DANMAP 2002

DANMAP 2002 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark

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

Editors:
Hanne-Dorthe Emborg
Ole Eske Heuer
Danish Zoonosis Centre
Danish Veterinary Institute
Bülowsvej 27
DK - 1790 Copenhagen V

DANMAP board:
Danish Veterinary Institute:
Henrik C. Wegener
Frank Aarestrup
Danish Veterinary and Food Administration:
Jeppe Boel
Statens Serum Institut:
Dominique L. Monnet
Peter Gerner-Smidt
Niels Frimodt-Møller
Kåre Mølbak
Danish Medicines Agency:
Lasse Larsen

Layout:
Susanne Carlsson
Danish Zoonosis Centre
Printing: Datagraf Auning AS

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Reprints can be ordered from:
Danish Veterinary Institute
Bülowsvej 27
DK - 1790 Copenhagen V
Phone: +45 35 30 01 48
Fax: +45 35 30 01 20
e-mail: dzc@dzc.dk

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Contents

The DANMAP 2002 was written by the following persons:

Ole Eske Heuer
Vibeke Frøkjær Jensen
Hanne-Dorthe Emborg
Danish Zoonosis Centre
Danish Veterinary Institute
Bülowsvej 27
DK - 1790 Copenhagen V

Berit Müller-Pebody
Anette Marie Hammerum
Hans Ulrik Kjærem Nielsen
Niels Frimodt-Møller
Dominique L. Monnet
National Center for Antimicrobials and Infection Control
Statens Serum Institut
Artillerivej 5
DK – 2300 Copenhagen S

Sigrid Andersen
Institute of Food Safety and Nutrition
Mørkhøj Bygade 19
DK – 2860 Søborg

The following persons were involved in providing data for the report:

Danish Veterinary Institute:
Karl Pedersen
Jens Christian Østergaard Jørgensen
Eva Haarup Sørensen
Kirsten Christensen
Erik Jacobsen
Frank Møller Aarestrup
Anne Mette Seyfarth
Eva Møller Nielsen
Lone Jannok Porsbo
Jakob Neimann

Institute of Food Safety and Nutrition:

Rikke Kubert
Sigrid Andersen
Jeppe Boel
Naseer M. Shukri
Flemming Kæreby
Peter Saadbye
Dorthe Laugesen
Amer Mujezinovic
Winnie Grebell
Kate Vibefeldt
Anne Arvedlund Olsen
Catia Andersen

Statens Serum Institut:
Anette Arndt
Kristiane B. Beicher
Jens Jørgen Christensen
Jørgen Engberg
Steen Ethelberg
Alice Grassy
Ingrid B. Jensen
Annemarie Jørgensen
Helle B. Konradsen
Anders R. Larsen
Lena Lisbeth Mejlby
Robert Skov

H:S Hvidovre Hospital:
Henrik Westh
Bettina Lundgren
Elly Keller Kristensen

H:S Rigshospitalet:
Michael Tvede
Niels Høiby

Herlev Hospital:
Jens Otto Jarløv

Roskilde Hospital:
Inge Kolle

Slagelse Hospital:
Henrik M. Friis
Hanne Bagge

Næstved Hospital:
Hans Erik Busk

Odense University Hospital:
Hans Jørn Kolmos
Per Søgaard

Sønderborg Hospital:
Poul Kjældgaard

Esbjerg Hospital:
Kjeld Truberg Jensen

Herning Hospital:
Steen S. Schrøder

Aarhus Municipality Hospital:
Jens K. Møller

Viborg Hospital:
Jørgen Prag
Birgitte Tønning

Aalborg Hospital:
Henrik C. Schønheyder
Lena Mortensen

Danish Medicines Agency:
Karin Hovgaard

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Sammendrag

Forbrug af antibiotika

DANMAP giver en samlet fremstilling af anvendelsen af antibiotika til dyr såvel som til mennesker. Alle 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 på besætningsniveau, herunder forbruget til de enkelte husdyrarter og aldersgrupper.

