									
	Pigs	0 [0.0-0.7]								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

Typhimurium isolates from poultry, pigs and humans are presented.

The frequency of resistance was similar among *S*. Typhimurium isolates from poultry, cattle and pigs. In general, similar frequencies of resistance were observed in isolates from domestically acquired human cases and isolates from food animals. In *S*. Typhimurium isolates from travel associated human cases the level of resistance was generally higher than in domistically acquired cases.

As the proportion of multi-resistant *S*. Typhimurium among the isolates has a strong influence on the frequency of resistance, resistance in *S*. Typhimurium belonging to phage types other than DT104/104a/104b and DTU302, are presented in Table 22**.**
In this group the frequency of resistance in isolates from poultry, cattle and pigs was similar to the frequency of resistance in domestically acquired human cases.

Table 20. Comparison of resistance (%) among Salmonella *Enteritidis from food animals, imported foods and human cases acquired domestically or associated with travel abroad, Denmark* DANMAP 2003

Compound	Poultry	Broiler meat	Humans	
	Danish %	Imported %	Domestic a) %	Travel abroad %
Tetracyclines	0	3	2	1
Chloramphenicol	0	0	0	0
Ampicillin	0	3	3	1
Ceftiofur	0	0	0	0
Sulfonamide	0	6	2	2
Trimethoprim	0	0	<1	1
Apramycin	0	0	0	0
Gentamicin	0	6	0	0
Spectinomycin	0	6	<1	0
Streptomycin	0	14	<1	0
Nalidixic acid	14	49	15	24
Colistin	0	0	0	0
Number of isolates	14	35	326	86

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

DANMAP 2003

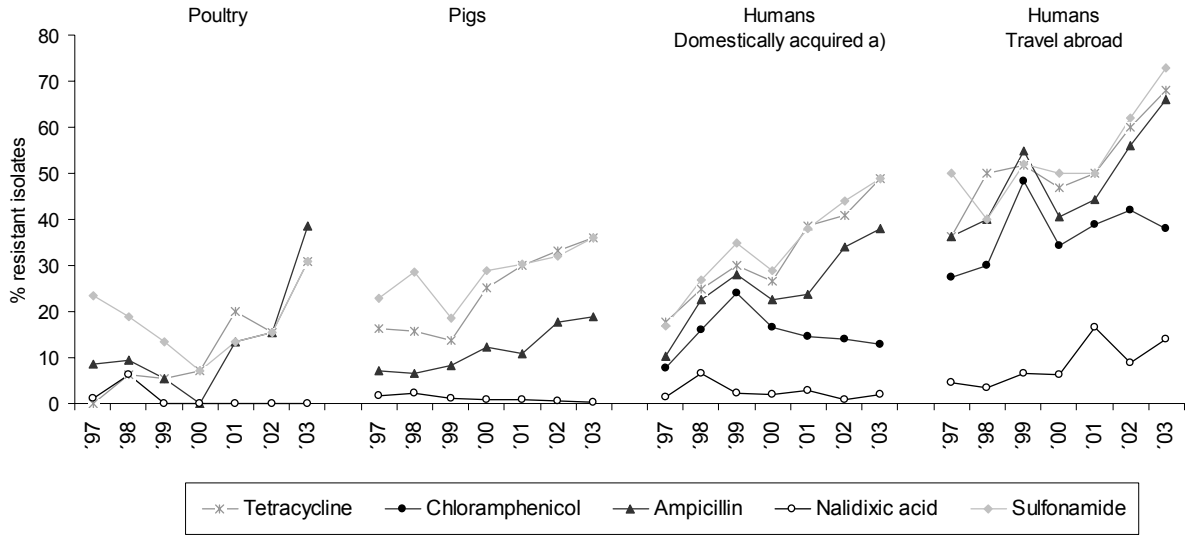

Figure 7. Trends in resistance to selected antimicrobials among Salmonella *Typhimurium isolated from poultry and pigs and from human cases, Denmark*

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

Table 21. Comparison of resistance (%) among Salmonella *Typhimurium from food animals, imported foods and human cases acquired domestically or associated with travel abroad, Denmark*

DANMAP 2003

Compound	Poultry	Cattle	Pigs	Pork	Humans	
	Danish %	Danish %	Danish %	Imported %	Domestic a) %	Travel abroad %
Tetracycline	31	39	36	93	49	68
Chloramphenicol	8	7	9	26	13	38
Florfenicol	8	7	5	24	10	30
Ampicillin	39	25	19	60	38	66
Ceftiofur	0	0	0	0	0	2
Sulfonamide	31	39	36	64	49	73
Trimethoprim	8	0	9	14	3	13
Apramycin	0	0	2	0	1	2
Gentamicin	0	0	2	0	2	11
Spectinomycin	8	11	14	33	15	45
Streptomycin	23	36	34	60	49	68
Nalidixic acid	0	4	<1	7	2	14
Colistin	0	0	<1	0	0	0
Number of isolates	13	28	547	42	392	56

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

Table 22. Comparison of resistance (%) among Salmonella *Typhimurium other than DT104, DT104a, DT104b and DTU302 from food animals and human cases acquired domestically, Denmark*

DANMAP 2003

Compound	Poultry	Cattle	Pigs	Humans
	Danish %	Danish %	Danish %	Domestically acquired a) %
Tetracyclines	18	39	34	37
Chloramphenicol	0	0	5	4
Ampicillin	27	22	16	20
Ceftiofur	0	0	0	0
Sulfonamide	18	39	34	35
Trimethoprim	9	0	9	4
Apramycin	0	0	2	1
Gentamicin	0	0	2	2
Spectinomycin	0	4	11	8
Streptomycin	9	35	32	37
Nalidixic acid	0	0	<1	2
Colistin	0	0	0	0
Number of isolates	11	23	502	283

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

Table 18b. Distribution of MICs and occurrence of resistance in Salmonella *Typhimurium from pork (n=51), Denmark*

DANMAP 2003

Compound	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	84	[71.4-93.0]							15.7			2.0	11.8	70.6					
Chloramphenicol	22	[11.3-35.3]							2.0	47.1	29.4				21.6				
Florfenicol	20	[9.8-33.1]							3.9	68.6	7.8		11.8		7.8				
Ampicillin	57	[42.2-70.7]						37.3	5.9					56.9					
Amoxicillin/clavulanic acid a)	2	[0.05-10.4]							43.1	9.8	17.6	27.5	2.0						
Cephalothin	0	[0.0-7.0]							25.5	47.1	25.5	2.0							
Ceftiofur	0	[0.0-7.0]					56.9	43.1											
Sulfonamide	59	[44.2-72.4]												39.2	2.0				58.8
Trimethoprim	12	[4.4-23.9]								88.2				11.8					
Apramycin	0	[0.0-7.0]								100									
Gentamicin	0	[0.0-7.0]						100											
Neomycin	0	[0.0-7.0]							100										
Spectinomycin	28	[15.9-41.7]											70.6	2.0		27.5			
Streptomycin	57	[42.2-70.7]								2.0	39.2	2.0	2.0	11.8	43.1				
Ciprofloxacin	6	[1.2-16.2]	92.2	2.0	5.9														
Nalidixic acid	3	[1.2-16.2]									92.2	2.0				5.9			
Colistin	0	[0.0-7.0]								100									

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

Campylobacter

In 2003, there were 3,542 laboratory confirmed cases of human campylobacteriosis in Denmark making it the most common bacterial cause of diarrheal illness. This corresponds to an incidence rate of 65.8 per 100,000 inhabitants and reflects a drop in the number of cases by 19% over the previous year. *Campylobacter* isolates from human infections were predominantly *Campylobacter jejuni* and the remaining were *Campylobacter coli.* Among *Campylobacter* isolates from poultry and cattle *C. jejuni* remains the most common species, while in pigs *C. coli* is the most common species.

Campylobacter from food animals

Table 23 presents the MIC distributions and occurrence of antimicrobial resistance among *C. jejuni* from broilers and cattle in 2003 and Table 24 presents data for *C. coli* from pigs in 2003. Trends in resistance to selected antimicrobials among *C. jejuni* and *C. coli* from 1996 to 2003 are presented in Figures 8 and 9, respectively. In *C. jejuni* isolates from broilers and cattle low levels of antimicrobial resistance were observed and no significant changes in the occurrence of antimicrobial resistance were observed in 2003. In *C. coli* isolates from pigs resistance to erythromycin (24%) and streptomycin (49%) was most frequently observed. The proportions of resistant isolates remained unchanged from 2002 to 2003 for all antimicrobials tested.

Campylobacter from food

Eighty-nine *C. jejuni* isolates obtained from poultry meat samples collected at retail outlets were subjected to susceptibility testing. The results are presented in Table 25. Resistance to tetracycline, nalidixic acid, streptomycin and ciprofloxacin was detected. The occurrence of resistance to tetracycline was significantly higher in isolates from imported broiler meat (42%) than in broiler meat of Danish origin (5%)(Table 26).

Campylobacter in humans

Species determination and serotyping were available for 115 (3%) of all *Campylobacter* isolates reported to the Unit of Gastrointestinal Infections at the Statens Serum Institut. Among these, 107 (93%) were *C. jejuni.* Table 26 shows the occurrence of resistance among *C. jejuni* isolates from humans by origin of infection. Due to the low number of *C. coli* isolates, resistance data for this species are not presented. Trends in resistance to selected antimicrobials among *C. jejuni* in domestically acquired cases are shown in Figure 8.

In previous years resistance in *C. jejuni* was generally higher in isolates from infections associated with travel abroad than in isolates from infections acquired in Denmark. In 2003, resistance to tetracycline, gentamicin, nalidixic acid and ciprofloxacin was higher in *C. jejuni* isolates from infections acquired abroad than from infections acquired in Denmark (Table 26). Isolates of *C. jejuni* were generally susceptible to erythromycin. Most *Campylobacter* infections do not

Table 23. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *isolates from broilers (n=84) and cattle (n=53), Denmark*

DANMAP 2003

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs													
				0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	Broilers	2	[0.3-8.3]					97.6						2.4			
	Cattle	0	[0.0-6.7]					100									
Chloramphenicol	Broilers	0	[0.0-4.3]						1.2	47.6	50.0	1.2					
	Cattle	0	[0.0-6.7]						1.9	30.2	67.9						
Erythromycin	Broilers	0	[0.0-4.3]					8.3	67.9	22.6	1.2						
	Cattle	0	[0.0-6.7]					7.5	56.6	34.0	1.9						
Gentamicin	Broilers	0	[0.0-4.3]					84.5	15.5								
	Cattle	0	[0.0-6.7]					90.6	7.5	1.9							
Neomycin	Broilers	1	[0.03-6.5]						79.8	17.9	1.2				1.2		
	Cattle	0	[0.0-6.7]						75.5	22.6	1.9						
Streptomycin	Broilers	0	[0.0-4.3]						34.5	57.1	7.1	1.2					
	Cattle	2	[0.05-10.1]						41.5	49.1	7.5				1.9		
Ciprofloxacin	Broilers	4	[0.7-10.1]		2.4	66.7	25.0	2.4					3.6				
	Cattle	8	[2.1-18.2]		3.8	45.3	43.4					1.9	5.7				
Nalidixic acid	Broilers	4	[0.7-10.1]								41.7	52.4	2.4			1.2	2.4
	Cattle	8	[2.1-18.2]								32.1	56.6	3.8			1.9	5.7

Vertical lines indicate breakpoints for resistance

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

Table 24. Distribution of MICs and occurrence of resistance among Campylobacter coli *isolates from pigs (n=98), Denmark*

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs													
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	1	[0.03-5.6]					91.8	6.1	1.0					1.0		
Chloramphenicol	0	[0.0-3.7]						3.1	28.6	45.9	18.4	4.1				
Erythromycin	24	[15.5-33.1]				6.1	7.1	10.2	25.5	22.4	5.1			23.5		
Gentamicin	0	[0.0-3.7]					35.7	50.0	14.3							
Neomycin	0	[0.0-3.7]						8.2	65.3	13.3	13.3					
Streptomycin a)	48	[38.2-58.8]						3.1	15.5	28.9	4.1			7.2	41.2	
Ciprofloxacin	3	[0.6-8.7]		9.2	45.9	35.7	5.1	1.0				2.0	1.0			
Nalidixic acid	3	[0.6-8.7]								7.1	75.5	14.3			2.0	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) 97 isolates from pigs were tested against streptomycin

Table 25. Susceptibility and occurrence of resistance among Campylobacter jejuni *from broiler meat (n=89), Denmark*

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs														
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline	10	[4.7-18.3]					87.6		1.1		1.1			10.1				
Chloramphenicol a)	0	[0.0-4.1]						2.3	54.5	37.5	4.5	1.1						
Erythromycin	0	[0.0-4.1]					2.2	30.3	61.8	5.6								
Gentamicin	0	[0.0-4.1]					100											
Streptomycin a)	1	[0.03-6.2]						96.7	2.3						1.1			
Ciprofloxacin	2	[0.7-9.5]	2.2	4.5	50.6	36.0	1.1	2.2	1.1		2.2							
Nalidixic acid	2	[0.3-7.9]							16.9	69.7	11.2					2.2		

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
a) 88 isolates from broiler meat were tested against chloramphenicol and streptomycin

require antimicrobial treatment, however, these results should be taken into account prior to prescribing any necessary antimicrobial treatment to patients with *Campylobacter* infections. Doctors should inquire into the patient's travel history before considering treatment with fluoroquinolones because of the high probability of resistance to these antimicrobials in *Campylobacter* infections acquired outside Denmark.

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 26. In 2003, resistance to tetracycline, streptomycin, ciprofloxacin and nalidixic acid was noted in isolates from food animals, food and human cases with infection acquired in Denmark (Table 26).

The occurrence of resistance to chloramphenicol, ciprofloxacin and nalidixic acid was significantly higher in isolates from domestically acquired human cases compared with isolates from Danish broiler meat.

One explanation could be that information about travel is not systematically registered and some cases reported as domestically acquired have actually been acquired abroad. For isolates from Danish broiler meat and domestically acquired human cases differences were significant for chloramphenicol, ciprofloxacin and nalidixic acid. This may indicate that sources other than Danish broiler meat contribute to *C. jejuni* infections in humans.
In 2003, resistance to tetracycline among isolates from human cases with infection acquired in Denmark continued to decrease (Figure 8). Among *C. jejuni* isolates in cattle a significant decrease in tetracycline resistance was noted in 2003 compared to 2002. Resistance to erythromycin was noted in 1% of *C. jejuni* isolates from humans with infection acquired in Denmark. As in the previous year, no resistance to erythromycin was detected in *C. jejuni* isolates from cattle and broilers in 2003 (Figure 8).

Table 26. Susceptibility and occurrence of resistance among Campylobacter jejuni *from Danish food animals, foods of Danish origin, imported foods and from human cases acquired domestically or associated with travel, Denmark*

DANMAP 2003

Compound	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestic a)	Travel abroad
	%	%	%	%	%	%
Tetracycline	0	2	5	42	13	43
Chloramphenicol b)	0	0	0	0	6	7
Erythromycin	0	0	0	0	1	7
Gentamicin	0	0	0	0	1	21
Streptomycin b)	2	0	1	0	3	14
Ciprofloxacin	8	4	1	8	17	64
Nalidixic acid	8	4	1	8	17	64
Number of isolates	53	84	77	12	93	14

a) Includes cases where origin of infection is non-documented and may therefore include some isolates acquired abroad but not documented as such
b) 76 isolates from Danish broiler meat were tested against chloramphenicol an streptomycin

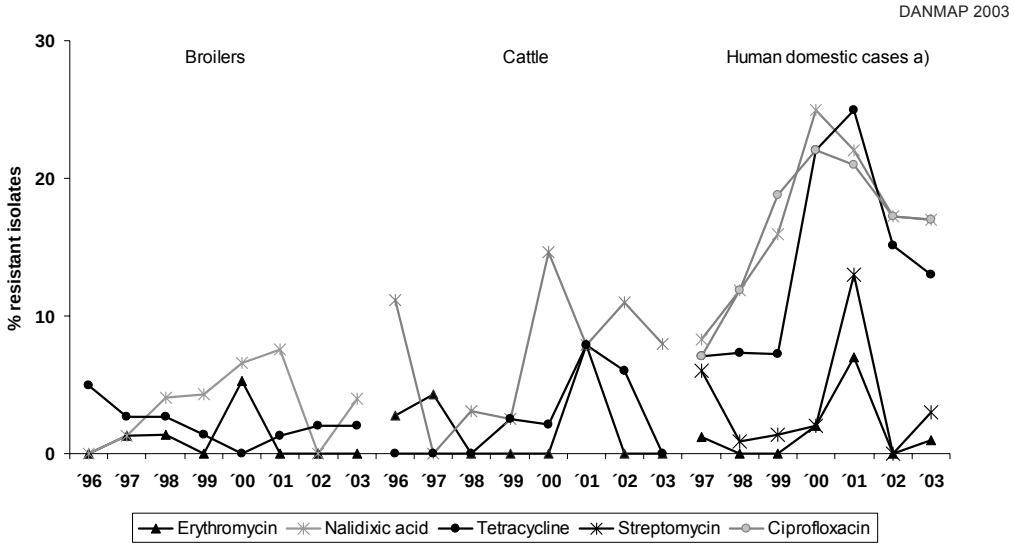

Figure 8. Trends in resistance to selected antimicrobials among Campylobacter jejuni *isolates from broilers, cattle and human domestic cases, Denmark*

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

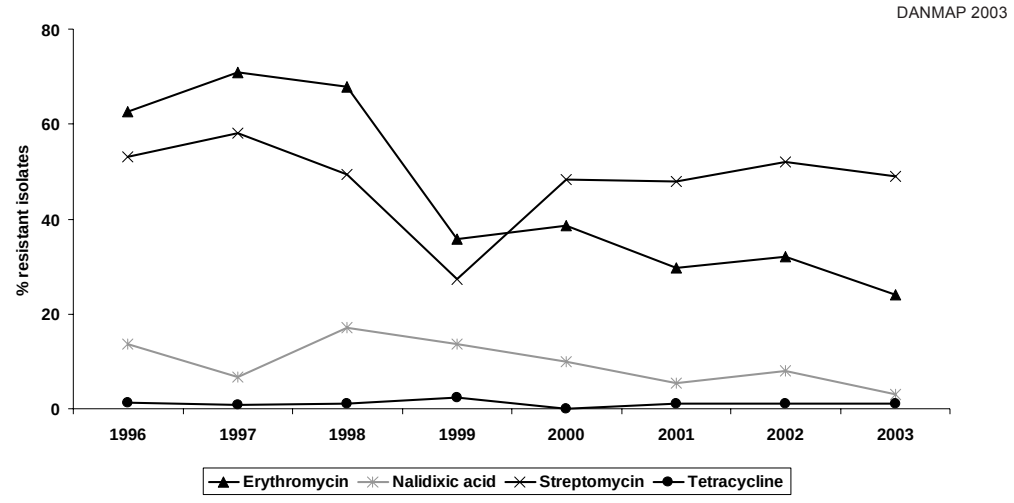

Figure 9. Trends in resistance to selected antimicrobials among Campylobacter coli *isolates from pigs, Denmark*

Resistance in indicator bacteria

Enterococci from food animals

Enterococci from food animals were isolated from faecal samples from cattle and pigs, and cloacal swabs from broilers. All samples were collected at slaughter.

The MIC distribution and the occurrence of resistance among enterococci from food animals are shown in Tables 27 and 28.

Among *Enterococcus faecium* isolates from broilers, cattle and pigs the proportions of resistant and susceptible isolates remained unchanged from 2002 to 2003, except for a significant decrease in resistance to erythromycin (from 30% in 2002 to 21% in 2003) among isolates from pigs (Table 27 and Figures 10-15). This decrease in occurrence of resistance

Table 27.Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broilers (n=123), cattle (n=27) and pigs (n=175), Denmark*

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs														
				0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	5	[1.8-10.3]			95.1					3.3	1.6						
	Cattle	0	[0.0-12.8]			100												
	Pigs	50	[42.6-57.9]			48.0		1.1	0.6		6.3	44.0						
Chloramphenicol	Broilers	0	[0.0-3.0]				0.8	29.3	69.9									
	Cattle	0	[0.0-12.8]					18.5	81.5									
	Pigs	<1	[0.01-3.1]				2.9	31.4	65.1		0.6							
Florfenicol	Broilers	<1	[0.02-4.4]				39.0	60.2					0.8					
	Cattle	0	[0.0-12.8]				14.8	85.2										
	Pigs	0	[0.0-2.1]				27.4	72.6										
Penicillin	Broilers	54	[44.4-62.7]				19.5	13.8	13.0	7.3	37.4	8.9						
	Cattle	0	[0.0-12.8]				48.1	33.3	18.5									
	Pigs	39	[32.1-47.1]				24.6	20.6	15.4	25.7	13.7							
Erythromycin	Broilers	17	[10.9-24.9]		13.8	53.7	11.4	4.1	3.3	5.7	4.1	4.1						
	Cattle	4	[0.1-19.0]			40.7	33.3	22.2	3.7									
	Pigs	21	[14.8-27.3]			16.6	36.0	26.9	2.9	0.6		17.1						
Gentamicin	Broilers	0	[0.0-3.0]					3.3	15.4	1.6			79.7					
	Cattle	0	[0.0-12.8]										100					
	Pigs	0	[0.0-2.1]										99.4		0.6			
Kanamycin	Broilers	0	[0.0-3.0]										35.0	47.2	16.3	1.6		
	Cattle	0	[0.0-12.8]										59.3	22.2	11.1	7.4		
	Pigs	19	[13.4-25.5]										49.7	18.3	12.0	1.1		18.9
Streptomycin	Broilers	7	[3.4-13.4]										91.9	0.8				7.3
	Cattle	0	[0.0-12.8]										100					
	Pigs	19	[13.8-26.1]										74.9		0.6	5.1	5.1	14.3
Teicoplanin	Broilers	3	[0.5-7.0]		95.8	0.8				0.8	2.5							
	Cattle	0	[0.0-12.8]		100													
	Pigs	2	[0.6-5.7]		96.0	1.1				0.6	2.3							
Vancomycin	Broilers	3	[0.9-8.1]				95.9	0.8			0.8	2.4						
	Cattle	0	[0.0-12.8]				96.3	3.7										
	Pigs	3	[0.9-6.5]				92.0	2.3	2.9			2.9						
Quinupristin/dalfopristin	Broilers	25	[17.8-33.8]		22.0	15.4	37.4	18.7	6.5									
	Cattle	0	[0.0-12.8]		48.1	3.7	48.1											
	Pigs	9	[4.9-13.7]		19.4	8.6	63.4	8.0		0.6								
Avilamycin	Broilers	7	[2.8-12.4]			5.7	32.5	43.9	11.4	4.1	0.8	1.6						
	Cattle	0	[0.0-12.8]				77.8	22.2										
	Pigs	0	[0.0-2.1]			25.1	58.3	16.6										
Salinomycin	Broilers	<1	[0.02-4.4]			7.3	3.3	8.9	79.7	0.8								
	Cattle	0	[0.0-12.8]			88.9	11.1											
	Pigs	0	[0.0-2.1]			91.4	8.0		0.6									
Linezolid	Broilers	0	[0.0-3.0]		2.4	13.8	83.7											
	Cattle	0	[0.0-12.8]				100											
	Pigs	0	[0.0-2.1]		0.6	9.1	90.3											

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

coincided with a 12% reduction in macrolide consumption in pigs from 2001 to 2003 (Figure 2). In contrast, the substantial increase in consumption of tetracycline and penicillins in pigs did not have any immediate effect on the occurrence of 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%) since 1999. In 2003, 54% of *E. faecium* isolates from broilers were resistant to penicillin. The consumption of penicillins (amoxicillin) in broilers was reduced by almost 50% from 2002 to 2003.

Penicillins remain the most widely used group of antimicrobials in broilers (Table 9a), and the overall consumption of antimicrobials in broilers was substantially reduced from 2002 to 2003.

Among *E. faecium* isolates from cattle, valid comparison between years is hampered by low sample size. No significant changes in the proportions of resistant and sensitive isolates were observed from 2002 to 2003. The consumption of penicillins, macrolides and aminoglycosides in cattle (calves < 12 months) increased in 2003, without any immediate effect on the occurrence of resistance in *E. faecium* isolates.

Among *Enterococcus faecalis* isolates from broilers and pigs no significant change in the occurrence of resistance were observed from 2002 to 2003. Resistance to tetracycline, erythromycin and streptomycin remains common among *E. faecalis* isolates from pigs (Table 28).

Enterococci from food

Isolation of enterococci from samples of broiler meat, beef and pork from retail outlets yielded 199 isolates of *E. faecium* and 213 isolates of *E. faecalis* in 2003.

The MIC distribution and occurrence of resistance among enterococci from foodstuff are shown in Tables 29 and 30.

Among *E. faecium* and *E. faecalis* isolates from broiler meat, pork and beef the proportions of resistant and sensitive isolates remained unchanged from 2002 to

Table 28. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broilers (n=66) and pigs (n=207), Denmark*

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs														
				0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	46	[34.0-58.9]			50.7	1.5	1.5			22.4	23.9						
	Pigs	82	[75.7-86.7]			17.4	0.5	0.5		4.3	14.0	63.3						
Chloramphenicol	Broilers	0	[0.0-5.4]				1.5	55.2	41.8	1.5								
	Pigs	3	[1.1-6.2]				0.5	5.8	87.4	3.4		1.9	1.0					
Florfenicol	Broilers	0	[0.0-5.4]				85.1	14.9										
	Pigs	0	[0.0-1.8]				17.4	82.6										
Penicillin	Broilers	0	[0.0-5.4]				24.2	74.2	1.5									
	Pigs	<1	[0.01-2.7]				15.9	82.6	1.0	0.5								
Erythromycin	Broilers	18	[9.6-29.2]			43.3	25.4	13.4		3.0	4.5	10.4						
	Pigs	41	[33.8-47.6]			39.6	18.8	1.0			0.5	40.1						
Gentamicin	Broilers	0	[0.0-5.4]										100					
	Pigs	9	[5.6-14.0]										90.8			1.9	1.4	5.8
Kanamycin	Broilers	0	[0.0-5.4]										100					
	Pigs	20	[15.0-26.4]										79.2	0.5				20.3
Streptomycin	Broilers	3	[0.4-10.4]										91.0	6.0				3.0
	Pigs	35	[28.8-42.2]										48.8	14.0	0.5	1.4	4.3	30.9
Teicoplanin	Broilers	0	[0.0-5.4]		100													
	Pigs	0	[0.0-1.8]		100													
Vancomycin	Broilers	0	[0.0-5.4]				97.0	3.0										
	Pigs	0	[0.0-1.8]				96.6	3.4										
Avilamycin	Broilers	0	[0.0-5.4]			16.4	77.6	4.5	1.5									
	Pigs	<1	[0.01-2.7]			23.2	70.5	5.3	0.5	0.5								
Flavomycin	Broilers	3	[0.4-10.4]		17.9	47.8	25.4	6.0				3.0						
	Pigs	<1	[0.01-2.7]		34.3	50.7	13.0	1.0	0.5			0.5						
Salinomycin	Broilers	0	[0.0-5.4]			67.2	6.0	20.9	6.0									
	Pigs	0	[0.0-1.8]			100												
Linezolid	Broilers	0	[0.0-5.4]		3.0	58.2	37.3	1.5										
	Pigs	0	[0.0-1.8]			19.8	80.2											

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

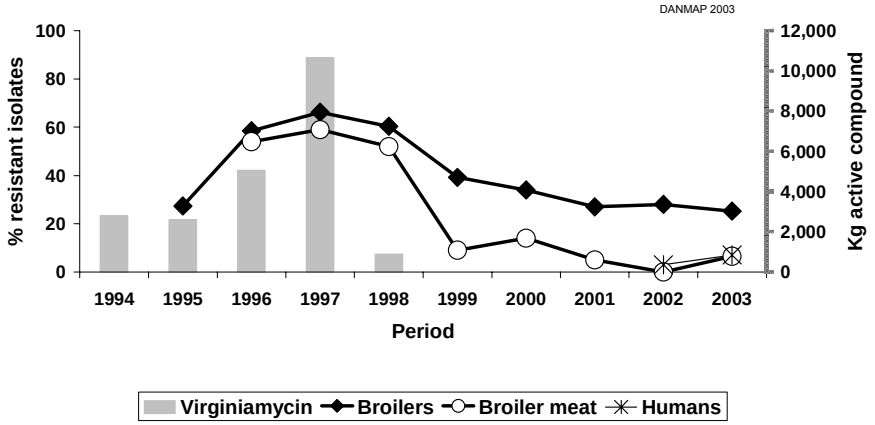

Figure 10. Trends in streptogramin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

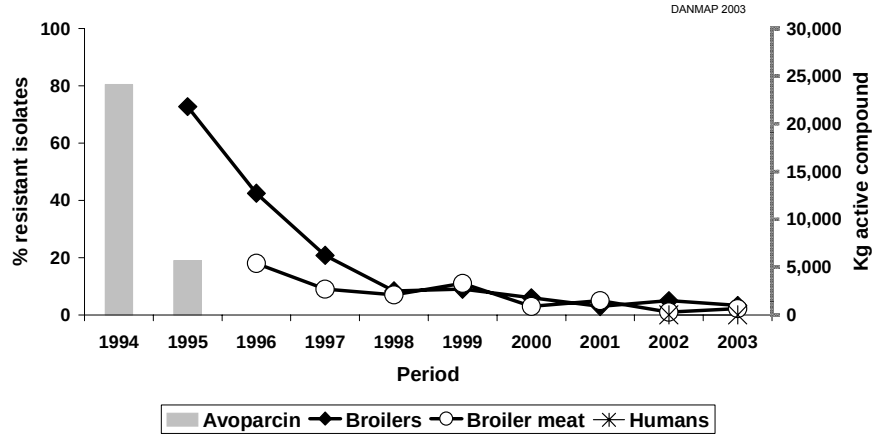

Figure 11. Trends in glycopeptide resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avoparcin in animals, Denmark*

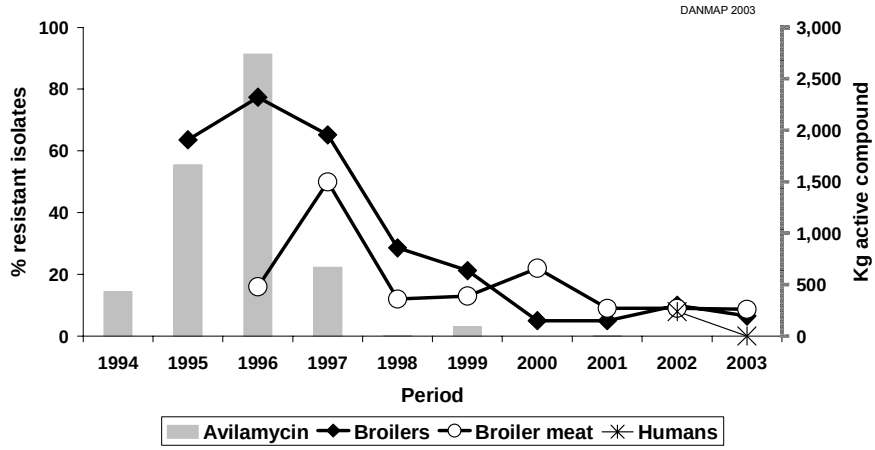

Figure 12. Trends in avilamycin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avilamycin in animals, Denmark*

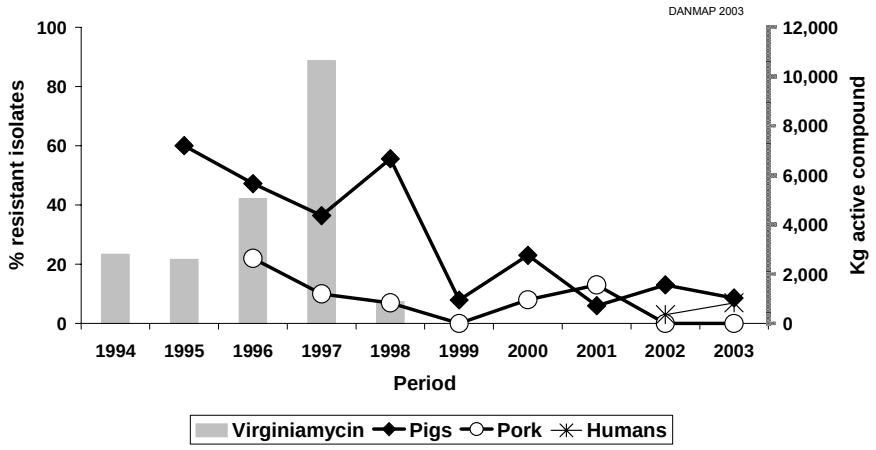

Figure 13. Trends in streptogramin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

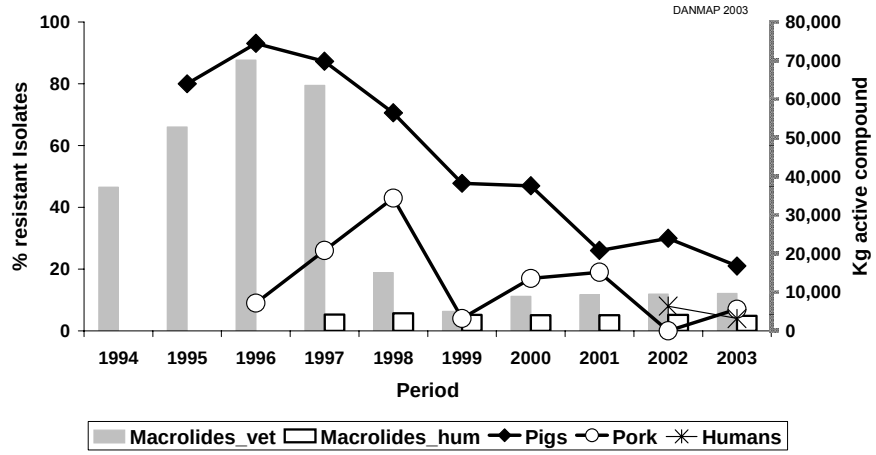

Figure 14. Trends in erythromycin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the total consumption of macrolides, both as growth promoters in animals and therapeutics in animals and humans, Denmark*

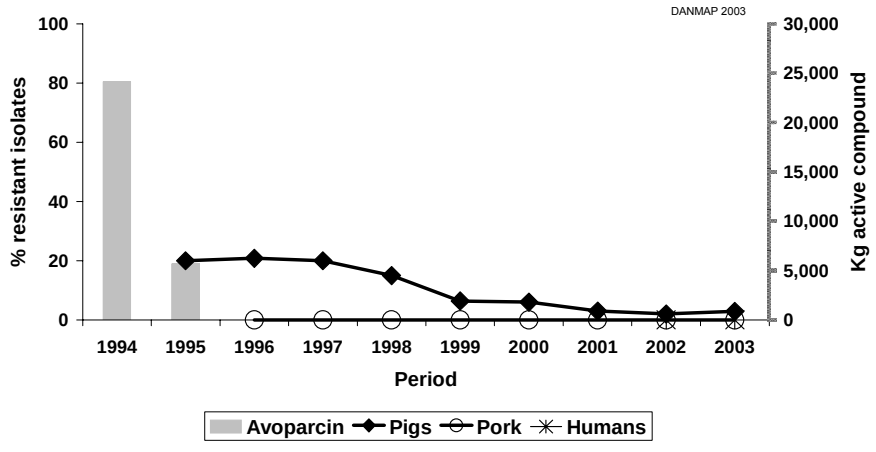

Figure 15. Trends in glycopeptide resistance among Enterococcus faecium *from pigs and pork and the consumption of the growth promoter avoparcin, Denmark*

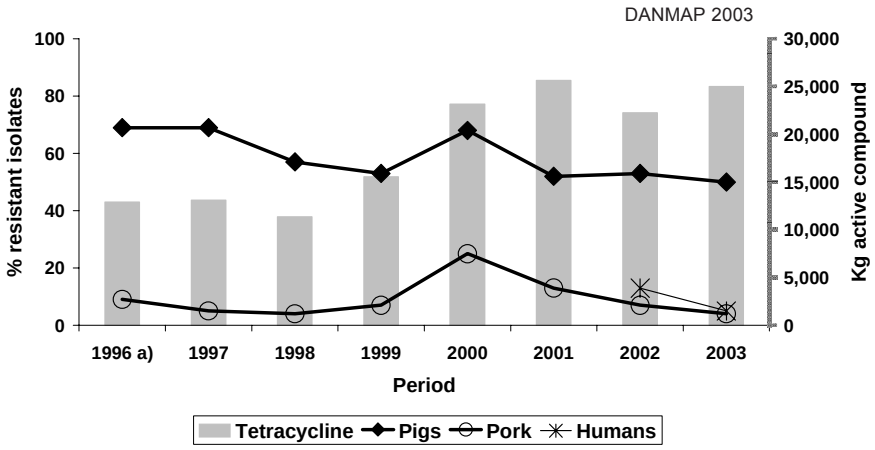

Figure 16. Trends in tetracycline resistance among Enterococcus faecium from pigs, pork and healthy humans and the comsumption of tetracycline in pig production, Denmark

Table 29. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broiler meat (n=92), beef (n=62) and pork (n=45), Denmark*

DANMAP 2003

Compound	Food type	% 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	Broiler meat	10	[4.6-17.8]			89.1			1.1			9.8						
	Beef	2	[0.04-8.7]			98.4						1.6						
	Pork	4	[0.5-15.1]			95.6						4.4						
Chloramphenicol	Broiler meat	0	[0.0-3.9]				32.6	39.1	28.3									
	Beef	0	[0.0-5.8]				16.1	37.1	46.8									
	Pork	2	[0.1-11.8]				31.1	35.6	31.1		2.2							
Florfenicol	Broiler meat	0	[0.0-3.9]				75.0	25.0										
	Beef	0	[0.0-5.8]				71.0	29.0										
	Pork	0	[0.0-7.9]				73.3	26.7										
Penicillin	Broiler meat	19	[11.1-27.9]				64.1	9.8	7.6	7.6	10.9							
	Beef	0	[0.0-5.8]				79.0	21.0										
	Pork	2	[0.1-11.8]				84.4	13.3		2.2								
Erythromycin	Broiler meat	21	[12.9-30.4]			65.2	10.9	3.3	2.2	4.3	1.1	13.0						
	Beef	3	[0.4-11.2]			64.5	27.4	4.8	1.6			1.6						
	Pork	7	[1.4-18.3]			48.9	31.1	13.3	4.4	2.2								
Gentamicin	Broiler meat	0	[0.0-3.9]										100					
	Beef	0	[0.0-5.8]										100					
	Pork	0	[0.0-7.9]										100					
Kanamycin	Broiler meat	0	[0.0-3.9]										34.8	39.1	22.8	3.3		
	Beef	2	[0.04-8.7]										45.2	29.0	21.0	3.2		1.6
	Pork	0	[0.0-7.9]										51.1	28.9	15.6	4.4		
Streptomycin	Broiler meat	2	[0.3-7.6]										95.7	1.1		1.1	1.1	1.1
	Beef	2	[0.04-8.7]										98.4					1.6
	Pork	0	[0.0-7.9]										100					
Teicoplanin	Broiler meat	2	[0.3-7.6]		97.8						2.2							
	Beef	0	[0.0-5.8]		100													
	Pork	0	[0.0-7.9]		95.6	4.4												
Vancomycin	Broiler meat	2	[0.3-7.6]				96.7	1.1				2.2						
	Beef	0	[0.0-5.8]				98.4	1.6										
	Pork	0	[0.0-7.9]				100											
Quinupristin/dalfopristin	Broiler meat	7	[2.4-13.7]		47.8	15.2	30.4	2.2	2.2	1.1	1.1							
	Beef	3	[0.4-11.2]		40.3	12.9	43.5	1.6			1.6							
	Pork	0	[0.0-7.9]		15.6	26.7	57.8											
Avilamycin	Broiler meat	9	[3.8-16.4]			19.6	40.2	25.0	6.5		3.3	5.4						
	Beef	0	[0.0-5.8]			24.2	32.3	37.1	6.5									
	Pork	2	[0.1-11.8]			37.8	37.8	20.0	2.2			2.2						
Salinomycin	Broiler meat	0	[0.0-3.9]			32.6	6.5	28.3	32.6									
	Beef	0	[0.0-5.8]			88.7	9.7	1.6										
	Pork	0	[0.0-7.9]			88.9	6.7		4.4									
Linezolid	Broiler meat	0	[0.0-3.9]		7.6	39.1	52.2	1.1										
	Beef	0	[0.0-5.8]		3.2	29.0	61.3	6.5										
	Pork	0	[0.0-7.9]	2.2	6.7	35.6	46.7	8.9										

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

2003, except for a significant decrease in resistance to tetracycline (from 28% in 2002 to 10% in 2003) among isolates from broiler meat.

Enterococci from healthy human volunteers

In 2003, stool samples from 124 healthy human volunteers were collected. In total 55 *E. faecium* isolates and 73 *E. faecalis* isolates were obtained. Please see Appendix 1 for details on specimen collection.

The MIC distribution and occurrence of resistance among enterococci from humans are shown in Tables 31 and 32.

Resistance towards penicillin (11%) was most common among *E. faecium* isolates.
Resistance to tetracycline (21%) was most common among *E. faecalis* isolates.
None of the randomly selected enterococcal isolates were resistant to vancomycin or teicoplanin. A single *E. faecalis* isolate was resistant to gentamicin, whereas none of the *E. faecium* isolates were gentamicin resistant.

Using a vancomycin-resistant enterococcus selective method a single vancomycin resistant *E. faecium* isolate was detected in stool samples from healthy humans (Textbox 1, page 38).

Table 30. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broiler meat (n=40), beef (n=95) and pork (n=78), Denmark*

DANMAP 2003

Compound	Food type	% 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	Broiler meat	35	[20.6-51.7]			65.0					10.0	25.0						
	Beef	18	[10.8-27.1]			82.2					2.1	15.8						
	Pork	24	[15.3-35.4]			75.6					2.6	21.8						
Chloramphenicol	Broiler meat	0	[0.0-8.8]				17.5	40.0	42.5									
	Beef	2	[0.3-7.4]				15.8	41.1	41.1			2.1						
	Pork	1	[0.03-6.9]				19.2	37.2	42.3			1.3						
Florfenicol	Broiler meat	0	[0.0-8.8]				72.5	27.5										
	Beef	0	[0.0-3.8]				67.4	32.6										
	Pork	0	[0.0-4.6]				73.1	26.9										
Penicillin	Broiler meat	0	[0.0-8.8]				50.0	50.0										
	Beef	0	[0.0-3.8]				65.3	34.7										
	Pork	0	[0.0-4.6]				65.4	34.6										
Erythromycin	Broiler meat	28	[14.6-43.9]			47.5	22.5	2.5	2.5		2.5	22.5						
	Beef	6	[2.4-13.2]			67.4	24.2	2.1		1.1		5.3						
	Pork	5	[1.4-12.6]			92.3	1.3	1.3				5.1						
Gentamicin	Broiler meat	0	[0.0-8.8]										100					
	Beef	2	[0.3-7.4]										97.9					2.1
	Pork	0	[0.0-4.6]										100					
Kanamycin	Broiler meat	0	[0.0-8.8]										100					
	Beef	6	[2.4-13.2]										90.5	3.2				6.3
	Pork	4	[0.8-10.8]										96.2				1.3	2.6
Streptomycin	Broiler meat	3	[0.1-13.2]										82.5	7.5	7.5			2.5
	Beef	5	[1.7-11.9]										94.7					5.3
	Pork	6	[2.1-14.3]										89.7	2.6	1.3			6.4
Teicoplanin	Broiler meat	0	[0.0-8.8]		100													
	Beef	0	[0.0-3.8]		98.9		1.1											
	Pork	0	[0.0-4.6]		100													
Vancomycin	Broiler meat	0	[0.0-8.8]				95.0	5.0										
	Beef	0	[0.0-3.8]				97.9	2.1										
	Pork	0	[0.0-4.6]				98.7	1.3										
Avilamycin	Broiler meat	0	[0.0-8.8]			22.5	75.0	2.5										
	Beef	0	[0.0-3.8]			45.3	45.3	8.4	1.1									
	Pork	0	[0.0-4.6]			43.6	52.6	3.8										
Flavomycin	Broiler meat	0	[0.0-8.8]		12.5	57.5	27.5	2.5										
	Beef	5	[1.7-11.9]		16.8	43.2	23.2	7.4	4.2			5.3						
	Pork	0	[0.0-4.6]		20.5	38.5	35.9	5.1										
Salinomycin	Broiler meat	0	[0.0-8.8]			65.0	5.0	27.5	2.5									
	Beef	0	[0.0-3.8]			97.9	1.1		1.1									
	Pork	0	[0.0-4.6]			100												
Linezolid	Broiler meat	0	[0.0-8.8]	5.0	10.0	40.0	45.0											
	Beef	0	[0.0-3.8]	1.1	11.6	36.8	48.4	2.1										
	Pork	0	[0.0-4.6]	5.1	11.5	30.8	51.3	1.3										

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

Text box 1

Vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected seven years after the ban of avoparcin, is possibly related to pig isolates

The occurrence of vancomycin-resistant *Enterococcus faecium* (VREF) in faeces from food animals and from meat has been associated with the use of the glycopeptide, avoparcin, for growth promotion. Tn*1546*, the *vanA* gene cluster encoding vancomycin resistance, has been detected both in VREF of human and animal origin. The use of avoparcin was banned in Denmark in May 1995, and in all of the European Union in 1997. Between 1995 and 2002 the occurrence of VREF isolated from Danish pigs declined from 20% to 2% [DANMAP 2002]. The aim of the present study was to investigate if Danish healthy humans were carrying VREF seven years after the ban of avoparcin.