I DANMAP 2002, anvendes enheden standardiserede daglige doser til dyr (ADD). Disse er beregnet for hvert enket præparat og modsvarer den nødvendige daglige dosis af det pågældende præparat til at behandle et givet dyr af en bestemt vægt. Introduktionen af ADD gør det muligt at sammenligne forbruget mellem forskellige dyrearter, aldersgrupper og antibiotika. På trods af en stigning på 68% i forbruget af terapeutisk antibiotika til dyr efter vækstfremmerophøret, fra 57.300 kg aktivt stof i 1998 til 96.202 kg i 2001, blev det totale forbrug af antibiotika til dyr reduceret med mere end 50% efter vækstfremmerophøret. Fra 2001 til 2002 forblev det samlede forbrug af antibiotika til dyr næsten uændret. En mindre stigning i forbruget på 866 kg, fra 96.202 kg i 2001 til 97.068 kg i 2002, kunne næsten udelukkende tilskrives et øget forbug i dambrug som følge af øget sygdomsforekomst. På trods af at det samlede forbrug var uændret, skete der betydelige ændringer i forbruget af de enkelte antibiotika. Forbruget af fluorokinoloner faldt kraftigt som følge af en lovændring i foråret 2002, der havde til formål at begrænse brugen af netop dette antibiotika. Det samlede forbrug af antibiotika til fravænningsgrise faldt, medens forbruget til slagtesvin og smågrise steg. Fra 1998 til 2001 blev der observeret en betydelig stigning i forbruget af tetracyklin, der væsentligst anvendes til svin. I 2002 faldt forbruget imidlertid i forhold til 2001.

I 2002 var det totale forbrug af systemisk anvendt antibiotika til mennesker 14,8 Definerede Døgn Doser (DDD) / 1000 indbygger dage. Dette modsvarer ca. 44.000 kg aktivt stof og udgør en stigning sammenlignet med forbruget i 2001. I primærsektoren steg forbruget med 3% fra 2001 til 2002. Penicilliner, såvel beta-lactamase følsomme som resistente, udgjorde mere en 50% af denne stigning. Årsagen til stigningen i forbruget i primærsektoren er ukendt. Forbruget af antibiotika på hospitaler i Danmark har været stigende siden 1997. I 2002 udgjorde penicillin 57% af det totale antibiotikaforbrug på hospitaler. Ordinationen af antibiotika på danske hospitaler kan stadig beskrives som konservativ, men der er sket en stigning i ordinationen af bestemte klasser af antibiotika, herunder cephalosporiner, fluoroquinoloner og kombinationer af penicilliner incl. beta-lactamase inhibitorer. Brugen af disse tre klasser udgjorde 30% af stigningen i det totale antibiotikaforbrug fra 1997 til 2001. Dette langsomme, men vedholdende, skift mod et øget forbrug af bredspektrede antibiotika på danske hospitaler er bekymrende og bør overvåges nøje.

Resistens i zoonotiske bakterier

I 2002 havde spredning af en nalidixinsyre resistent klon af *Salmonella* Enteritidis i konsumægsproduktionen stor betydning for den samlede forekomst af kinolonresistens blandt *S.* Enteritidis fra fjerkræ. Den danske *Salmonella* handlingsplan for konsumægsproduktionen sikrer imidlertid, at æg eller kød fra en flok vil blive varmebehandlet såfremt der bliver fundet *Salmonella* i flokken. Der blev ikke observeret nogen samtidig stigning i forekomsten af nalidixinsyre resistente *S.* Enteritidis isolater fra humane *Salmonella* infektioner erhvervet i Danmark. I modsætning hertil steg andelen af nalidixinsyre resistente *S.* Enteritidis isolater fra rejseassocierede humane *Salmonella* infektioner markant fra 8% i 2001 til 28% i 2002.

Forbruget af fluoroquinoloner til svin og slagtekyllinger faldt kraftigt i 2002. Forekomsten af nalidixinsyre resistente *Campylobacter jejuni* fra slagtekyllinger faldt fra 8% i 2001 til 0% i 2002. Blandt *C. coli* isolater fra svin og *C. jejuni* isolater fra kvæg blev der i 2002 ikke observeret nogen signifikant ændring i nalidixinsyre resistensforekomsten sammenlignet med 2001.