Between May 2002 and May 2003, 149 faecal samples were obtained from healthy volunteers [Hammerum *et al.* 2004. J. Antimicrob. Chemother. 53: 547-549]. High-level vancomycin resistant *Enterococcus* spp. were isolated using pre-enrichment in enterococcosel broth overnight at 35ºC followed by spread on bile aesculin agar with 16 mg/l vancomycin and incubated for 2 days at 35ºC (Appendix 1). One *vanA* VREF isolate, 841V03, was obtained from a 35 years old female office worker. She had not received antibiotics, was not a vegetarian and had eaten pork, broiler meat and beef.

A base pair variation has been found in *vanX* in position 8234 of Tn*1546* GenBank M97287. VREF isolated from humans contain either the G-variant or the T-variant, whereas isolates from pigs have the G-variant and poultry has the T-variant. [Jensen (1998) Antimicrob. Agents Chemother. 42:2463-4]. Two fragments of 239 and 185 bp were obtained by restriction digesting of the *vanX* PCR-product from 841V03, indicating a pig origin of the strain (G-variant). [Palepou *et al.* (1998). J. Antimicrob. Chemother. 42:605-12]. Earlier studies have shown that occurrence of VREF in the Danish pig population was primarily caused by the presence of a single *E. faecium* clone. [Aarestrup (2000). J. Clin. Microbiol. 38:2774-7, Hammerum *et al.* (2000). J. Antimicrob. Chemother. 45:677-80]. E8sv3 was chosen as a representative of the *vanA* pig clone [Hammerum *et al.* (2000). J. Antimicrob. Chemother. 45:677-80]. 841V03, E8sv3 and four *vanA* positive VREF isolates (S163-1, S166-3, S328-2 and S658-3) obtained from pigs in 2002 [DANMAP 2002] were typed by PFGE using *Sma*I. The obtained PFGE-profiles showed that 841V03 was related to three VREF pig isolates obtained in 2002. These three pig isolates were similar to the VREF clone detected from 1995. After banning of avoparcin, tetracycline and tylosin were still extensively used therapeutically in pigs in 2002. All the *vanA* VREF isolates in this study were resistant to tetracycline. Additionally three of the four pig isolates were erythromycin/tylosin resistant. Although 841V03 and one of the pig isolates were not resistant to erythromycin, they contained the same mutation in *vanX* and had related PFGE-patterns. This could indicate a possible pig origin of 841V03.

The use of the macrolide, tylosin, as a growth promoter was banned in all of European Union in July 1999. A decrease in the occurrence of VREF in the pig herds has been detected following the termination of the use of tylosin as a growth promoter and it reached 2% in 2002. We suggest that the persistence of VREF at a low level in the pig herds seven years after the ban of avoparcin may, in part, be related to the therapeutic use of tetracycline and tylosin.

The Scientific Ethics Committee for Copenhagen and Frederiksberg municipalities has approved the protocol ((KF) 01-006/02).

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Comparison of resistance in enterococci from farm, table and healthy human volunteers

Comparisons of resistance in enterococci from Danish food animals, foods of Danish and imported origin and humans are presented in Tables 33-34.

Like in previous years, the most pronounced differences between the frequency of resistance in food animals and food were observed between *E. faecium*/*E. faecalis* isolates from pigs and from Danish pork, where resistance to tetracycline, penicillin (*E. faecium* only), erythromycin, kanamycin, streptomycin and quinupristin/dalfopristin (*E. faecium* only) were significantly higher in isolates from pigs than in isolates from Danish pork.

In broilers, resistance to penicillin and quinupristin/dalfopristin in *E. faecium* isolates was significantly higher than in Danish broiler meat.

In isolates from Danish and imported broiler meat, the frequency of resistance was similar for all antimicrobials, except for resistance to tetracycline, which was significantly higher in imported broiler meat.

With the exception of *E. faecium* from broiler meat, where the frequency of erythromycin resistance was significantly higher than in humans, resistance frequencies in *E. faecium* from humans were comparable to levels seen in isolates from food products. These observations are consistent with the

Table 31. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from healthy humans (n=55), Denmark*

DANMAP 2003

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	5	[1.1-15.1]			91.0	3.6			1.8		3.6						
Chloramphenicol	2	[0.1-9.7]					14.5	65.5	18.2	1.8							
Florfenicol	0	[0.0-6.5]				12.7	87.3										
Penicillin	11	[4.1-22.3]				41.8	20.0	27.3	9.1	1.8							
Erythromycin	4	[0.4-12.5]			40.0	32.7	23.7				3.6						
Gentamicin	0	[0.0-6.5]										100					
Kanamycin	2	[0.1-9.7]										43.6	38.2	14.6	1.8		1.8
Streptomycin	4	[0.4-12.5]										96.4				1.8	1.8
Teicoplanin	0	[0.0-6.5]		83.6	16.4												
Vancomycin	0	[0.0-6.5]				98.2	1.8										
Quinupristin/dalfopristin	7	[2.0-17.6]		23.6	14.6	54.5	7.3										
Avilamycin	0	[0.0-6.5]			3.6	34.5	45.5	16.4									
Salinomycin	0	[0.0-6.5]			47.3	49.1	3.6										
Linezolid	0	[0.0-6.5]				29.1	70.9										

Vertical lines indicate breakpoints for resistance

Flavomycin is not listed, since *Enterococcus faecium* is naturally resistant

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

Table 32. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from healthy humans (n=73), Denmark*

DANMAP 2003

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	21	[12.0-31.6]			79.5					8.2	12.3						
Chloramphenicol	0	[0.0-4.9]				1.4	16.4	67.1	15.1								
Florfenicol	0	[0.0-4.9]				37.0	61.6	1.4									
Penicillin	0	[0.0-4.9]				23.3	75.3	1.4									
Erythromycin	7	[2.3-15.3]			68.5	24.6					6.9						
Gentamicin	1	[0.03-7.4]										98.6					1.4
Kanamycin	4	[0.9-11.5]										95.9					4.1
Streptomycin	5	[1.5-13.4]										90.5	2.7		1.4	2.7	2.7
Teicoplanin	0	[0.0-4.9]		98.6	1.4												
Vancomycin	0	[0.0-4.9]				90.4	9.6										
Avilamycin	0	[0.0-4.9]			23.3	67.1	8.2	1.4									
Flavomycin	0	[0.0-4.9]		4.1	30.1	52.1	9.6	4.1									
Salinomycin	0	[0.0-4.9]			94.5	5.5											
Linezolid	0	[0.0-4.9]				83.6	16.4										

Vertical lines indicate breakpoints for resistance

Virginiamycin and quinupristin/dalfopristin are not listed, since *Enterococcus faecalis* is naturally resistant

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

Table 33. Occurence of resistance (%) among Enterococcus faecium *from food animals, foods of Danish and imported origin and healthy humans, Denmark*

DANMAP 2003

Compound	Pigs	Pork	Cattle	Beef	Broilers	Broiler meat		Healthy humans
	Danish %	Danish %	Danish %	Danish %	Danish %	Danish %	Imported %	%
Tetracycline	50	4	0	2	5	3	32	5
Chloramphenicol	1	2	0	0	0	0	0	2
Florfenicol	0	0	0	0	<1	0	0	0
Penicillin	39	2	0	0	54	21	9	11
Erythromycin	21	7	4	2	17	20	23	4
Gentamicin	0	0	0	0	0	0	0	0
Kanamycin	19	0	0	2	0	0	0	2
Streptomycin	19	0	0	2	7	1	5	4
Teicoplanin	2	0	0	0	3	3	0	0
Vancomycin	3	0	0	0	3	3	0	0
Quinupristin/dalfopristin	9	0	0	4	25	6	9	7
Avilamycin	0	2	0	0	7	9	9	0
Salinomycin	0	0	0	0	<1	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Number of isolates	175	45	27	55	123	70	22	55

Table 34. Occurrence of resistance (%) among Enterococcus faecalis *from food animals, foods of Danish and imported origin and healthy humans, Denmark*

DANMAP 2003

Compound	Pigs	Pork	Beef	Broilers	Broiler meat	Healthy humans
	Danish %	Danish %	Danish %	Danish %	Danish %	%
Tetracycline	82	24	15	46	34	21
Chloramphenicol	3	1	2	0	0	0
Florfenicol	0	0	0	0	0	0
Penicillin	<1	0	0	0	0	0
Erythromycin	41	5	4	18	29	7
Gentamicin	9	0	1	0	3	1
Kanamycin	20	4	4	0	0	4
Streptomycin	35	6	4	3	3	5
Teicoplanin	0	0	0	0	0	0
Vancomycin	0	0	0	0	0	0
Avilamycin	<1	0	0	0	0	0
Flavomycin	<1	0	6	3	0	0
Salinomycin	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0
Number of isolates	207	76	81	66	38	73

assumption that resistance found in isolates from food are reflected in the occurrence of resistance in humans (Table 33 and 34).

The differences in the occurrence of resistance in *E. faecium* isolates from animals and humans were observed particularly between pigs and humans. Resistance to tetracycline, penicillin, erythromycin, kanamycin and streptomycin was significantly higher in isolates from pigs than in isolates from humans.

In *E. faecium* isolates from broilers and humans, resistance to penicillin and quinupristin/dalfopristin was higher in isolates from broilers. In isolates from cattle and humans no differences were observed.

The frequency of resistance in *E. faecalis* isolates from humans was not different from resistance found in pork. In *E. faecalis* isolates from broiler meat and humans, the only difference was in resistance to erythromycin, which was higher in broiler meat than in humans. In *E. faecalis* isolates from pigs and humans, resistance to tetracycline, erythromycin, streptomycin and kanamycin was higher in pigs than in humans. In *E. faecalis* isolates from broilers and humans, the only difference was in resistance to tetracycline, which was higher in broilers than in humans (Table 33 and 34).

Escherichia coli from food animals

Table 35 presents the MIC distribution and occurrence of resistance in *E. coli* isolates from animals at slaughter (indicator *E. coli*). A total of 523 isolates from broilers, cattle and pigs were included.

In indicator *E. coli* isolates from broilers and cattle only minor changes were observed in 2003, except for a significant increase in resistance to tetracycline among isolates from broilers (from 5% in 2002 to 14% in 2003). In isolates from broilers resistance to sulfonamide (20%) and ampicillin (15%) remains most frequently observed. In poultry, the consumption of these antimicrobials, and the consumption of tetracycline was reduced substantially in 2003. The level of resistance in isolates from cattle was generally low and did not exceed 4% for any of the antimicrobials tested.

In contrast to previous years, resistance to several antimicrobials in indicator *E. coli* isolates from pigs increased significantly in 2003. Resistance to

Table 35. Distribution of MICs and occurrence of resistance among Escherichia coli *from broilers (n=138), cattle (n=86) and pigs (n=317), Denmark*

DANMAP 2003

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	14	[8.5-20.7]							86.2				1.4	12.3					
	Cattle	4	[0.7-9.9]							96.5					3.5					
	Pigs	31	[26.6-36.6]							67.8	0.3	0.6	0.6	2.8	27.8					
Chloramphenicol	Broilers	0	[0.0-2.6]							7.2	69.6	23.2								
	Cattle	1	[0.03-6.3]							3.5	32.6	62.8				1.2				
	Pigs	7	[4.1-9.9]							5.0	46.1	40.1	2.2	3.2	0.3	3.2				
Florfenicol	Broilers	0	[0.0-2.6]							9.4	76.8	13.8								
	Cattle	0	[0.0-4.2]							4.7	29.1	66.3								
	Pigs	0	[0.0-1.2]							6.0	47.0	43.5	3.5							
Ampicillin	Broilers	15	[9.1-21.5]						15.9	51.4	15.9	1.4	0.7		14.5					
	Cattle	2	[0.3-8.1]						3.5	34.9	57.0	2.3			2.3					
	Pigs	23	[18.2-27.7]						6.9	43.2	24.6	2.2	0.3	0.3	22.4					
Amoxicillin/clavulanic acid a)	Broilers	<1	[0.02-4.0]							59.4	24.6	15.2		0.7						
	Cattle	0	[0.0-4.2]							20.9	72.1	7.0								
	Pigs	<1	[0.01-1.7]							34.1	41.0	22.7	1.9	0.3						
Cephalothin	Broilers	2	[0.5-6.2]							10.1	37.0	34.8	15.9	1.4		0.7				
	Cattle	1	[0.03-6.3]							1.2	12.8	64.0	20.9	1.2						
	Pigs	4	[2.0-6.5]							3.2	18.6	48.3	26.2	2.5	0.9	0.3				
Ceftiofur	Broilers	0	[0.0-2.6]					98.6	1.4											
	Cattle	0	[0.0-4.2]					98.8	1.2											
	Pigs	0	[0.0-1.2]					99.4	0.6											
Sulfonamide	Broilers	20	[13.9-28.0]												79.7					20.3
	Cattle	4	[0.7-9.9]												91.9	4.7				3.5
	Pigs	31	[25.6-36.0]												69.1	0.3				30.6
Trimethoprim	Broilers	1	[0.2-5.1]								98.6				1.4					
	Cattle	2	[0.3-8.1]								97.7				2.3					
	Pigs	14	[10.0-17.8]								86.1	0.3			13.6					
Apramycin	Broilers	0	[0.0-2.6]								91.3	8.7								
	Cattle	0	[0.0-4.2]								95.3	4.7								
	Pigs	<1	[0.2-2.7]								95.6	3.5				0.9				
Gentamicin	Broilers	0	[0.0-2.6]						99.3	0.7										
	Cattle	0	[0.0-4.2]						100											
	Pigs	<1	[0.1-2.3]						97.5	1.6		0.3	0.3		0.3					
Neomycin	Broilers	2	[0.5-6.2]							97.1	0.7			0.7	1.4					
	Cattle	2	[0.3-8.1]							97.7				2.3						
	Pigs	6	[3.4-8.8]							92.7	1.6		0.3	1.9	3.5					
Spectinomycin	Broilers	1	[0.02-4.0]									5.8	89.1	4.3		0.7				
	Cattle	0	[0.0-4.2]										90.7	7.0	2.3					
	Pigs	34	[28.6-39.3]								0.3	0.3	52.4	9.1	4.1	10.4	23.3			
Streptomycin	Broilers	4	[1.6-9.2]								46.4	47.8	1.4	2.2	1.4	0.7				
	Cattle	4	[0.7-9.9]								61.6	33.7	1.2	1.2		2.3				
	Pigs	44	[38.3-49.5]								26.8	24.3	5.0	9.8	17.7	16.4				
Ciprofloxacin	Broilers	10	[5.7-16.4]	89.9		5.8	3.6	0.7												
	Cattle	0	[0.0-4.2]	100																
	Pigs	0	[0.7-4.1]	97.8	0.3	0.3	0.9	0.3	0.3											
Nalidixic acid	Broilers	10	[5.7-16.4]									89.1	0.7	0.7	4.3	4.3	0.7			
	Cattle	0	[0.0-4.2]									100								
	Pigs	2	[0.7-4.1]									98.1			0.6	1.3				
Colistin	Broilers	0	[0.0-2.6]								100									
	Cattle	0	[0.0-4.2]								100									
	Pigs	0	[0.0-1.2]								100									

Vertical lines indicate breakpoints for resistance

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

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

Table 36. Distribution of MICs and occurrence of resistance among Escherichia coli *from broiler meat (n=175), beef (n=192) and pork (n=123), Denmark*

DANMAP 2003

Compound	Food Type	%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	15	[10.4-21.6]							82.3	2.3		0.6	5.7	9.1					
	Beef	9	[5.2-13.8]							90.1		1.0	0.5	1.0	7.3					
	Pork	22	[15.0-30.3]							76.4	1.6		1.6	5.7	14.6					
Chloramphenicol	Broiler meat	2	[0.6-5.7]							24.0	50.9	22.9		0.6		1.7				
	Beef	1	[0.01-2.9]							33.3	34.4	31.8				0.5				
	Pork	4	[1.3-9.2]							39.0	29.3	26.0	1.6	0.8		3.3				
Florfenicol	Broiler meat	0	[0.0-2.1]							28.6	55.4	16.0								
	Beef	0	[0.0-1.9]							34.4	44.8	20.8								
	Pork	0	[0.0-3.0]							41.5	35.8	22.8								
Ampicillin	Broiler meat	11	[7.1-17.1]						46.9	34.9	6.9				11.4					
	Beef	6	[3.3-10.7]						34.4	45.3	13.5	0.5			6.3					
	Pork	15	[8.9-22.1]						30.1	43.1	11.4		0.8		14.6					
Amoxicillin/clavulanic acid a)	Broiler meat	0	(0.0-2.1)							75.4	18.9	5.7								
	Beef	0	(0.0-1.9)							69.3	27.6	2.6	0.5							
	Pork	1	(0.02-4.4)							61.8	29.3	6.5	1.6	0.8						
Cephalothin	Broiler meat	2	[0.6-5.7]							17.7	28.0	36.6	15.4	2.3						
	Beef	3	[1.2-6.7]							4.7	22.4	56.3	13.5	3.1						
	Pork	6	[2.3-11.4]							8.9	19.5	48.8	17.1	4.9		0.8				
Ceftiofur	Broiler meat	0	[0.0-2.1]					99.4		0.6										
	Beef	0	[0.0-1.9]					100												
	Pork	0	[0.0-3.0]					99.2	0.8											
Sulfonamide	Broiler meat	14	[9.0-19.7]												85.7	0.6		0.6		13.1
	Beef	5	[2.5-9.4]												94.8			0.5		4.7
	Pork	21	[14.3-29.4]												78.9			0.8		20.3
Trimethoprim	Broiler meat	6	[3.2-11.0]								93.7				6.3					
	Beef	3	[1.2-6.7]								96.9				3.1					
	Pork	13	[7.6-20.3]								87.0				13.0					
Apramycin	Broiler meat	1	[0.01-3.1]								92.6	6.9	0.6							
	Beef	1	[0.01-2.9]								92.2	7.3	0.5							
	Pork	1	[0.02-4.4]								91.1	8.1	0.8							
Gentamicin	Broiler meat	0	[0.0-2.1]						97.7	2.3										
	Beef	0	[0.0-1.9]						99.5	0.5										
	Pork	0	[0.0-3.0]						96.7	3.3										
Neomycin	Broiler meat	1	[0.01-3.1]							96.6	2.3	0.6		0.6						
	Beef	1	[0.01-2.9]							98.4	1.0			0.5						
	Pork	4	[1.3-9.2]							94.3	1.6			1.6	2.4					
Spectinomycin	Broiler meat	2	[0.4-4.9]								2.3	17.1	72.6	5.7	0.6	1.1	0.6			
	Beef	2	[0.6-5.2]								1.0	26.6	64.6	5.7			2.1			
	Pork	15	[8.9-22.1]								4.1	19.5	52.0	8.9	0.8	5.7	8.9			
Streptomycin	Broiler meat	9	[5.3-14.4]								45.1	42.9	2.9	2.9	2.9	3.4				
	Beef	8	[4.4-12.6]								37.5	50.5	4.2	0.5	1.6	5.7				
	Pork	24	[16.4-32.1]								37.4	30.1	8.9	3.3	7.3	13.0				
Ciprofloxacin	Broiler meat	8	[4.4-13.1]	90.3	1.7	2.3	4.6	1.1												
	Beef	0	[0.0-1.9]	99.5	0.5															
	Pork	1	[0.5-7.0]	97.6		0.8	0.8					0.8								
Nalidixic acid	Broiler meat	9	[5.3-14.4]									90.9		1.1	2.3	3.4	2.3			
	Beef	0	[0.0-1.9]									100								
	Pork	2	[0.2-5.8]									96.7	1.6			0.8	0.8			
Colistin	Broiler meat	0	[0.0-2.1)								100									
	Beef	0	[0.0-1.9]								100									
	Pork	0	[0.0-3.0]								100									

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

ampicillin, sulfonamide, trimethoprim, spectinomycin and streptomycin increased from 2002 to 2003, and were the most frequently observed resistance in indicator *E. coli* isolates from pigs in 2003. Resistance to chloramphenicol increased from 2001 to 2003. These observations coincided with increased consumption of penicillins, sulfonamides / trimethoprim, tiamulin and amphenicols in pigs in 2003. The substantial increase in consumption of tetracycline in pigs in 2003, was not reflected in the occurrence of resistance to tetracycline among indicator *E. coli* in pigs in 2003. Figure 17 presents the trend in resistance to selected antimicrobials from 1996 to 2003.

Escherichia coli from food

A total of 490 isolates of *E. coli* were collected from broiler meat, beef and pork at retail outlets during 2003. In 2003 resistance to ampicillin, tetracycline, sulfonamide, trimethoprim and streptomycin was most frequently observed (Table 36). Similar observations were made in 2002, and the proportions of resistant isolates remained unchanged from 2002 to 2003. Thus,

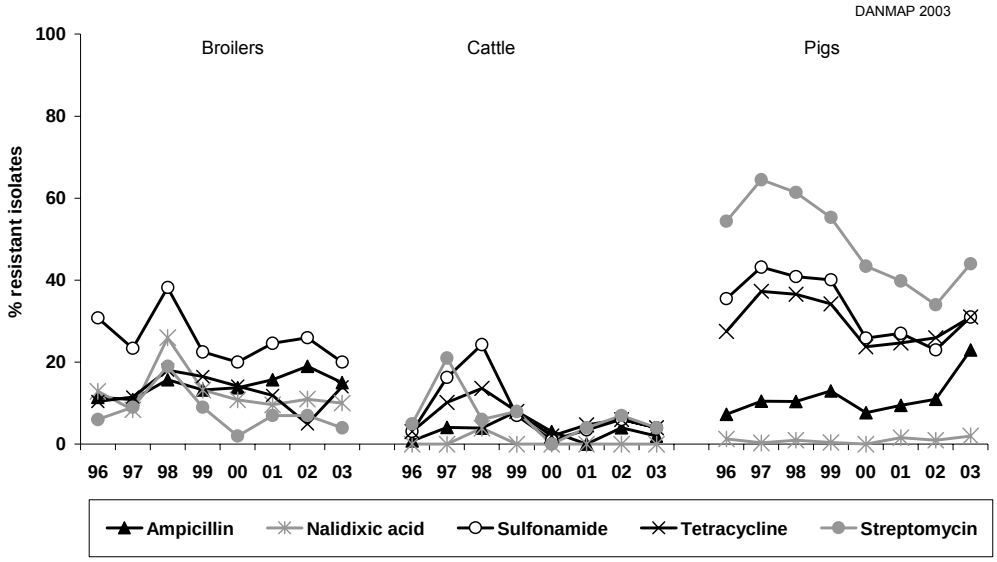


Figure 17. Trends in resistance to some selected antimicrobials among Escherichia coli *from food animals, Denmark*

the increased consumption of antimicrobials in pigs, and the concurrent rise in the occurrence of resistance in indicator *E. coli* from pig, was not reflected in the occurrence of resistance in *E. coli* from pork.

Escherichia coli from healthy human volunteers

In 2003, stool samples from 124 healthy human volunteers were collected and 107 *E. coli* isolates were subsequently isolated. Table 37 presents the MIC distribution and occurrence of resistance of the 107 isolates. Like in 2002, resistance to sulfonamide, ampicillin, tetracycline and streptomycin were most common. None of the isolates were resistant to gentamicin. No significant changes in resistance were observed between 2002 and 2003.
Ciprofloxacin/nalidixic acid resistance was observed in one percent of the isolates, but none exceeded the NCCLS breakpoint for ciprofloxacin. In all indicator *E. coli* isolates a breakpoint of 0.125 µg/ml was used (see Appendix 1).

Comparison of *Escherichia coli* from farm, table and healthy human volunteers

The occurrence of resistance in food animals, food and healthy humans volunteers are compared in Table 38. Trends in resistance among *E. coli* isolates from pigs, pork and humans to tetracycline, sulfonamide and ampicillin are presented in figures 18-20. Resistance to sulfonamide and tetracycline in *E. coli* from pork and humans has been below the resistance levels for pigs, except in pork from 2001.

In 2003, the frequency of resistance in *E. coli* isolates were similar for Danish meat and food animals, except for resistance to streptomycin and spectinomycin in isolates from Danish pork, which were significantly higher in pigs than in pork.
In Danish and imported meat, the frequency of resistance was similar for all antimicrobials, except for resistance to tetracycline, which was significantly higher in imported broiler meat compared to Danish broiler meat.
The frequency of resistance in *E. coli* from healthy human volunteers was similar to resistance in *E. coli* from Danish meat.

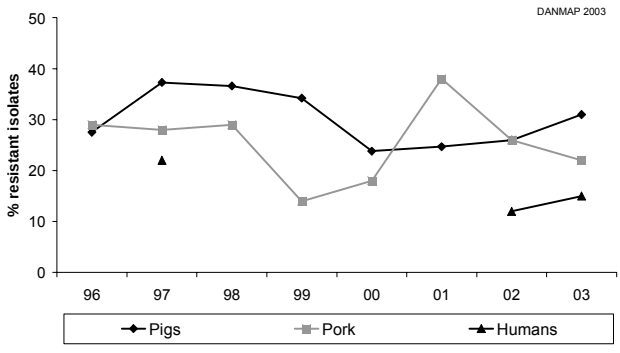


Figure 18. Trends in tetracycline resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

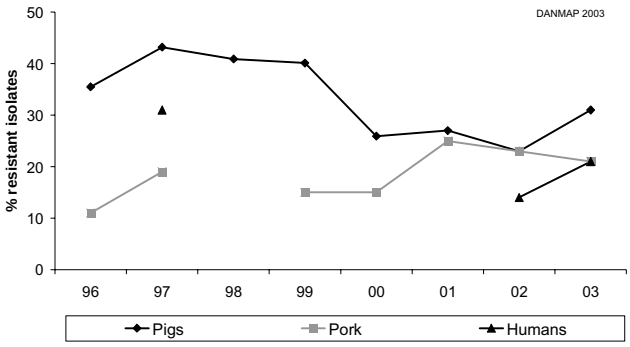


Figure 19. Trends in sulfonamide resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

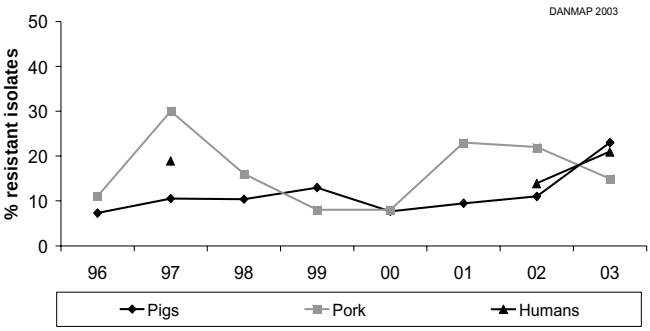


Figure 20. Trends in ampicillin resistance among Escherichia coli *from pigs, pork and healthy humans in the community, Denmark*

Table 37. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy humans (n=107), Denmark*

DANMAP 2003

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
			0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	15	[8.8-23.1]							85.1				0.9	14.0					
Chloramphenicol	3	[0.6-8.0]							2.8	29.0	63.6	1.8			2.8				
Florfenicol	0	[0.0-3.4]							8.4	57.0	34.6								
Ampicillin	21	[13.4-29.5]						5.6	38.3	34.6	0.9			20.6					
Amoxicillin/clavulanic acid	0	[0.0-3.4]							20.6	53.3	20.6	5.6							
Cephalothin	2	[0.2-6.6]							1.8	23.4	46.8	26.2	1.8						
Ceftiofur	0	[0.0-3.4]					98.2	1.8											
Sulfonamide	21	[13.4-29.5]												79.4					20.6
Trimethoprim	7	[3.3-14.2]								92.5				7.5					
Apramycin	0	[0.0-3.4]								89.7	10.3								
Gentamicin	0	[0.0-3.4]						99.1	0.9										
Neomycin	1	[0.05-5.1]							98.2	0.9			0.9						
Spectinomycin	4	[1.0-9.3]								0.9	11.3	76.7	5.6	1.8	0.9	2.8			
Streptomycin	20	[12.6-28.4]								47.7	29.0	3.7	2.8	3.7	13.1				
Ciprofloxacin	1	[0.05-5.1]	99.1			0.9													
Nalidixic acid	1	[0.05-5.1]									99.1				0.9				
Colistin	0	[0.0-3.4]								100									

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

Table 38. Occurrence of resistance (%) among Escherichia coli *from Danish animals, foods of Danish and imported origin and humans, Denmark*

DANMAP 2003

Compound	Broilers	Broiler meat		Cattle	Beef		Pigs	Pork	Humans
	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	Danish %	Danish %	%
Tetracycline	14	9	59	4	7	16	31	22	15
Chloramphenicol	0	1	9	1	1	0	7	4	3
Florfenicol	0	0	0	0	0	0	0	0	0
Ampicillin	15	10	18	2	5	13	23	15	21
Amoxicillin/clavulanic acid	<1	0	0	0	0	0	<1	1	0
Cephalothin	2	2	0	1	4	0	4	6	2
Ceftiofur	0	0	0	0	0	0	0	0	0
Sulfonamide	20	14	14	4	4	13	31	21	21
Trimethoprim	1	6	9	2	2	10	14	13	7
Apramycin	0	1	0	0	1	0	<1	1	0
Gentamicin	0	0	0	0	0	0	<1	0	0
Neomycin	2	1	0	2	1	0	6	4	1
Spectinomycin	1	2	0	0	2	0	34	15	4
Streptomycin	4	8	18	4	7	10	44	24	20
Ciprofloxacin	0	0	0	0	0	0	0	1	1
Nalidixic acid	10	10	5	0	0	0	2	2	1
Colistin	0	0	0	0	0	0	0	0	0
Number of isolates	120	153	22	86	161	31	317	123	107

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 poultry, cattle and pigs, *Staphylococcus aureus* from cattle, and *Staphylococcus hyicus* from pigs. Most isolates from diagnostic submissions originate from animals already in antimicrobial therapy, or animals with a history of previously antimicrobial therapy. For this reason a higher frequency of resistance is expected in bacterial isolates from diagnostic submissions, than in isolates of indicator bacteria originating from animals sampled at slaughter.

Escherichia coli

In 2003, *E. coli* isolates from diagnostic submissions from cattle and pigs were included, whereas no isolates of *E. coli* from diagnostic submissions from poultry were included. The MIC distribution and the occurrence of resistance in isolates from cattle and pigs are presented in Table 39**.**

The relatively small number of isolates of *E. coli* available from diagnostic submissions from poultry during the previous years makes it difficult to demonstrate changes in resistance levels from year to year. With some caution it is possible to evaluate the trend in occurrence of resistance over time. Generally *E. coli* isolates from diagnostic submissions from poultry have become less resistant over time and

Table 39. Distribution of MICs and occurrence of resistance among Escherichia coli *from diagnostic submissions from cattle (n=57) and pigs (n=77), Denmark*

DANMAP 2003

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	75	[62.2-85.9]							24.6				5.3	70.2					
	Pigs	73	[61.4-82.3]							24.7	2.6			10.4	62.3					
Chloramphenicol	Cattle	14	[6.3-25.8]								40.4	45.6			1.8	12.3				
	Pigs	26	[16.6-37.2]							9.1	51.9	10.4	2.6	1.3	3.9	20.8				
Florfenicol	Cattle	4	[0.4-12.1]								63.2	33.3				3.5				
	Pigs	1	[0.03-7.0]							16.9	58.4	19.5	3.9		1.3					
Ampicillin	Cattle	75	[62.2-85.9]						8.8	7.0	8.8				75.4					
	Pigs	43	[31.6-54.6]						10.4	39.0	7.8			1.3	41.6					
Amoxicillin/clavulanic acid a)	Cattle	4	[0.4-12.1]							14.0	21.1	45.6	15.8	3.5						
	Pigs	4	[0.8-11.0]							39.0	24.7	31.2	1.3	3.9						
Cephalothin	Cattle	7	[1.9-17.0]								21.1	49.1	22.8	7.0						
	Pigs	7	[2.1-14.5]								24.7	53.2	15.6	2.6		3.9				
Ceftiofur	Cattle	0	[0.0-6.3]					100												
	Pigs	0	[0.0-4.7]					100												
Sulfonamide	Cattle	65	[51.1-77.1]												35.1				1.8	63.2
	Pigs	73	[61.4-82.6]												27.3				2.6	70.1
Trimethoprim	Cattle	53	[39.0-66.0]								47.4			52.6						
	Pigs	36	[25.7-48.1]								63.6			36.4						
Apramycin	Cattle	4	[0.4-12.1]								84.2	12.3	1.8			1.8				
	Pigs	9	[3.7-17.8]								89.6	1.3				9.1				
Gentamicin	Cattle	4	[0.4-12.1]						93.0	3.5			1.8	1.8						
	Pigs	7	[2.1-14.5]						89.6			3.9	2.6	1.3	2.6					
Neomycin	Cattle	16	[7.5-27.9]							84.2				1.8	14.0					
	Pigs	31	[21.1-42.7]							68.8				10.4	20.8					
Spectinomycin	Cattle	16	[7.5-27.9]										64.9	8.8	10.5	7.0	8.8			
	Pigs	55	[42.8-65.9]									1.3	28.6	6.5	9.1	9.1	45.5			
Streptomycin	Cattle	68	[54.8-80.1]								10.5	14.0	7.0	8.8	10.5	49.1				
	Pigs	66	[54.6-76.6]								19.5	5.2	9.1	19.5	16.9	29.9				
Ciprofloxacin	Cattle	2	[0.04-9.4]	86.0		8.8	3.5					1.8								
	Pigs	0	[0.0-4.7]	70.1		14.3	13.0	1.3	1.3											
Nalidixic acid	Cattle	14	[6.3-25.8]									86.0			1.8	7.0	5.3			
	Pigs	27	[17.7-38.6]									72.7		1.3	10.4	14.3	1.3			
Colistin	Cattle	0	[0.0-6.3]								100									
	Pigs	0	[0.0-4.7]								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

resistance to sulfonamide, tetracycline and nalidixic acid have decreased (Figure 21).
Among *E. coli* isolates from cattle resistance to tetracycline, ampicillin, sulfonamide and streptomycin was most frequently observed. The proportion of isolates resistant to ampicillin and tetracycline has remained unchanged since 1996, whereas resistance to sulfonamide and gentamicin declined significantly from 2001 to 2003 (Figure 21).
Between 1999 and 2003, ampicillin resistance increased significantly among *E. coli* isolates from pigs (Figure 21). The consumption of penicillins in pigs has increased concurrently (Table 3), and in 2003 an increase in consumption for all age-groups of pigs was observed (Table 7). Resistance to tetracycline, sulfonamide, gentamicin, streptomycin and nalidixic acid remained unchanged from 2000.

Staphylococci
Isolates of *S. aureus* originated from cases of bovine mastitis while *S. hyicus* originated from skin infections in pigs.The MIC distribution and the occurrence of resistance among *S. aureus* from cattle, and among *S. hyicus* from pigs, are presented in Tables 40 and 41, respectively. Trends in resistance to some selected antimicrobials among staphylococci from diagnostic submissions from cattle and pigs are presented in Figure 22.

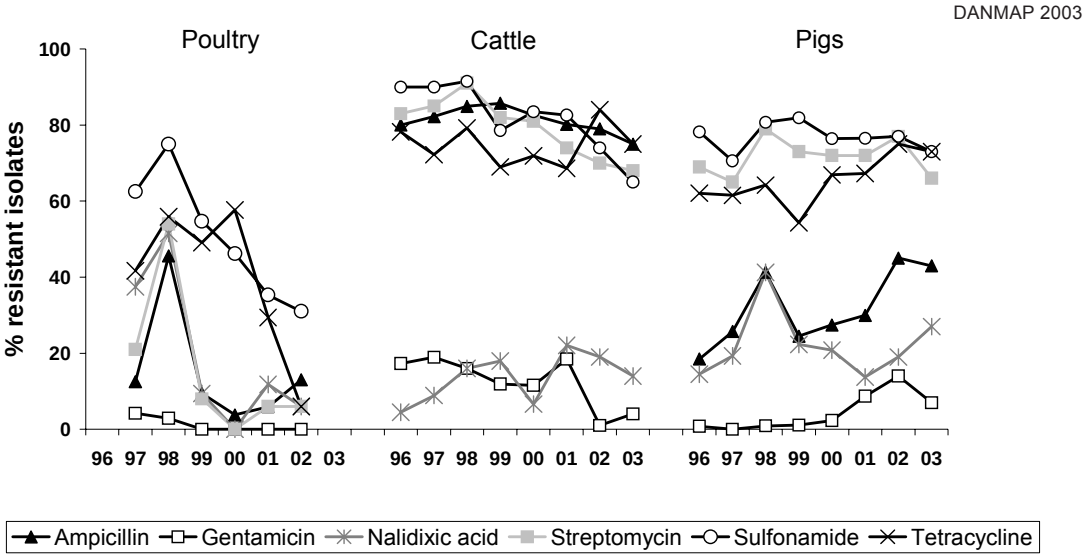


Figure 21. Trends in resistance to selected antimicrobials among Escerichia coli *from diagnostic submissions from animals, Denmark*

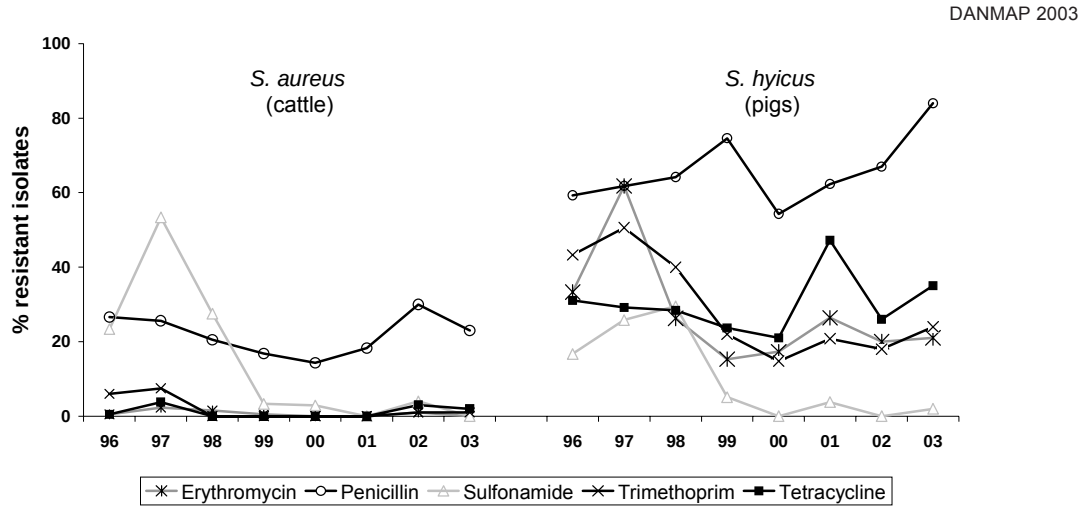


*Figure 22. Trends in resistance to selected antimicrobials among staphylococci from diagnostic submissions from cattle (*S. aureus*) and pigs (*S. hyicus*), Denmark*

Table 40. Distribution of MICs and occurrence of resistance among Staphylococcus aureus *from cattle (n=99), Denmark*

DANMAP 2003

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	2	[0.2-7.1]				94.9	1.0	1.0		1.0	1.0	1.0					
Chloramphenicol	0	[0.0-3.7]							23.2	76.8							
Florfenicol	0	[0.0-3.7]					1.0	44.4	54.5								
Penicillin	23	[15.3-32.8]	76.8			3.0	4.0	4.0	9.1	3.0							
Ceftiofur	0	[0.0-3.7]		4.0	13.1	53.5	29.3										
Sulfonamide	0	[0.0-3.7]								26.3	30.3	34.3	7.1	2.0			
Trimethoprim	1	[0.03-5.5]					83.8	12.1	2.0	1.0			1.0				
Erythromycin	0	[0.0-3.7]		2.0	53.5	44.4											
Gentamicin	0	[0.0-3.7]						94.9	5.1								
Spectinomycin	9	[4.2-16.6]										3.0	87.9	9.1			
Streptomycin	1	[0.03-5.5]						13.1	67.7	18.2				1.0			
Ciprofloxacin	0	[0.0-3.7]		50.5	37.4	12.1											
Vancomycin	0	[0.0-3.7]						100									
Quinupristin/dalfopristin	1	[0.03-5.5]					93.9	5.1		1.0							
Avilamycin	0	[0.0-3.7]						34.3	59.6	6.1							
Tiamulin	0	[0.0-3.7]			3.0	48.5	47.5	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

Table 41. Distribution of MICs and occurrence of resistance among Staphylococcus hyicus *from pigs (n=68), Denmark*

DANMAP 2003

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	35	[24.1-47.8]				61.8	2.9				8.8	20.6	5.9				
Chloramphenicol	0	[0.0-5.3]							72.1	27.9							
Florfenicol	0	[0.0-5.3]						83.8	16.2								
Penicillin	84	[79.9-91.6]	16.2	1.3	1.5	4.4	8.8	10.3	5.9	14.7	19.1	19.1					
Ceftiofur	0	[0.0-5.3]				30.9	64.7	2.9	1.5								
Sulfonamide	2	[0.04-7.9]								19.1	32.4	30.9	7.4	5.9	2.9	1.5	
Trimethoprim	24	[14.1-35.4]					20.6	29.4	20.6	5.9			23.5				
Erythromycin	21	[11.7-32.1]		1.3	38.2	39.7	1.5					20.6					
Gentamicin	0	[0.0-5.3]						100									
Spectinomycin	13	[6.2-23.6]										14.7	72.1			13.2	
Streptomycin	44	[32.1-56.7]						10.3	36.8	7.4	1.5	5.9	8.8	14.7	14.7		
Ciprofloxacin	4	[0.9-12.4]		76.5	11.8	7.4			1.5	2.9							
Vancomycin	0	[0.0-5.3]					38.2	61.8									
Quinupristin/dalfopristin	3	[0.4-10.2]				51.5	29.4	16.2	1.5	1.5							
Avilamycin	0	[0.0-5.3]						57.4	42.6								
Tiamulin	13	[6.2-23.6]			2.9	41.2	35.3			1.5	5.9		13.2				

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

Isolates of *S. aureus* from cattle were generally susceptible to antimicrobials in the test panel, except for penicillin for which the frequency of resistance has remained between 14% and 30% since 1996. No significant change was observed in 2003.

The resistance of isolates of *S. hyicus* from pigs to penicillin increased significantly from 54% in 2000 to 84% in 2003. For all other antimicrobials in the test panel, the frequency of resistance was unchanged from 2000, except from an increase in resistance to tetracycline in 2001.The consumption of penicillins in pigs increased concurrently from 2001 to 2003, and the consumption of tetracycline reached a maximum in 2001 (Figure 16).