I 2002 var 79% af alle *C. jejuni* isolater erhvervet ved udlandsrejse resistente overfor ciprofloxacin og nalidixinsyre, hvorimod kun 17% af isolaterne fra infektioner erhvervet i Danmark var resistente overfor disse to antibiotika. Forekomsten af resistens overfor nalidixinsyre i *C. jejuni, S.* Typhimurium og *S.* Enteritidis isolater var signifikant højere i isolater fra infektioner erhvervet i udlandet sammenlignet med isolater fra infektioner erhvervet i Danmark. De fleste mave-tarm infektioner kræver ikke behandling, men når behandling er påkrævet bør det afklares om infektionen er associeret med udlandsrejse, idet der er observeret en høj forekomst af resistens overfor nalidixinsyre og ciprofloxacin i isolater fra infektioner erhvervet i udlandet.

Resistens i indikatorbakterier

Siden 1996 har enterokokker fra raske dyr og fødevare været inkluderet i DANMAP. Fra og med 2002 er *Enterococcus faecium, E. faecalis og Escherichia coli* fra ikke hospitaliserede mennesker ligeledes inkluderet i overvågningen. Dette muliggør fuld jord til bord sammenligning af resistensforekomst blandt indikatorbakterier. Forekomsten af resistens i enterokokker fra mennesker er på nogenlunde samme niveau som forekomsten af resistens i enterokokker fra fødevarer. Dette synes at stemme overens med antagelsen om, at resistensforekomsten i bakterier fra fødevarer afspejles i resistensforekomsten i bakterier fra mennesker. Forekomsten af resistens i enterokokker fra dyr stemmer imidlertid kun i ringe grad overens med forekomsten af resistens i enterokokker fra fødevarer, dette gælder især for svin og svinekød. Disse observationer kan indikere at fødevarer bliver forurenet med enterokokker fra miljøet eller fra personer der håndterer varerne, selv om disse formodentlig ikke er de eneste mulige kilder. Det er derfor muligt, at enterokokker i fødevarer kun delvis afspejler enterokokpopulationerne i de dyr, de pågældende fødevarer stammer fra. Derimod er der for *E. coli* noget bedre overensstemmelse mellem resistensforekomsten i isolater fra dyr, fødevare og mennesker. Ti procent af *E. coli* isolaterne fra raske mennesker besidder den samme resistensprofil som ofte findes i isolater fra hospitaler. Der eksisterer således et reservoir af resistensgener i bakterier fra raske mennesker. Tilstedeværelsen af disse resistensgener vil kunne forårsage behandlingssvigt, hvis der opstår infektion, eller hvis de overføres til patogene *E. coli.*

Resistens i bakterier fra diagnostiske indsendelser

Resistensforekomsten i *E. coli* isolater fra diagnostiske indsendelser fra fjerkræ har været faldende siden 1998. Dette gælder især resistens overfor sulfonamid, tetracyklin og nalidixinsyre. Blandt *E. coli* isolater fra kvæg blev der observeret et signifikant fald i forekomsten af resistens overfor gentamicin, fra 19% i 2001 til 1% i 2002. I 2001 steg forekomsten af resistens overfor gentamicin blant *E. coli* isolater fra svin for første gang. Denne tendens fortsatte i 2002.

I 2002 var *Staphylococcus aureus* isolater fra mastitis hos kvæg følsomme overfor de fleste af de antibiotika der blev udført resistensbestemmelse overfor. Den eneste signifikante ændring fra 2001 til 2002 var en stigning i forekomst af resistens overfor penicillin. Dette faldt sammen med en stigning i forbruget af penicillin til kvæg i 2002.