Bacteria from humans

In this report, data on resistance levels in *Streptococcus pneumoniae* and *Staphylococcus aureus* isolates, as well as *Salmonella* spp. and *Campylobacter* spp. isolates (see pages 25 and 29) cover all 16 counties in Denmark. For *Escherichia coli* and coagulase-negative staphylococci, this report includes data from clinical microbiology laboratories of 12 counties, namely Copenhagen and Frederiksberg municipalities (which also have the status of counties) and the counties of Copenhagen, Roskilde, West Zealand, Storstroem, Funen, Ribe, Ringkoebing, Aarhus, Viborg and North Jutland, representing 81% of the Danish population. Data on penicillin and macrolide resistance in *Streptococcus pyogenes* cover approximately 65% of the population. Demographic data is presented in Table 2, page 11.

Escherichia coli

Results from invasive isolates and urine isolates of *E. coli* in hospitals were obtained from 12 counties. Eleven counties contributed data on urine isolates in primary health care. The results for the period 1995-2003 are presented for each county in Figures 25-27, showing resistance in blood and urine isolates in *E. coli* to selected antimicrobials.

In 2003, the generally high level of ampicillin resistance in *E.coli* blood isolates remained between 40 and 50%, with only two counties reporting levels below 35 % (Figure 25). Similar to what has been reported in recent years, gentamicin resistance in *E. coli* blood isolates remained at around 2%. *E. coli* resistance to cefuroxime was 2.3%. In counties reporting levels of resistance to cefuroxime above average, limited sample size and data from the national referral hospital was considered the most likely reason.

In 2003, *E. coli* urine isolates from primary health care did not show any further increase in resistance to ampicillin compared to 2002 and remained at around 40% (37.5 to 43.7%) (Figure 26). Sulfonamide resistance levels in *E. coli* urine isolates from primary health care remained between 35 and 40%. Despite these high resistance levels, sulfonamides still represent the drug of choice for treating urinary tract infections in Denmark. One should be aware that the reported resistance levels may be biased because a significant proportion of urine samples are submitted for microbiological diagnosis following failure of empirical treatment. As reported in DANMAP 2002, ampicillin and sulfonamide resistance in *E. coli* isolates from uncomplicated urinary tract infections in primary health care is at around 20% (Kerrn et al. J. Antimicrob. Chemother. 2002;50:513-6).

Data on ciprofloxacin resistance in *E. coli* urine isolates were available from seven counties, representing 43% of the Danish population. Because there has been an increase in fluoroquinolone consumption during the past year (see Table 13 and Figure 5, pages 21 and 22), we compared the variations of fluoroquinolone consumption in these seven counties with variations in resistance. In these seven counties, consumption of fluoroquinolones in primary health care increased from 0.19 to 0.26 DDD per 1,000 inhabitant-days between 2002 and 2003. A significant increase from 1.3% in 2002 to 2% in 2003 ($p<0.0001$). Although resistance to fluoroquinolones in *E. coli* is still low, the present increase in consumption due to a large change in market structure and price drop warrants closer surveillance of fluoroquinolone resistance in the years to come. (See Figure 6).

Data on resistance in urine isolates from hospitals in all participating counties are presented in Figure 27. For the seven counties that reported data on ciprofloxacin resistance, there was no significant increase between 2002 (1.9%) and 2003 (2.1%). This is consistent with the very moderate increase (less than 0.5%) in hospital consumption of fluoroquinolones observed in these counties.

Methicillin-Resistant *Staphylococcus aureus*

In 2003, the number of Methicillin-Resistant *Staphylococcus aureus* MRSA infections in Denmark more than doubled from 2002 to reach 204 cases.The number of MRSA infections imported from abroad did not change between 2002 and 2003. Thus, the increase in MRSA infections was due to an increase in infections acquired in Denmark. More than 50% of MRSA infections in 2003 were community onset (See textbox 2).

Coagulase-negative staphylococci

In 2003, the average level of penicillin resistance in coagulase-negative staphylococci blood isolates remained around 80% (70 to 92%). Erythromycin resistance averaged 38 % (13 to 59.5%). Methicillin resistance varied widely among counties from 11% to 70%. It is possible that these differences and large variations were merely the consequences of the procedure for selection of isolates that are submitted for susceptibility testing. Caution is therefore warranted when trying to make comparisons of resistance levels among counties.

Streptococcus pneumoniae

The *Streptococcus* Unit, which is the national reference centre at Statens Serum Institut, performs typing and susceptibility testing on *S. pneumoniae* isolates referred by the local clinical microbiology laboratories in Denmark. In 2003, susceptibility testing was performed on 1,215 non-duplicate isolates from blood or cerebrospinal fluid samples.

Non-susceptibility (resistant plus intermediate isolates) to penicillin in *S. pneumoniae* reached a peak of approximately 4% in 1999. This is still much lower than levels reported outside Denmark. Since 1999, non-susceptibility to penicillin has decreased to 2.2% in 2003 (Figure 23). Resistance to macrolides reached 5% among *S. pneumoniae* invasive isolates (blood and cerebrospinal fluid) in 2000 and has since remained at the same level (Figure 23 and 24). With 2.1 DDD/1,000 inhabitant-days, macrolides were the third most used class of antimicrobials in primary health care in 2003 (Table 13). There has been no major change in the level of total macrolide use since 1994. However, since 2001 there have been small changes in the distribution of the macrolides used, with an increasing use of roxithromycin on behalf of azithromycin and erythromycin. Previously the increase in azithromycin consumption until 1999 was followed by an increase in resistance (Figure 24). Consumption of azithromycin, and of clarithromycin, has remained fairly stable during the past years. This could be the explanation for the now stable level of macrolide resistance in *S. pneumoniae* in Denmark (Figure 24).

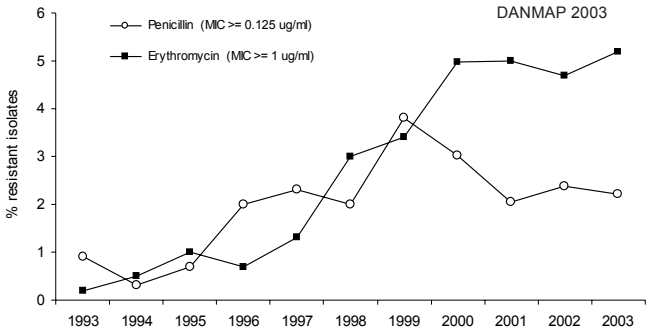


Figure 23. Resistance (%) to penicillin and macrolides in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

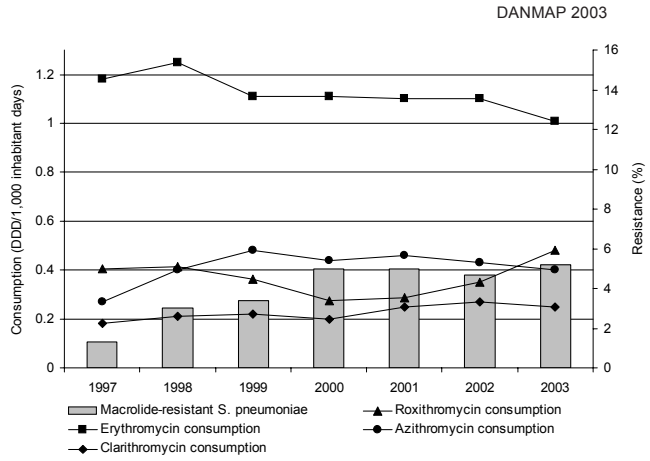


Figure 24. Macrolide resistance in Streptococcus pneumoniae *blood and spinal fluid isolates from humans and consumption of the individual macrolides, Denmark. Resistance (%) is shown on the left Y-axis and consumption (DDD/1,000 inhabitant-days) on the right Y-axis.*

Streptococcus pyogenes

Although macrolide resistance in *S. pyogenes* (Group A streptococci or GAS) is not considered a problem in Denmark, the increasing resistance worldwide has since 2001 called for closer monitoring in Denmark. In 2003, data were reported on 164 invasive and 6,757 non-invasive GAS isolates from clinical samples in 10 counties. Resistance to macrolides in invasive GAS isolates was 1.8%. Resistance to macrolides in non-invasive GAS isolates decreased significantly from 2.5% in 2002 to 1.8 % in 2003. However, there were county-to-county variations ranging from 0.6% to 3.4% in 2003. As in previous years, resistance to penicillin in GAS was not reported in 2003. Penicillin remains the drug of choice for the treatment of GAS tonsillitis.

Figure 25. Resistance (%) to ampicillin, gentamicin and cefuroxime in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2003.*

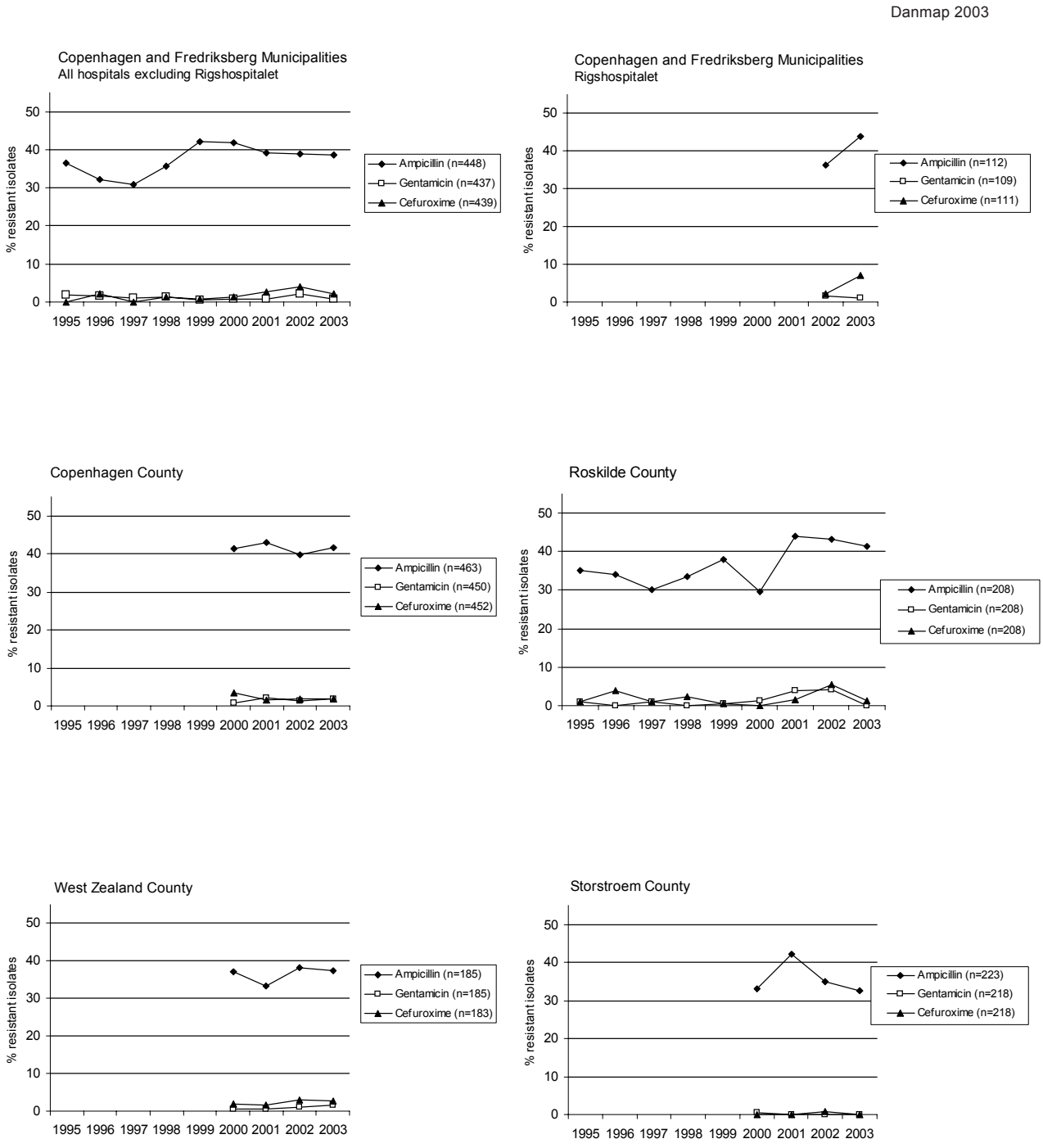
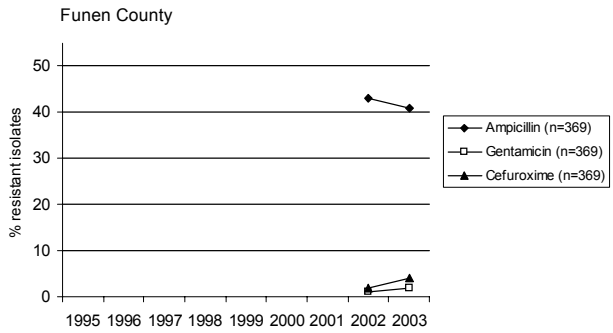
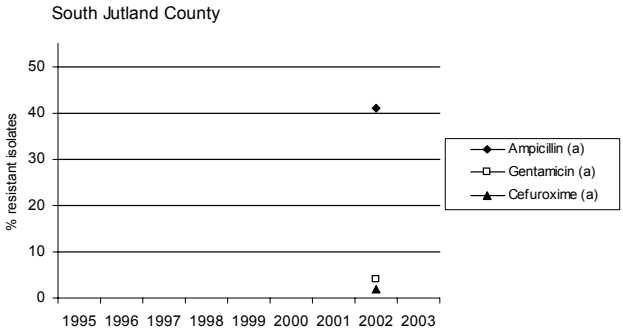
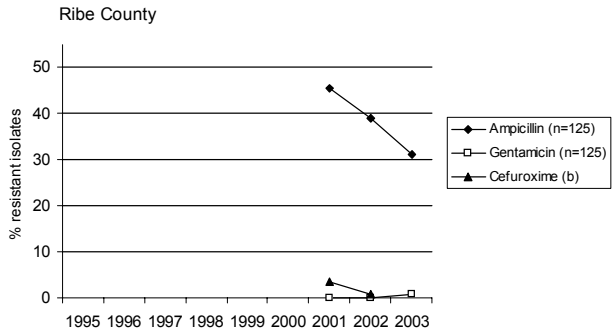
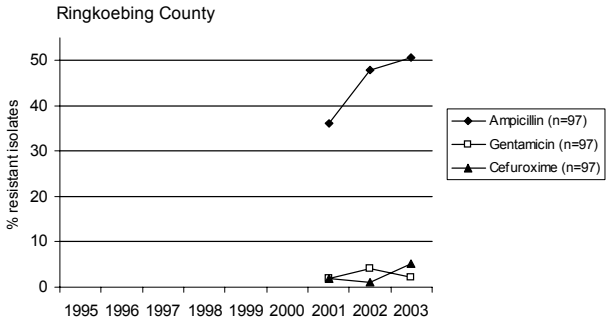


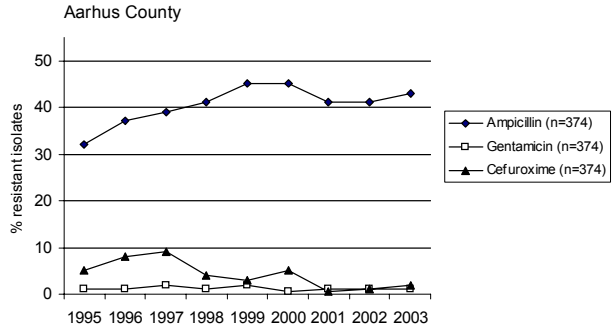
Figure 25. (continued) Resistance (%) to ampicillin, gentamicin and cefuroxime in Escherichia coli *blood isolates from humans presented by county, Denmark. Copenhagen and Frederiksberg share the same clinical microbiological laboratories. Rigshospitalet is the national referral hospital. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2003.*

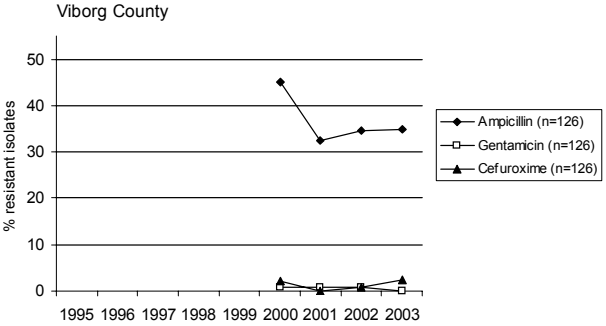


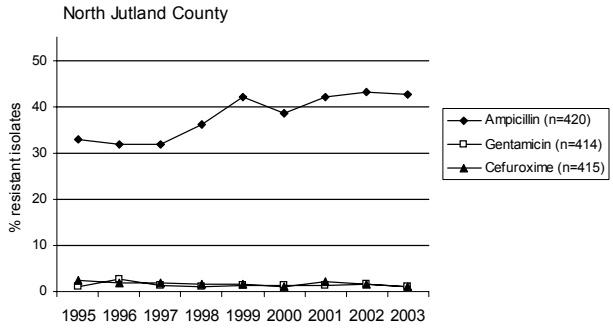








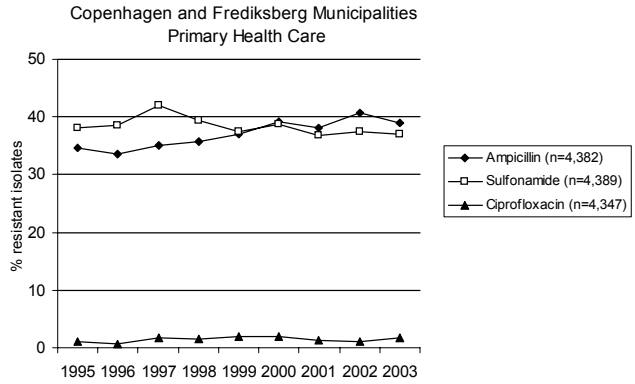


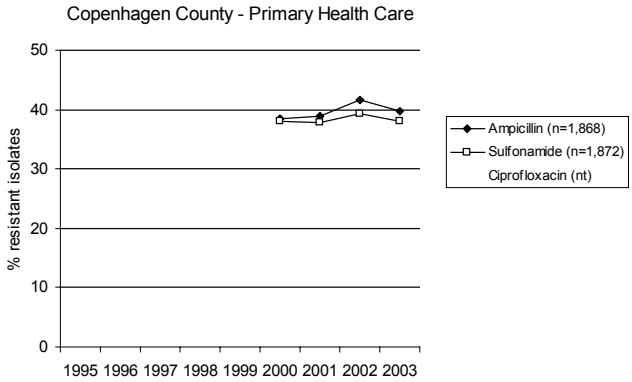


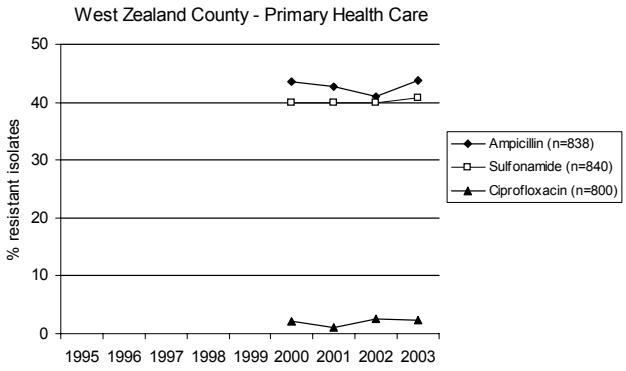
(a) data for 2002 only

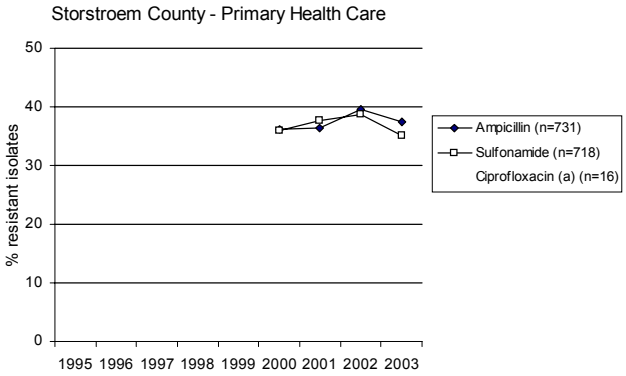
(b) data for 2001 and 2002 only

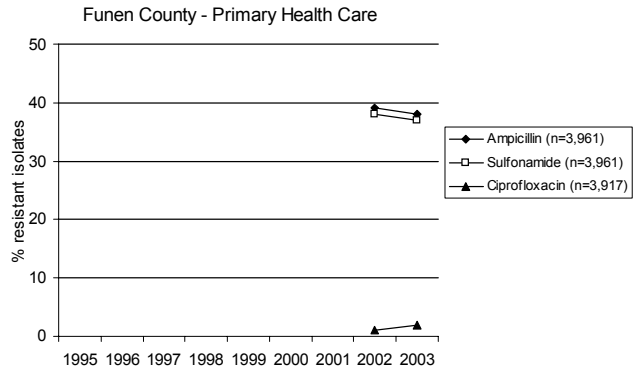
Figure 26. Resistance (%) to ampicillin, sulfonamides and ciprofloxacin in Escherichia coli *urine isolates from humans in primary health care by county, Denmark. The number (n) in parentheses represents the number of isolates tested for susceptibility in 2003.*

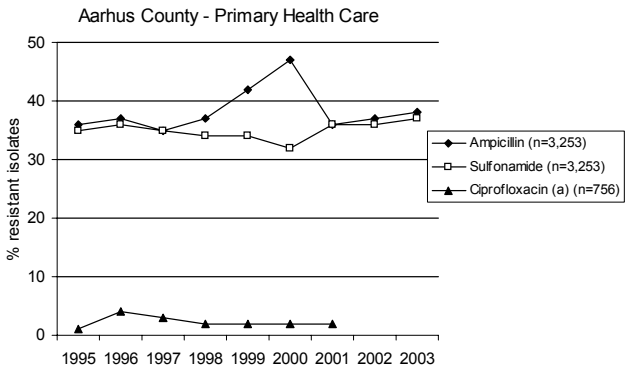


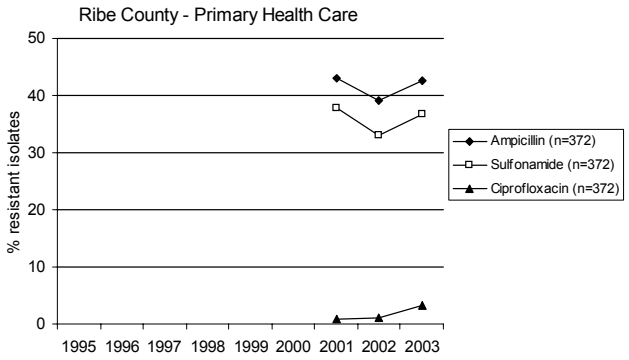


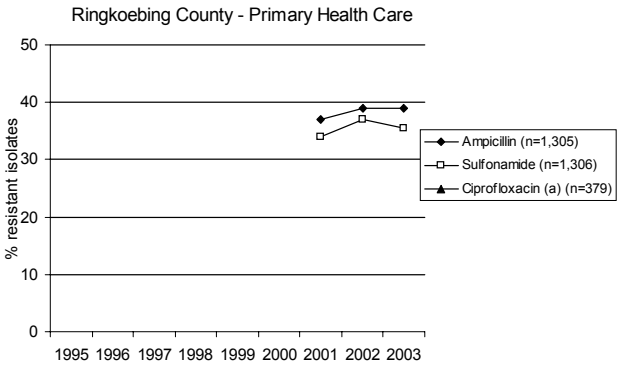


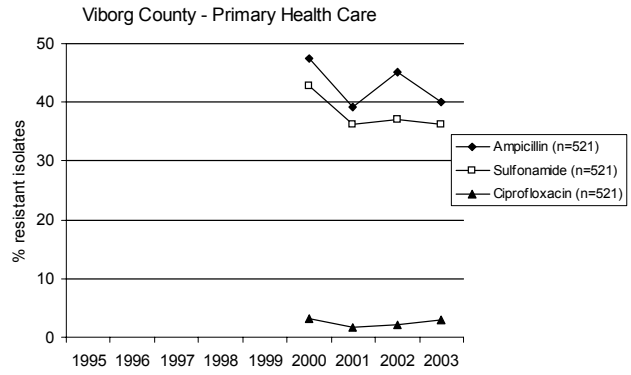


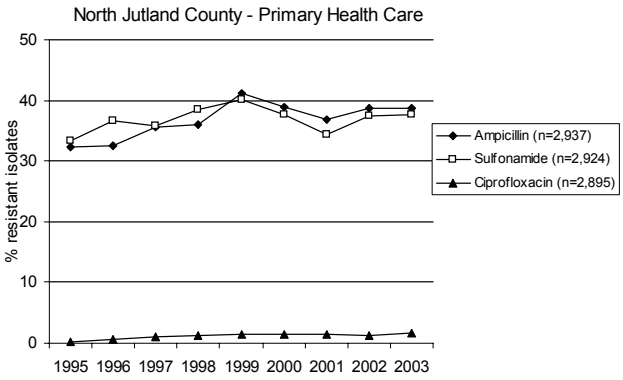






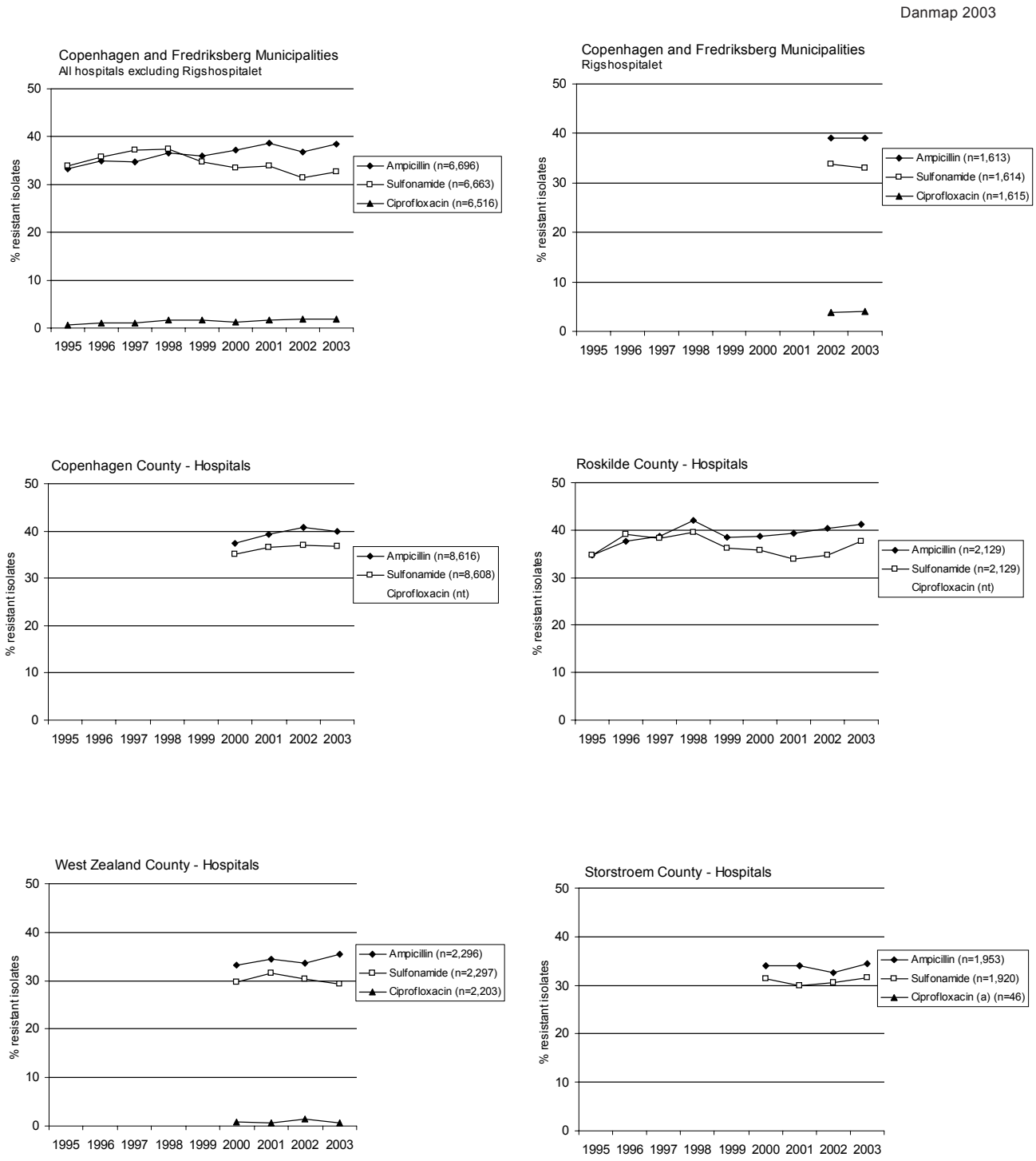






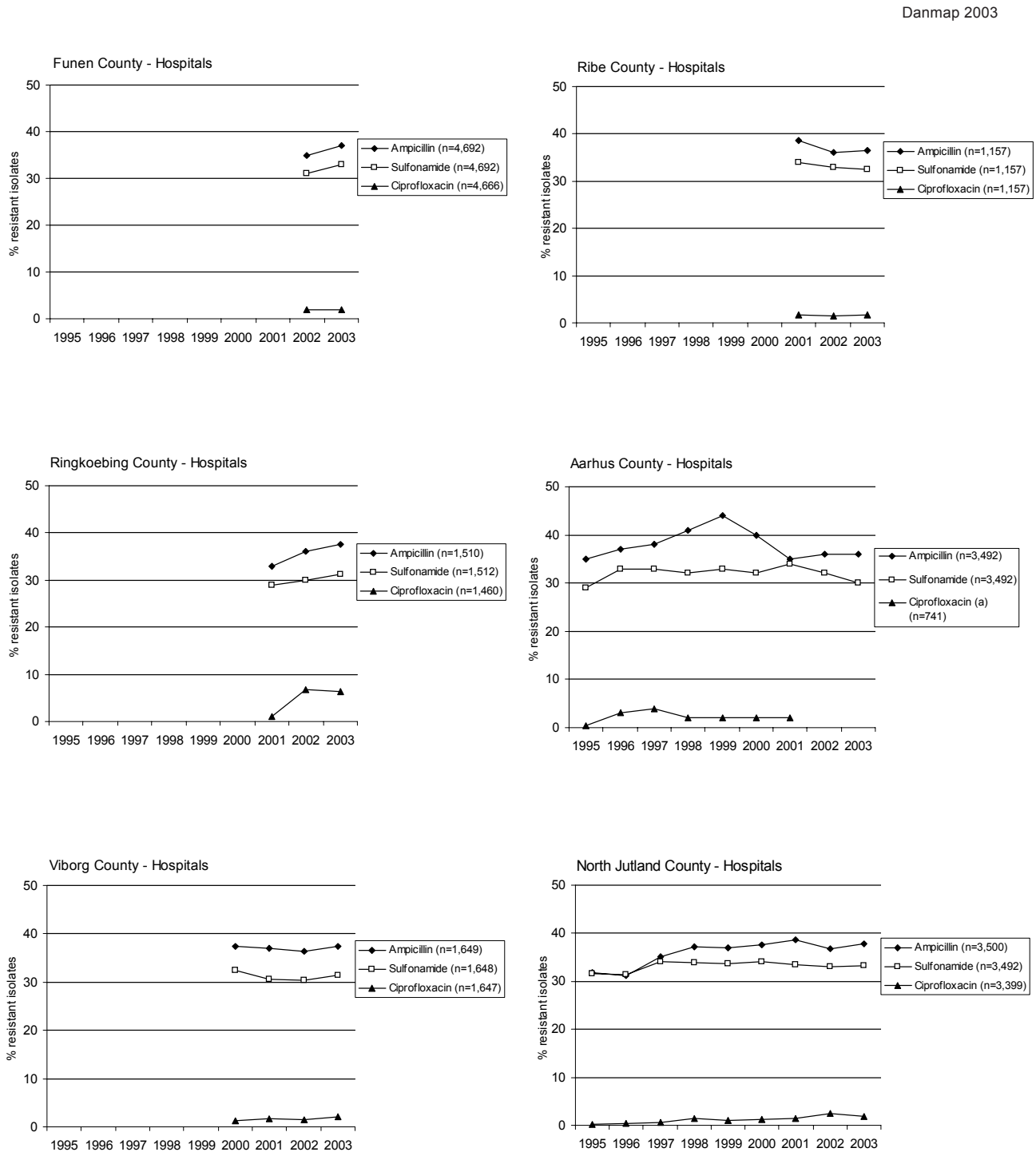
(a) Data on ciprofloxacin is not shown where tests were carried out on selected number of isolates only
(nt) = not tested

Figure 27. Resistance (%) to ampicillin, sulfonamides and ciprofloxacin 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 2003.*



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

Figure 27. Continued. Resistance (%) to ampicillin, sulfonamides and ciprofloxacin 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 2003.*



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

Text box 2

Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Denmark

All *Staphylococcus aureus* isolates from blood cultures from 15 out of 16 counties, as well as all methicillin-resistant *S. aureus* (MRSA) isolates, are referred to the Staphylococcus Laboratory at the Statens Serum Institut (national reference centre) for typing and susceptibility testing. Furthermore, discharge summaries of all reported *S. aureus* from blood and all MRSA are requested from the hospitals or from general practitioners. Data on *Staphylococcus aureus* bacteraemias are published yearly on the SSI website at: http://www.ssi.dk/sw3425.asp.

Based on discharge summary data, reported MRSA are classified as:

- Active screening for MRSA colonisation, or
- Infections, i.e.:
  - imported infection (acquired outside Denmark),
  - hospital acquired infection (no sign of infection at admission)
  - community onset infection with risk factor (infection diagnosed outside a hospital, but prior found positive for MRSA, being a close relative to a MRSA patient or has had contact with a hospital during the past six months)
  - community onset infection without identified risk factor

The number of reported MRSA in Denmark more than doubled between 2002 and 2003 (Figure 1). The distribution of reported MRSA according to origin is shown in Figure 2. Eighteen percent of MRSA in 2003 were identified by active screening, which represented a minor decrease in percentage as compared to previous years.
Overall, the number of MRSA infections more than doubled from 2002 to reach 204 cases in 2003. The number of MRSA infections imported from abroad did not change between 2002 and 2003. The increase in MRSA infections was therefore due to an increase in infections acquired in Denmark. More than 50% of MRSA infections in 2003 were community onset. In 29 % of community onset infections, no risk factor was identified; however, this percentage is probably an overestimate since it is based on discharge summaries that may lack information on risk factors.
Preliminary results of pulsed field gel electrophoresis (PFGE) typing of the MRSA from 2003 reveals that clone ST80-IV (formely known in Denmark as EDK97-1) is still the most frequent clone responsible for community onset infections in Denmark. However, this clone accounted for the same number of MRSA infections in 2002 and 2003 and therefore cannot explain the recent increase in the number of MRSA infections. A prospective case-control study was initiated on 1st January 2004 to investigate the increase in MRSA infections, study the epidemiology of community onset MRSA infections and identify the risk factors associated with MRSA infections in Denmark.

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

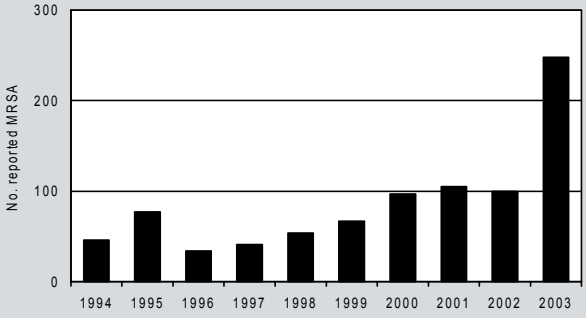


Figure 1. Reported methicillin-resistant Staphylococcus aureus *(MRSA), Denmark, 1994-2003.*

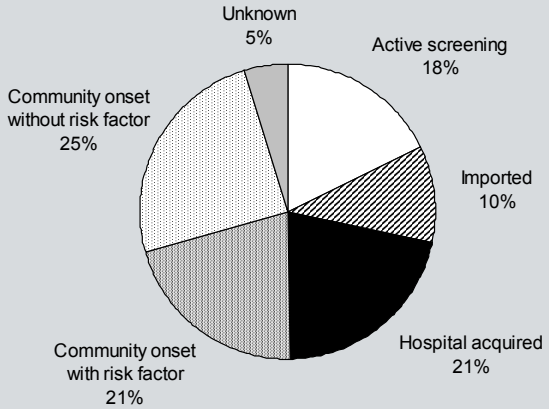


Figure 2. Distribution of reported methicillin-resistant Staphylococcus aureus *(MRSA) according to origin, Denmark, 2003.*

Appendix 1

Materials and Methods

Materials and methods

Data on consumption of antimicrobials

Consumption of antimicrobials in animals

In DANMAP reports prior to 2001, data on antimicrobial usage in animals were based on sales figures reported by the pharmaceutical industry. The monitoring program, VetStat now collects data on all medicine prescribed by veterinarians for use in animals, and on the consumption of coccidiostatics and antimicrobial growth promoters. VetStat contains detailed information about source and usage for each prescription item, the data comprise: date of sale, source, drug identity and amount, and the recipient animal. Data on the recipient animal is the farm-identity code (CHR-ID) within the Danish Central Husbandry Register (CHR), target animal species, age-group, and disease category. The age-group also to some extent represents animal production classes (Table 4). Knowledge of the target animal species enables the presentation of usage statistics in Defined Animal Daily Doses (ADD). The ATCvet/ADD system is a veterinary national equivalent to the international ATC/DDD system used for human consumption (Jensen et al., 2004)

The data source may be the dispensing pharmacy, feed mill, or the veterinarian using the drug. In Denmark, virtually all therapeutic drugs are prescription-only. All prescription medicines are sold through a pharmacy (96% of the antimicrobials) except premixes used in medicated feed, which is sold through feed mills (4% of the antimicrobials). 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, and thus they have little financial incentive to sell medicines. At the pharmacies, the data entry is inevitably linked to billing and electronic transfer to VetStat, thus minimizing the risk of human errors (ensuring the validity of identity and quantity of the drug). Data from the feed mills are subject to logical validation of the codes used at data entry; that is, only valid commodity numbers, units, disease category, animal species, and age-groups may be entered. The total national annual usage (data from feed mills and pharmacies) of antimicrobial drugs is validated against the wholesalers' statistics. In 2002, a deviation of 1.2 % in kg active compound was caused by erroneous reporting of a wholesaler's export, confirming that the pharmacy and feedmill data are reliable (DANMAP 2002). Major technical difficulties in the complex system[1] for transferring data from the veterinary practitioners have been overcome within 2002-2003, and a system enabling the practitioner to correct erroneous data records detected by a full logical validation was introduced in January 2003. The system validates the commodity number, the CHR-ID (if registered) and whether the combination of animal species, age-group and disease category[2].

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. Validation of the amounts of drugs used in mixed practices is restricted to drugs that are primarily used in production animals, because drugs used in pet animals only are reported from the pharmacies – not when used in practice (Stege et al., 2003).

Due to legal regulation of prescription practices, a large part of drugs used in cows (>90%) are used by the practitioner, while the majority of drugs used in poultry (66%) and veal calves (~ 80%), and nearly all drugs used in pigs (>98%), are sold directly by the pharmacies or by the feed mills to the farm. About two-thirds of the antibiotics used in poultry is distributed through veterinary practice by less than 10 practitioners. Errors in their reporting were corrected in 2002, providing a good data quality for poultry. For other species reporting from veterinary practice is still not fully validated. The correction of technical errors caused considerably improvement of data from practitioners during 2003 - affecting primarily the validity of cattle-usage statistics. Validation has shown that the transfer of data is still incomplete for 2003, while the data for 2004 are expected to be almost complete. Antibacterials used in humans and/or animals are presented in Table A1.

[1] The veterinary practices use different soft ware packages and companies to report the data in connection with billing. Fewer veterinarians report directly to VetStat – either on the VetStat web page or by up-load of discs sent to VetStat. The use of different software companies has given rise to many of technical errors (in particular incorrect coding of package size) affecting the drug amounts.

[2] The age-groups are species specific. The disease groups are partially species specific (Stege et al., 2003)

Consumption of antimicrobials in humans

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

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

The present report includes data on the consumption of antibacterials for systemic use, or group J01 of the 2004 update of ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antimicrobials in primary health care is expressed as a number of DDD per 1,000 inhabitants and per day (DDD/1,000 inhabitant-days) and consumption of antimicrobials in hospitals is expressed as a number of DDD per 1,000 beds and per day (DDD/1,000 bed-days). Data on the number of bed-days in each hospital were obtained from the National Board of Health (http://www.sst.dk).

Table A1. Antibacterials used in humans and/or in animals in Denmark a). Antibacterials, which are only used in animals are mentioned in italics (animal growth promoters used before 1999 are mentioned in parentheses). Antibacterials, which are used in humans and in animals are underlined. Danmap 2003

ATC/ATCvet codes	Therapeutic group	Names of antibacterials in group
J01AA/*QJ01AA/QJ51AA*	Tetracyclines	Doxycycline, *chlortetracycline* , lymecycline, oxytetracycline, tetracycline
J01BA/*QJ01BA*	Amphenicols	*Florfenicol*
J01CA/*QJ01CA*	Penicillins with extended spectrum	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, piperacillin
J01CE/*QJ01CE*	β-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, *procaine penicillin* , *penethamate hydroiodide*
J01CF/*QJ51CF*	β-lactamase resistant penicillins	Dicloxacillin, *cloxacillin* , flucloxacillin, *nafcillin*
J01CR/*QJ01CR*	Comb. of penicillins, incl. β-lactamase inhibitors	Amoxicillin/clavulanate, piperacillin/tazobactam
J01DA/*QJ01DA/QJ51DA*	Cephalosporins	Cefalexin, cefalotin, *cefadroxil* , *cefapirine* , cefuroxime, cefotaxime, ceftazidime, ceftriaxone, *cefoperazone, ceftiofur, cefquinom*
J01DF	Monobactams	Aztreonam
J01DH	Carbapenems	Meropenem, imipenem/cilastatin
J01EA	Trimethoprim and derivatives	Trimethoprim
J01EB/*QJ01EQ/QP51AG*	Sulfonamides	Sulfamethizole, *sulfadimidine, sulfaclozine*
J01EE/*QJ01EW*	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfamethoxazole/trimethoprim, *sulfadiazine/trimethoprim* , *sulfadoxine/trimethoprim* , *sulfatroxazole/trimethoprim*
J01FA/*QJ01FA*	Macrolides	Erythromycin, *spiramycin* , roxithromycin, clarithromycin, azithromycin, *tylosin* , *tilmicosin*
J01FF/*QJ01FF*	Lincosamides	Clindamycin, *lincomycin*
J01FG/*QJ01XX*	Streptogramins	*(Virginiamycin)* b)
J01G/A07AA/*QJ01G/QA07AA* c)	Aminoglycosides	*Streptomycin* , *dihydrostreptomycin* , tobramycin, gentamicin, *neomycin* , netilmicin, *apramycin*
J01MA/*QJ01MA*	Fluoroquinolones	Ofloxacin, ciprofloxacin, norfloxacin, moxifloxacin, *enrofloxacin* , *danofloxacin* , *marbofloxacin, difloxacin*
QJ01MB	Other quinolones	*Oxolinic acid*
QJ01MQ	Quinoxalines	*(Carbadox, olaquindox)*
J01XA	Glycopeptides	Vancomycin, teicoplanin, *(avoparcin)*
J01XB/A07AA/*QA07AA* c)	Polypeptides (incl. polymyxins)	Colistin, *(bacitracin)*
J01XC	Steroid antibacterials	Fusidic acid
J01XD/P01AB/*QJ01XD* c)	Imidazole derivatives	Metronidazole
J01XE/*QJ01XE*	Nitrofurane derivatives	Nitrofurantoin
J01XX/*QJ01XX*	Other antibacterials	*Spectinomycin* , methenamine, linezolid, *tiamulin, valnemulin*
QP51AH	Pyranes and hydropyranes (ionophores)	*(Monensin, salinomycin)*
Not in ATCvet	Oligosaccharides	*(Avilamycin)*
Not in ATCvet	Flavofosfolipols	*(Flavomycin)*

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

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 and *Staphylococcus aureus* from examination of cattle for mastitis and *E. coli* from diarrhoea in cattle and pigs and septicaemia in poultry). Finally, *Salmonella* isolates from sub-clinical infections as well as from cases of clinical salmonellosis are included.

The samples from animals at slaughter are collected by meat inspection staff or company personnel and sent to the Danish Institute for Food and Veterinary Research (DFVF) for examination. The number of samples for each plant has been determined in proportion to the number of animals slaughtered per year. Each sample represents one herd or flock. They are collected once a month (weekly for broilers). The broiler, cattle and pig slaughter plants included in the surveillance programme account for 95%, 90% and 95%, respectively, of the total production of these animal species in Denmark. Accordingly, the bacterial isolates may be regarded as representing a stratified random sample of the respective populations, so that the occurrence of resistance provides an estimate of the true occurrence in the populations.

Among all *Salmonella* isolates serotyped at DFVF only one isolate of each serotype per farm is selected for the DANMAP report. The DFVF is the national reference laboratory with respect to *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, Steins Laboratorium and the laboratory of the Federation of Danish Pig Producers and Slaughterhouses in Kjellerup. Accordingly, the programme achieves nationwide coverage for these pathogens.

Isolates from food

All food samples were collected at wholesale and retail outlets by the Regional Veterinary and Food Control Authorities (RFCA) during the course of routine inspection carried out by the authorities, or on request specifically for the DANMAP surveillance programme.

The collection of food samples for analyses of indicator bacteria (enterococci and *E. coli*) was planned and coordinated by the Danish Veterinary and Food Administration (DVFA). The collected material consisted of Danish and imported foods. The food samples were collected according to the guidelines for microbiological examination of foods from the 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.

Collection of *E. faecium*, *E. faecalis*, vancomycin-resistant enterococci and *E. coli* isolates from health volunteers in the community (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. With a calculated response rate of 20% in total, 1055 individuals were invited to participate in the study in 2003. 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 samples in 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 are subjected to pulsed-field gel electrophoresis (PFGE) typing.

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

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

Isolation of bacteria

Examination of samples from animals

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

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

***Campylobacter* spp.** The samples were examined by direct inoculation of selective agar as well as by selective enrichment. As selective agar we used CCD agar, which was incubated in micro-aerophilic atmosphere with 3-6% hydrogen for 1-3 days at 42°C. Selective enrichment was done by inoculation of Preston broth at a ratio of 1:10, followed by incubation in microaerophilic atmosphere for 24 h at 42°C.Ten µl of this enrichment culture was inoculated onto CCD agar and incubated 1 – 3 days at 42°C.

Campylobacter-like colonies were identified by their catalase activity, by their ability to hydrolyse hippurate and indoxyl acetate, and by their susceptibility to cephalothine. For isolates from cattle and pigs, also oxidase activity and nalidixic acid susceptibility were tested.

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

Enterococci from pigs and cattle were isolated and identified by the following procedure. One drop of faecal material suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz-Bartley agar and incubated for 2 days at 42°C. Up to three colonies showing a morphology typical of *E. faecalis* and *E. faecium* were then sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified according to the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose.

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

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

Examination of samples from food

The isolation of indicator organisms from food samples was performed by the Regional Veterinary and Food Control Authorities. Subsequently, the isolates were transferred to standard transport media and shipped to the Danish Veterinary and Food Administration. Verifications of species identity and MIC-determinations were performed by the DVFA. Only one

isolate of *E. coli* and / or *Enterococcus* from each food sample was tested for antimicrobial susceptibility.

E. coli. The isolation method for *E. coli* employed 5 g of food, which was incubated at 44°C for 18-24 hours in 45 ml of MacConkey- or laurylsulfphate-broth. The broth culture was streak-inoculated onto violet red bile agar and incubated for 48 h at 44°C. Presumptive *E. coli* were sub-cultured onto blood agar, transferred to standard transport medium and shipped to DVFA. The isolates were identified as *E. coli* by standard morphological examinations and biochemical tests, including AP80 test (Sensititre).

Enterococci. Analysis for enterococci was carried out by adding 5 g of the sample to 45 ml of azide dextrose broth, which was incubated at 44°C for 18-24 hours, and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours the plates were examined for growth, and typical red colonies were sub-cultured on blood agar, then transferred to transport medium and shipped to the DVFA. After subcultivation on Slanetz-Bartley agar the isolates were identified by PCR with the procedure described for isolates from humans. Only *E. faecium* and *E. faecalis* were included in the surveillance.

A few of the *Enterococcus* and *E. coli* strains were isolated in accordance with the Nordic Committee on Food Analysis (NMKL) No. 68, 2nd ed., 1992 (*Enterococcus*) and NMKL No. 125, 3rd ed., 1996 (*E. coli*).

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

***Campylobacter* spp.** were isolated by a semi-quantitative method. Twenty-five g food sample was mixed 1:4 with Mueller-Hinton broth supplemented with sodium pyrovate 0.25 mg/l, sodium metabisulphite 0.25 mg/l, ferrous sulphate 0.25 mg/l, cefaperazone 30 mg/l, and trimethoprim lactate 50 mg/l and the sample was stomachated. Dilutions 1:10 were prepared, and 1 ml from each dilution was enriched under micro-aerophilic conditions for 24 hours at 42°C in 9 ml of Mueller-Hinton bouillon with supplement (as described above). After pre-enrichment 10 µl was striked on mCCDA and further incubated under microaerophilic conditions for 24-48 hours at 42°C. mCCDA plates were examined for the presence of *Campylobacter*-like colonies. Suspect colonies were verified by phase-contrast microscopy, positive oxidase reaction, and hydrolysis of hippurate- and indoxyl acetate. Species identification was performed according to NMKL No. 119, 2nd ed., 1990. Only isolates of *C. jejuni* were included in the surveillance.

Examination of samples from humans

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

***Campylobacter* spp.** were isolated from faecal samples using modified CCDA (SSI Diagnostika). Species identification was performed with the hippurate test and the indoxyl acetate tests.

Enterococci 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 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. *Escherichia coli* from healthy humans in the community (NorMat study) were isolated and identified by the following procedure. One spoonful

of faeces suspended in 2 ml sodium chloride (0.9%) was spread on the SSI Enteric Medium. Presumptive *E. coli* isolates were sub-cultured on 5% blood agar plates. The isolates were identified as *E. coli* using API 20E test (BioMérieux, France).

Susceptibility testing

Isolates from animals and foods

Agar dilution MIC was used to test the susceptibility of *Campylobacter* isolates to all animicrobials. All other susceptibility testing was done with Sensititre (Trek Diagnostic Systems Ltd.), a commercially available MIC technique using dehydrated antimicrobials in microtitre wells. The wells were inoculated and incubated according to NCCLS guidelines. The MIC was defined as the lowest concentration of antimicrobial with no visible growth. The breakpoints used are shown in Table A2.

Table A3. Breakpoints used for Campylobacter *spp. from humans. Isolates were considered resistant if they had an inhibition zone less than shown in the table.* DANMAP 2003

Antimicrobial agent	*Campylobacter*
Apramycin	24 mm
Chloramphenicol	33 mm
Colistin	18 mm
Ciprofloxacin	27 mm
Erythromycin	27 mm
Gentamicin	30 mm
Kanamycin	22 mm
Nalidixic acid	27 mm
Spectinomycin	30 mm
Streptomycin	32 mm
Tetracycline	32 mm

The following strains were used for quality control: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212. In Sensititre, weekly quality control was performed by inoculation and incubation of a set of wells with the control strains. The MIC values for the strains were evaluated in

Table A2. Breakpoints and range of dilutions used for testing bacteria from animals, foods and humans. Isolates with MIC higher than or equal to the figures shown were considered resistant DANMAP 2003

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

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

accordance to NCCLS guidelines (except for ciprofloxacin, see below). In agar dilution MIC (for *Campylobacter*) all control strains and *Campylobacter jejuni* ATCC 35360 were included on each agar plate.

Isolates from humans

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

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

***Staphylococcus aureus*.** Susceptibility testing was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar (Resistensplade, SSI Methicillin susceptibility was screened for by a cefoxitin 60 mg tablet using <31 mm as a break-point. Methicillin resistance was confirmed by EVIGENE™ (SSI) (Skov RL, et al. J. Antimicrob. Chemother. 1999; 43: 467-475).

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

***Escherichia coli*, coagulase-negative staphylococci and *Streptococcus pyogenes*.** In 2003, the clinical microbiology laboratories serving Ribe, Roskilde, Storstroem and Viborg counties, and Rigshospitalet, which is the national referral hospital and serves part of the municipality of Copenhagen, used the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by A/S Rosco. The clinical microbiology laboratory serving North Jutland county used the same 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 2003, 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 laboratory serving 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.ltkronoberg.se/ext/raf/ZONTAB/Zontab.htm).

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

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

Quinolone resistance and breakpoint

The current National Committee for Clinical Laboratory standards (NCCLS) breakpoint for resistance to the fluoroquinolone ciprofloxacin is ≥ 4 mg/ml. There is now compelling evidence that the treatment efficacy of fluoroquinolones is reduced in humans infected with strains of *Salmonella enterica* with what is regarded as decreased susceptibility to fluoroquinolones (MIC values ≥ 0.125 mg/ml). Thus, to reduce the risk for humans, it has been recommend 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].

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

Danmap 2003

Antimicrobial agent	*E. coli*		*Salmonella* spp.		*Enterococcus* spp.	
	S + I a)	R	S + I	R	S + I	R
Penicillin	-	-	-	-	16/16	4/4
Ampicillin	15/15	10/10	15/15	10/10	-	-
Amoxicillin/Clavulanat	25/25	-	25/25	-	-	-
Cefalothin	24/25		25/25	-	-	-
Ceftiofur	25/25	-	25/25	-	-	-
Erythromycin	-	-	-	-	8/8	12/12
Tetracycline	15/15	10/10	15/15	10/10	4/4	16/16
Chloramfenicol	20/20	5/5	15/15	10/10	4/4	16/16
Vancomycin	-	-	-	-	20/20	-
Teicoplanin	-	-	-	-	20/20	-
Linezolid	-	-	-	-	20/20	-
Quinopristin/dalfopristin	-	-	-	-	8/8	12/12
Nalidixic acid	20/20	5/5	15/15	10/10	-	-
Ciprofloxacin	20/20	5/5	25/25	-	-	-
Neomycin	20/20	5/5	25/25	-	-	-
Streptomycin	-	-	-	-	16/16	4/4
Apramycin	25/25	-	20/20	5/5	-	-
Gentamicin	25/25	-	20/20	5/5	16/16	4/4
Spectinomycin	25/25	-	15/15	10/10	-	-
Colistin	25/25	-	25/25	-	-	-
Sulfonamide	15/15	10/10	15/15	10/10	-	-
Trimethoprim	15/15	10/10	20/20	5/5	-	-
Florfenicol	25/25	-	20/20	5/5	-	-
Avilamycin	-	-	-	-	20/20	-
Flavomycin	-	-	-	-	12/12	8/8
Salinomycin	-	-	-	-	20/20	-
Total	339/340	60/60	320/320	80/80	184/184	76/76
	(99.7%)	(100%)	(100%)	(100%)	(100%)	(100%)

a) S + I: Susceptible and intermediate, R: resistant

In this DANMAP report a breakpoint of ≥ 0.125 mg/ml for fluoroquinolones is used for *Salmonella* and indicator *E. coli* isolates.

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 Unit of Gastrointestinal Infections and the National Center for Antimicrobials and Infection Control at SSI, as well as the Section for Antimicrobial Resistance and Section of Poultry at DFVF, and Institute of Food Safety and Nutrition at DVFA , received five *E. coli* strains, five *Salmonella* spp. and five *Enterococcus* spp. All five laboratories tested the strains in micro-broth dilution test according to recommendations by TREK diagnostics.
A total of 1060 antimicrobial-bacterium susceptibility tests were performed and the overall results were 0.09% failures. The detailed results are shown in Table A4.

Data handling

Data on animal isolates

The results from the primary examination of slaughterhouse samples for the bacteria of interest – positive as well as negative findings – and of the susceptibility testing were stored in an Oracle Database 8i Enterprise Edition®.The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant breakpoints. Each isolate was identified by the bacterial species, including subtype as applicable and by the date and place of sampling and the species of animal. Information on the herd or flock of origin was also recorded. All handling and evaluation of results was carried out using SAS® Software, version 8 of the SAS System for Microsoft® Windows.

Data on food isolates

Results from the analysis of food samples were reported via the Food Microbiology Database or mailed as written data sheets. 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. This information was stored in a relational database (Microsoft® Access) and the data were combined with the susceptibility results (stored as MIC values) in a resistance database (Microsoft® Excel).

Data on human isolates

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

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

Streptococcus pneumoniae. Data on susceptibility testing of *Streptococcus pneumoniae* isolates are stored as MICs in a Microsoft® Access database at the Streptococcus Unit at the Statens Serum Institut.

Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed with Microsoft®Excel.

Escherichia coli, coagulase*-negative staphylococci and *Streptococcus pyogenes. Twelve clinical microbiology laboratories provided aggregated data on resistance levels in *E. coli* blood and urine isolates. Eleven laboratories provided data on 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), Ringkoebing county (Herning Hospital), Aarhus county (Skejby Sygehus) and Viborg county (Viborg 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 tested for each patient. In cases of urine samples, data on ciprofloxacin resistance in *E. coli* were excluded if susceptibility to this antimicrobial was tested on only a selected number of isolates.

Calculation of confidence limits and differences between proportions

Estimation of exact 95% (two-sided) confidence intervals for proportions were based on binomial probability distributions as described in Armitage & Berry (2001), Statistical Methods in Medical Research, 4th ed. 2001, Oxford: Blackwell Scientific Publications. Significance tests of differences between proportions of resistant isolates were calculated using StatCalc in EpiInfo™ v. 6. Yates continuity correction or Fisher's exact test (2-tailed) was applied when appropriate.

Appendix 2

DANMAP Publications

Danmap Publications

2002

Aarestrup FM, Hasman H, Jensen LB, Moreno M, Herrero IA, Domínguez L, Finn M, Franklin A. 2002. Antimicrobial resistance among enterococci from pigs in three European countries. Appl. Environ. Microbiol. 68:4127-4129.

Aarestrup FM, McNicholas PM. 2002. Incidence of high level evernimicin resistance in *Enterococcus faecium* among food animals and humans. Antimicrob. Agents Chemother. 46: 3088-3090.

Aarestrup FM, Jensen LB. 2002. Trends in antimicrobial susceptibility and presence of resistance genes in *Staphylococcus hyicus* isolated from exudative epidermitis in pigs. Vet. Microbiol. 89: 83-94.

Aarestrup FM, Butaye P, Witte W. 2002. Non-human reservoirs of enterococci. In: Gilmore M (ed.). The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance. 1st Edition. ASM Press, Washington DC, 55-99.

Aarestrup FM. 2002. Veterinary drug use in farm animal production and the antibiotic resistance problem. In: Smulders FJM, Collins JD (eds.). Food safety assurance and veterinary public health. Vol. 1 – Food safety assurance in the pre-harvest phase. Wageningen Academic Publishers, The Netherlands, 153-170.

Agersø Y, Jensen LB, Givskov M, Roberts MC. 2002. The identification of a tetracycline resistance gene *tet*(M), on a Tn*916*-like transposon, in the *Bacillus cereus* group. FEMS Microbiol. Lett. 214: 251-256.

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Frimodt-Møller N. 2002. Correlation between pharmacokinetic/pharmacodynamic parameters and efficacy for antibiotics in the treatment of urinary tract infection. Int. J. Antimicrob. Agents. 19: 546-553.

Frimodt-Møller N. 2002. How predictive is PK/PD for antibacterial agents? Int. J. Antimicrob. Agents 19: 333-339.

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Hammerum AM, Jensen LB. 2002. Prevalence of the *esp* gene, encoding the enterococcal surface protein, in *Enterococcus faecalis* and *Enterococcus faecium* isolates from hospital patients, poultry and pigs in Denmark. J. Clin. Microbiol. 40: 4396.

Hansen LH, Aarestrup F, Sørensen SJ. 2002. Quantification of bioavailable chlortetracycline in pig feces using a bacterial whole-cell biosensor. Vet. Microbiol. 87: 51-57.

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Hasman H, Aarestrup FM. 2002. *tcrB*, a gene conferring transferable copper resistance in *Enterococcus faecium*: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. Antimicrob. Agents Chemother. 46: 1410-1416.

Helms M, Vastrup P, Gerner-Smidt P, Mølbak K. 2002. Excess mortality associated with antimicrobial drug resistant *Salmonella* Typhimurium. Emerg. Infect. Dis. 8: 490-495.

Heuer OE, Pedersen K, Andersen JS, Madsen M. 2002. Vancomycin resistant enterococci (VRE) in broiler flocks 5 years after the avoparcin ban. Microb. Drug Resist. 8: 133-138.

Heuer OE, Pedersen K, Jensen LB, Madsen M, Olsen JE. 2002. Persistence of vancomycin resistant enterococci (VRE) in broiler houses after the avoparcin ban. Microb. Drug Resist. 8: 355-362.

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Appendix 3

Summary Research Reports

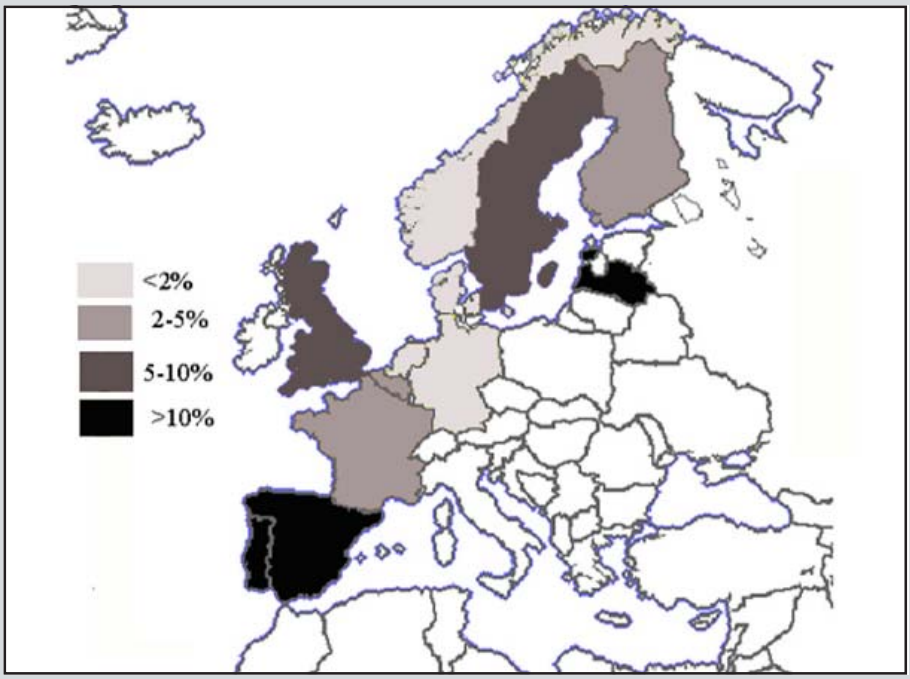
Text box 3

Antibiotic Resistance in Bacteria of Animal Origin - (ARBAO II)

Antimicrobial resistance is considered one of the largest emerging public health problems worldwide. Antimicrobial resistant bacteria spread among different countries as a consequence of travelling and trading of food products and breeding animals. Antimicrobial resistance in one country can therefore become a problem for other countries. Today there is, as yet, no international overview of the occurrence of antimicrobial resistance in different European countries. In addition it is not known whether results from one country are comparable to results from other countries. Recognising these concerns, the European Union (EU) has, in the period 1998-2001, funded a concerted action (FAIR PL 97-3654) that should examine ongoing monitoring of antimicrobial resistance in the EU. This concerted action found a low degree of standardisation regarding the susceptibility testing performed in the veterinary laboratories in the different European countries, making comparison between countries difficult. Thus, as a continuation of this work a new concerted action coordinated by DFVF with participation of the national veterinary reference laboratories in 18 countries was funded in 2003. The ARBAO II-project (FAIR5-QLK2-2002-01146) has, as its final aim, the establishment of continuous monitoring of antimicrobial susceptibility by the veterinary laboratories in all European countries based on validated and harmonised methodologies. It is not possible to implement new guidelines or methodologies in the different laboratories or a harmonisation for all pathogens at the same time. Thus, this concerted action will gradually implement an external quality control system for the most important bacterial pathogens. In addition, summary data of susceptibility testing of the most important bacteria from the different laboratories will be collected and made publicly available. Not all laboratories perform susceptibility testing of the same bacterial species. Thus, laboratories are only expected to participate if they routinely examine the bacterial species in question.

External quality control has been performed for *Salmonella*, *E. coli*, staphylococci and streptococci. Over all, the percentage of correct susceptibility testing of *E. coli*, *Salmonella* and staphylococci was close to 95%. Whether this performance should be deemed acceptable can, and should be discussed within the Community. Participating laboratories scored just above 80% correct results for antimicrobial susceptibility testing of streptococci. Currently there is no common agreement on quality thresholds for acceptable or satisfactory performance, but clearly this should be considered unsatisfactory. There were large differences in the performance of laboratories, ranging from less than 1% to more than 35% incorrect results. This indicates that there is a clear need for harmonisation of methodologies within the EU.

Summary data from the routine susceptibility testing was also collected for 2002. A total of 17 of 19 laboratories responded and provided data from between one to 20 groups out of 22 possibilities. None of the laboratories were able to provide data on all the potential bacterial groups. Not all laboratories provided data on all the antimicrobial agents asked for. Major differences in the occurrence of resistance can be observed between the different countries. Of immediate public health concern is the frequent apparent occurrence of methicillin resistant *Staphylococcus aureus,* occurrence of quinolone and cephalosporin resistance in *Salmonella*, and seemingly emergence of cephalosporin resistance in *E. coli*. However, it should also be noted that many of the reported resistances may have been detected using techniques such as the disc diffusion test and may not have been confirmed using other phenotypic or genetic tests. ARBAO-II is proving very useful in highlighting areas where the test procedures may need careful consideration in the overall evaluation of results. Preliminary results are available on: http://www.dfvf.dk/Default.asp?ID=9753



Floroquinolone resistance among E. coli *from pigs*

The Zoonosis Directive, adopted on 29 September 2003, requires that EU Member States implement a monitoring programme that provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents and, insofar as they present a threat to public health, other agents. Thus, the ARBAO-II concerted action forms a solid basis for this in most countries.

Frank M. Aarestrup, Rene S. Hendriksen, Danilo M. A. Lo Fo Wong

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Text box 4

Terrestial and aquatic environments as source to antimicrobial resistance in the human and animal reservoir

Antimicrobial resistant bacteria are part of the indigenous bacterial community of both soil and water, a large part of these bacteria contains unknown, and possibly mobile resistance mechanisms and genes. Bacteria from terrestic and aquatic environments can be transferred to animals and humans via crops and water, and thereby transfer resistance genes horizontally to pathogenic bacteria. Soil and aquatic environments may therefore be a source to antimicrobial resistance in bacteria that cause infections in humans and animals.

Antimicrobial agents have been used in Danish aquaculture since 1956. Currently, oxolinic acid, florphenicol, amoxicillin, and sulfadiazin and trimethoprim in combination, are used in aquaculture in Denmark. Investigations of the indigenous bacterial community in the aquatic environment have showed a higher level of oxytetracycline resistance at the outlet of four Danish fish farms compared to the inlet. This effect was not shown for any of the other antimicrobial agents, and the effect did not accumulate in the course of downstream fish farms. In contrast to this no significant antimicrobial resistance was found in any of the fish pathogenic bacteria [Schmidt *et al*. 2001. Appl. Environ. Microbiol. 67: 5675-82]. Resistance genes as well as integrons were detected in ubiquitous *Aeromonas* spp. from the aquatic environment. Some of the detected genes were located on mobile elements. Resistance genes from *Aeromonas* were transferred to *E. coli*, *Aeromonas* spp. and *Yersinia ruckeri* [Bruun *et al*. 2003. J. Aquat. Anim. Health. 15: 69-79]. Still, it is important to note that bacteria from the aquatic environment (and fish) in Denmark are adapted to low temperature and very rarely act as human pathogens or even survive at 37ºC.

Tetracyclines are the most used antimicrobial agents in Danish food animal production. It is therefore important to identify and characterise tetracycline resistance genes and mobile elements responsible for horizontal spread of these genes.
Isolates of the indigenous soil bacteria *Alcaligenes, Pseudomonas* and *Arthrobacter* were found to harbour tetracycline resistance genes and class I integrons. The isolates were resistant to up to eight antimicrobial agents including tetracyclines, streptomycin, sulphamethoxazole, trimethroprim, chloramphenicol and ciprofloxacin. Isolates of *Alcaligenes faecalis* had *tet*(A) and class I integron located on a 36 bp broad host-range plasmid capable of transfer *to E. coli, Pseudomonas putida* and *Yersinia ruckeri* at transfer frequencies of 10^{-3}-10^{-5} transconjugant/donor. *Pseudomonas* spp. with *tet*(C) or unidentified tetracycline resistance genes also showed co-transfer of tetracycline resistance gene and class I integrons at transfer frequencies 10^{-4}-10^{-5} transconjugant/donor. The isolates were from stable environment and manured farmland. Therefore, indigenous soil bacteria in close contact to manure and stable environment may contribute not only to spread of tetracycline resistance but other resistances as well.

Even though the occurrence of resistant bacteria in terrestrial and aquatic environments is low compared to the animal and humane reservoir, mobile resistance genes can be detected in both aquatic and terrestric environments that may transfer horizontally to pathogenic bacteria and cause treatment failure of bacterial infections. On this background DFVF and The Royal Veterinary and Agricultural University (RVAU) have an ongoing project focused on mobilisation and characterisation of tetracycline resistance genes in bacteria from terrestric and aquatic environments.

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Text box 5

Prevalence of β-lactamases among *E. coli* and *Salmonella* from food animals in Denmark

The share of β-lactam antibiotics consumed in the Danish farming industry has more than doubled since 1990. In 2002, the β-lactams accounted for 28% of the total amount of therapeutic antibiotics used for food animals in Denmark. Approximately 60% of the β-lactams consumed are β-lactamase-sensitive penicillins (e.g. ampicillin) while the remainder consists of cephalosporins and penicillins with extended spectrum or in combination with β-lactamase inhibitors (e.g. cephalothin and amoxicillin/clavulanic acid).

Extended spectrum β-lactamase (ESBL) resistance has emerged worldwide during the last decade and is today one of the fastest growing problems in public health.

Most studies on β-lactam resistance have focused on human isolates. This has shown that the most prevalent cause of β-lactam resistance in Gram-negative bacteria is due to the production of β-lactamases – enzymes capable of hydrolysing and inactivating the β-lactam antibiotics. Furthermore, the most widespread type of β-lactamases in Gram-negative bacteria is the plasmid-borne TEM-1 β-lactamase and descendants thereof. The preferred substrate of TEM-1 is ampicillin while its activity against cephalosporins is poor. However, an interesting feature of the TEM β-lactamases is that single point mutations in the bla_{TEM} gene can alter the range of β-lactams against which they are active, and hence their ability to escape β-lactamase inhibitors such as clavulanic acid. Today more than 130 variants of TEM β-lactamases are known to exist. The majority of these variants have increased activity against cephalosporins, while a smaller number are inhibitor resistant β-lactamases.

To investigate the occurrence of β-lactamases produced by Gram-negative bacteria isolated from food animals in Denmark, a collection of 160 ampicillin resistant *E. coli* and *Salmonella* isolates was obtained from all the ampicillin resistant *E. coli* and *Salmonella* isolates submitted to the Danish Institute for Food and Veterinary Research in 2002. PCR and sequencing with primers specific for different classes of β-lactamases revealed that 94% of the *Salmonella* isolates expressed the TEM-1 β-lactamase, while the remaining 6% expressed a PSE-type β-lactamase. With respect to the *E. coli* isolates, 86% produced TEM-1, 2% expressed TEM-30 (inhibitor resistant), 1% expressed an OXA-type β–lactamase, 2% expressed TEM-127 and TEM-128 (novel variants) while 9% were shown to hyper-produce the chromosomal AmpC β-lactamase.

In contrast to what is seen in neighbouring countries, no isolates expressing extended spectrum β-lactamase resistance were observed among isolates from Danish production animals.

β-lactam resistant animal pathogens and zoonotic bacteria constitute a potential threat to public health. The 'silent' evolution of the TEM β-lactamases, in spite of the restricted antibiotic usage in Denmark, emphasizes the importance of and need for continuous surveillance and research on this subject.

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Text box 6

Detection of *tet*(M), *tet*(O) and *tet*(S) in tetracycline/minocycline-resistant *Streptococcus pyogenes* bacteraemia isolates

Streptococcus pyogenes is the most common cause of bacterial pharyngitis and causes scarlet fever, impetigo and erysipelas. *S. pyogenes* may also give rise to severe invasive manifestations such as sepsis, necrotizing fasciitis and streptococcal toxic shock syndrome.
Tetracycline/minocycline resistance is often encoded by the *tet*(M) gene in Gram-positive bacteria and more rarely by the *tet*(O), *tet*(Q), *tet*(S), *tet*(T) and *tet*(W) genes, which all encode ribosomal protection proteins. Tetracycline resistance (Tc^R) alone is often encoded by the efflux genes: *tet*(K) and *tet*(L).

Ninety-two *S. pyogenes* isolates were received at the Department of Clinical Microbiology at Hvidovre Hospital, Denmark, between 1990-1999. Susceptibility to erythromycin, ciprofloxacin, penicillin, minocycline and tetracycline was assayed by Etest (AB Biodisk, Solna, Sweden). Thirty-one (33.7%) strains were resistant to tetracycline. This was surprising because tetracycline is not usually used to treat *S. pyogenes* infections in Denmark. Only one isolate from the first bacteremic episode from each patient was included in this study, except in one case where two isolates from one patient were obtained from two episodes of bacteremia two years apart. [Nielsen H. U., Kolmos H. J. & Frimodt-Moller N. Scand. Infect. Dis. 2002. 34:483-6.]

We have recently studied the genetic background for the Tc^R. [Hammerum *et al*. J. Antimicrob. Chemother. 2004. 53: 118-119].The 31 (Tc^R) *S. pyogenes* isolates were investigated for the presence of *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(Q), *tet*(S), *tet*(T) and *tet*(W) genes by PCR. Only one isolate was resistant to erythromycin, and this isolate was also investigated for the presence of *erm*(A), *erm*(B) and *mef*(A) by PCR. None of the isolates were resistant to ciprofloxacin or penicillin. All *S. pyogenes* isolates were T typed with 24 monovalent antisera from Statens Serum Institut and by using PFGE using *Sma*I. The nucleotide sequences of the amplification products of *tet*(S) were determined by cycle sequencing for three isolates.

In all isolates the tetracycline and minocycline resistance was encoded by the presence of a single gene, either *tet*(M), *tet*(O) or *tet*(S).
tet(S) was detected in seven of the 31 isolates. To our knowledge, *tet*(S) has not previously been reported in *S. pyogenes* isolates, although it has been detected in *Listeria* spp., *Enterococcus* spp. and *Lactococcus lactis*. All seven *tet*(S) isolates were collected in 1998 or 1999 and had the same PFGE type indicating a clonal relation ship and vertical spread of the *tet*(S) gene, but analysis of patient data did not indicate any epidemiological relationship.
tet(M) was detected in 23 of the 31 Tc^R *S. pyogenes* isolates. Twelve of the *tet*(M) positive strains were T-nontypeable and had the same PFGE type (type 3), which indicates a clonal relationship of these strains. It has been hypothesized that the transposon carrying the *tet*(M) gene, as typified by Tn*916*, was the original Gram-positive conjugative transposon. It is suggested that over time other antibiotic resistance genes were inserted directly into this family of transposons, creating larger units carrying two to four different antibiotic resistance genes. The remaining 13 *tet*(M) positive isolates had eight different PFGE types and three different T types indicating a horizontal transfer of the *tet*(M) gene.
tet(O) was present in a single isolate from 1999; this isolate was also resistant to erythromycin encoded by *erm*(A). Presence of *tet*(K), *tet*(L), *tet*(Q), *tet*(T) or *tet*(W) was not detected.

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Text box 7

Usage of growth promoters influence on regulation of resistance genes.

A high frequency of macrolide resistant Gram-positive bacteria is found among food animals in Denmark with *erm*(C) as the most prevalent resistance gene. Expression of *erm*(C) is regulated by formation of hairpin structures upstream of the *erm*(C) gene. Only 14- and 15 membered macrolides like erythromycin can induce expression of the gene while 16 membered macrolides like spiramycin, tylosin and streptogramin B will not induce expression [Lodder, C et al, Antimicrob. Agents Chemother. 1996; 40:215-217]. Constitutive expression of macrolide resistance is achieved by deletion of the regulatory DNA region forming a hairpin and will result in resistance to all macrolides. Deletions are believed to occur in environment with high concentrations of non-inducible macrolides such as tylosin. In 1996 68,350 kg of tylosin was used for growth promotion and 1,350 kg for therapy. No macrolides have been used for growth promotion for cattle but spiramycin and tylosin have been used therapeutically for treatment of mastitis. No 16-membered macrolides are used for human treatment.

Using PCR the presence and regulation of the *erm*(C) gene was investigated among macrolide resistant staphylococci of animal and human origin before and after the discontinued usage of growth promoter in 1998. A total of 185 macrolide resistant staphylococci were tested, twenty-nine from cattle (8 *Staphylococcus aureus* and 21 coagulase negative staphylococci (CNS), 111 *Staphylococcus hyicus* isolated from pigs and 33 *S. aureus* from non-hospitalised humans. All human, bovine and 96 porcine isolates were collected prior to 1998. The remaining 15 porcine isolates were collected in 2001, two years after the discontinued usage of growth promoters in Denmark. Results are presented in Table 1.

Table 1

Presence and regulation of *erm*(C)
Among staphylococci of human and animal origin (%)

Origin	Human	Cattle	Pigs	
	S. aureus	Staphylococci	*S. hyicus*	
Year	1995-98	1995-98	1995-98	2001
Usage of tylosin	None	644 kg	69 700 kg	14 300 kg
n =	45	29	96	15
erm(C) negative	14	0	1	8
erm(C) positive	31 (69)	29 (100)	95 (99)	7 (47)
Regulated	25 (81)	9 (31)	9 (9)	3 (43)
Constitutive	6 (19)	20 (69)	86 (91)	4 (57)

Using chi-square test the difference in occurrence of constitutive expressed *erm*(C) genes was compared between isolates from the different reservoirs. Significant differences were found for *S. hyicus* from pigs before and after discontinued usage of growth promoters (p=0.034), between staphylococci isolates from pigs and cattle (p= 0.013), isolates of bovine and human origin (p< 0.001) and isolates from pigs and humans (p<0.001)

The occurrence of constitutive expressed *erm*(C) genes could be related to the amount of tylosin used in the different reservoirs. This indicates that not only have usage of tylosin selected for macrolide resistant staphylococci but the regulation of expression of *erm*(C) gene has also changed.

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Text box 8

Multi-resistant *Salmonella*

Multiple antimicrobial resistant Salmonella isolates have emerged worldwide during the last decades. Especially the emergence of isolates resistant to quinolones and cephalosporins have caused concern, since quinolones are drug of choice for treatment of diarrhoea in humans, while cephalosporins are drug of choice for treatment of salmonellosis in children to whom quinolones must not be administered. Resistance to these groups of antimicrobials results in increased morbidity and mortality.

In Denmark, the occurrence of antimicrobial resistance among salmonella isolates from food animals has in general remained low. In addition, a very strict policy regarding the usage of antimicrobial agents has been implemented taking into consideration the potential consequences for human health. However, the increasing import of food products, breeding animals, travelling and other activities associated with the global economy constitutes a threat against the relatively good situation we have in Denmark. In many other countries antimicrobial agents are used more liberally resulting in a very high frequency of resistance and multiple resistance in several regions. So far this have not have measurable consequences for human health in Denmark.

However, in August 2003 the first extended-spectrum β-lactamase (ESBL) producing *Salmonella* isolate was isolated from the intestine of a boar imported from Canada in May 2003. The isolate was serotyped as *Salmonella* Heidelberg and the gene encoding resistance was located on a transferable plasmid. The isolate was also resistant to chloramphenicol, florphenicol, neomycin streptomycin, sulphonamides and tetracycline, but susceptible to quinolones and gentamicin. More details are given in Aarestrup et al. 2004. Antimicrob. Agents Chemother. 48: 1916-1917.

In October 2003 DFVF received three salmonella isolates found in quail's imported from France. The isolates were serotyped as *S.* Virchow and found resistant to ampicillin, cephalosporins, quinolones and tetracycline. Based on this finding the importer decided to with draw the product from the supermarkets.

In March 2004 three isolates from turkey imported from Germany were received. All isolates were identified as *S.* Saintpaul and found resistant to ampicillin, cephalothin, chloramphenicol, quinolones, spectinomycin, streptomycin, sulphonamides, tetracyclines and trimethoprim and had reduced susceptibility to amoxicillin + clavulanic acid and ceftiofur. The isolates were fully susceptible to apramycin/gentamicin, florphenicol and neomycin.

In April 2004 three isolates also from turkey imported from Germany were received. The isolates were serotyped as *S.* Anatum and found resistant to ampicillin, apramycin, cephalothin, chloramphenicol, gentamicin, neomycin, quinolones, spectinomycin, streptomycin, sulphonamides, tetracycline and trimethoprim and reduced susceptibility to amoxicillin + clavunic acid and ceftiofur. The isolates were only fully susceptible to florphenicol that is a drug only approved for veterinary usage. Thus, no antimicrobial approved for treatment of humans is available.

Especially the combination of resistance to both quinolones and cephalosporins is a matter of concern. These antimicrobial are in most cases drug of choice before results from susceptibility testing is available. In addition, several of the isolates imported from other countries are resistant to so many antimicrobial agents that it in practice could be almost impossible to find a treatment for the infection.

The examples given above clearly shows that food safety in the future has to be solved globally. Currently there is only a very limited control regarding which bacteria we import with food and other products traded across borders. In the future we can only expect to see an increased number of food products being imported from other countries and along with this a flow of multiple resistant bacteria that may give rise to infections that will be impossible to cure. The only way to control this is to build and implement systems and international networks that can predict which main problems we can expect in the near future, develop microbiological methods for rapid detection and characterisation of especially dangerous pathogens that will enable detection in fresh products before they reach the consumer and to implement rapid response systems that can act before it is too late.

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Text box 9

Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection

Besides antibiotics, a large number of other chemical substances are also used in the production of food of animal origin. These include disinfectants to reduce or eliminate environmental contamination and food preservatives to inhibit bacterial growth. In 2003, approximately 103 tons of antibiotics were used for the treatment of food animals in Denmark. Moreover, up to 100 tons of disinfectants for veterinary purposes and 600 tons of disinfectants in the food industry were also used [Aarestrup et al. Dansk Veterinær Tidsskrift 2001; 84(21): 6-13]. Thus, antimicrobial agents in general are more extensively used for disinfection than for treatment of animals. In addition, all feed for food animals are supplemented with copper sulphate and/or zinc chloride in various concentrations. As is the case for antibiotics used for treatment, bacteria may develop resistance to antimicrobial compounds used for disinfection as well as to metals.

The most commonly used disinfectants for veterinary purposes are formaldehyde, iodine, chloride-compounds and copper sulphate. In the food industry the most common compounds are chloride-compounds, acids and alcohols. Resistance to quaternary ammonium compounds is well known and several resistance genes have been identified. In addition, there have been reports of bacteria showing resistance or decreased susceptibility to other disinfectants. Concern has been expressed on the use of biocides possibly contributing to the development of antibiotic resistance in bacteria.

To date, there are only limited data on the susceptibility of different bacterial isolates to antimicrobial compounds used for disinfection. We, therefore, determined the susceptibility of different bacterial isolates (*Salmonella*, *E. coli*, *S. aureus*, *S. hyicus, E. faecalis*, *E. faecium*) from livestock in Denmark to copper sulphate, zinc chloride, benzalkonium chloride, formaldehyde, hydrogen peroxide and chlorhexidine [Aarestrup, Hasman, Vet. Microbiol. 2004; 100: 83-89]. Enterococcal isolates formed a bimodal distribution of MICs to copper sulphate, whereas the other bacterial species formed one large population. Large variations were observed in the susceptibility of the different bacterial species to the different compounds. Staphylococci were in general susceptible to all antimicrobial compounds tested. The *Salmonella* isolates were in general less susceptible to copper sulphate, benzalkonium chloride and chlorhexidine followed by *E. coli* and the Gram-positive species. The opposite was the case for zinc chloride. All isolates were susceptible to H_2O_2 and to formaldehyde. Acquired copper resistance was only found in enterococci. There were large differences in the intrinsic susceptibility of the different bacterial species to these compounds. The decreased susceptibility of *Salmonella* may have public health implications particularly where the use of copper sulphate is widespread. However, further studies are required to determine its impact.

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Text box 10

Has the spread of pig manure slurry an effect on the prevalence of antimicrobial resistant bacteria on Danish farmland?

Large amounts of antimicrobial resistant bacteria and antimicrobial compounds are spread with animal manure on Danish fields each year. We have studied the effects of spread of pig manure slurry on the prevalence of antimicrobial resistant bacteria in farm soil.

The level of tetracycline-, erythromycin- and streptomycin-resistant bacteria before and after spread of pig manure slurry on fields were investigated. The ratio, CFU of tetracycline resistant bacteria/ CFU of all bacteria, was significant higher immediately after pig manure slurry was spread. The number decreased rapidly showing no accumulation of tetracycline resistant bacteria one-year after the spread. No effect on erythromycin and streptomycin resistant bacteria in farmland soil was observed [Sengeløv et al. 2003. Environ. Int. 28: 587-595].

The presence of the *tet*(M) gene in the farmland soil was studied by direct detection of the gene. The *tet*(M) gene was most prevalent in farmland soil immediately after spread of pig manure slurry, but could be detected on farmland soil two years after the field had been treated. On soil not treated with animal manure *tet*(M) could only be detected after selective enrichment with tetracycline present in the media under anaerobic and aerobic conditions. The results indicate that *tet*(M) are spread with bacteria in the manure, but are also present in the indigenous soil bacterial flora possibly in the facultative anaerobic bacteria [Agersø et al. 2004. Environ. Int. 30: 117-22].

Horizontal transfer of the *tet*(M) gene present on a mobile Tn*916*-like element in one *B. cereus* group isolate from soil and two isolates from pig manure slurry was demonstrated. All *tet*(M) positive isolates were able to donate *tet*(M) to enterococci and other animal/human bacteria as well. Since the *tet*(M) gene frequently has been found in enterococci from animals and animal manure, *tet*(M) genes might have spread from bacteria of animal origin, such as enterococci, to the indigenous *B. cereus* group of soil bacteria. The *B. cereus* group isolates were able to donate the *tet*(M) and could therefore transfer the gene back to humans and animals [Agersø et al. 2002. FEMS Microbial. Lett. 214: 251-256].
Macrolide resistant *Bacillus cereus* group bacteria isolated from farmland soil prior to spread of pig manure slurry contained *erm*(B) genes with homology to *erm*(B) previously found in *Enterococcus hirae* and *Clostridium perfringens*. *Clostridium spp*. are frequently isolated from the intestine of pigs and transfer of *erm*(B) to different Gram-positive bacteria or animal/human origin have been demonstrated. Therefore, transfer of *erm*(B) to *B. cereus* group bacteria from bacteria of animal origin may have happened [Jensen et al. 2002. Environ. Int. 28: 487-491].

The overall conclusion is, that the addition of resistant bacteria and resistance genes to farm soil by spread of pig manure is of minor risk to human health in comparison with the risk of transfer of antimicrobial resistant bacteria and resistance genes from animals via the food chain to humans.

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