Denne DANMAP rapport indeholder resistensoplysninger vedrørende *Streptococcus pneumoniae* og *S. aureus* fra patienter fra hele landet. For *E. coli* og koagulase-negative staphylokokker indeholder rapporten data fra 13 af 16 amter. Data fra 2002 bekræfter, at den generelle stigning i resistens overfor ampicillin i *E. coli* isolater fra blod er aftaget og stabiliseret mellem 30 og 50%. Resistens overfor makrolider i *S. pneumoniae* isolater fra blod og spinalvæske har været stigende siden 1992 og nåede op på 5% i 2000. Denne stigning er tilsyneladende ophørt idet 4,7% af isolaterne fra 2002 var resistente overfor makrolid, hvilket var samme niveau som 2001. Stigningen i resistens overfor makrolid blandt *S. pneumoniae* startede efter introduktionen af azitromycin og har siden fulgt forbruget af azitromycin. Efter 1999 er stigningen i forbruget af azitromycin ophørt, hvilket kunne være årsag til stagnationen i forekomsten af makrolidresistente *S. pneumoniae*.
Igennem de sidste 20 år har methicillin resistente *S. aureus* (MRSA) udgjort mindre end 1% af *S. aureus* isolater fra blod og mere end halvdelen af disse MRSA isolater stammede fra udlandet. I 2002 udgjorde de importerede isolater 21% af alle MRSA isolater registreret i Danmark, sammenlignet med 45% i 1999. Andelen af MRSA infektioner erhvervet i Danmark er således steget i denne periode. Foreløbige resultater fra typning af MRSA isolater tyder på at en endemisk klon (EDK97-1), der var årsag til 2/3 af MRSA infektionerne i primærsektoren i 2001, stadig er tilstede. Undersøgelsen forventes at kunne give en bedre forståelse af stigningen i hospitalsrelaterede MRSA infektioner i 2002.

Summary

Antimicrobial usage

DANMAP presents data on the use of antimicrobials in animals and humans. In humans all prescription medicines have been monitored at the level of the individual patient since the early 1990's. Before 2001, only data on the total amount of antimicrobials consumed in animals were available. The VetStat programme, which was initiated in 2000, collects data on all medicines prescribed by veterinarians for use in animals. In DANMAP 2002, the usage of antimicrobials in animals is given as Animal Daily Dosages (ADD) for each compound, animal species and age group. ADDs are calculated for each formulation, and denote the daily dosage required to treat an animal of a certain weight. The introduction of ADDs makes it possible to compare usage between different animal species and compounds.

Despite an increase of 68% in the usage of therapeutic antimicrobials after the Antimicrobial Growth Promoters (AGP) withdrawal from 57,300 kg active compound in 1998 to 96,202 kg in 2001, the total consumption of antimicrobials in animal production was reduced by more than 50% after the AGP withdrawal. From 2001 to 2002 the total consumption of antimicrobials remained almost unchanged with an increase of 866 kg from 96,202 kg in 2001 to 97,068 kg active compound in 2002. This increase was related to increased usage in aquaculture, due to disease outbreaks. However, behind the almost unchanged antimicrobial usage there were changes in usage of individual groups of antimicrobials. In particular, the usage of fluoroquinolones decreased significantly during 2002, following restrictions imposed by the Danish Veterinary and Food Administration to reduce fluoroquinolone usage. A decline in the total usage of antimicrobials was observed for weaner pigs and an increase was observed for slaughter pigs and suckling pigs. The increase in usage of tetracyclines observed from 1998 to 2001 was a cause of concern; however, in 2002 the usage was reduced compared to 2001.

In 2002, total consumption of antimicrobials for systemic use in humans was 14.8 Defined Daily Doses (DDD) / 1,000 inhabitant-days. This corresponds to approximately 44,000 kg active compound and represents an increase as compared to 2001. In primary health care total consumption showed a 3% increase between 2001 and 2002. Beta-lactamase sensitive penicillins and beta-lactamase resistant penicillins were responsible for more than 50% of this increase. The reason for this increase in antimicrobial use in primary health care remains unexplained. In hospitals there has been a steady increase in antimicrobial consumption since 1997. In 2002, penicillins represented almost 57% of hospital antimicrobial use in Denmark. Although the prescription of antimicrobials in Danish hospitals can still be described as conservative, there has been an increase in the use of specific classes including cephalosporins, fluoroquinolones and combinations of penicillins incl. beta-lactamase inhibitors. Use of these three classes represented 30% of the increase in total antimicrobial use between 1997 and 2001. This slow but steady shift towards more use of so-called „broad-spectrum" antimicrobials in Danish hospitals is of concern and deserves further examination.

Resistance in zoonotic bacteria

In 2002, spread of a nalidixic acid resistant clone of *Salmonella* Enteritidis in poultry had a significant effect on the overall level of quinolone resistance in *S.* Enteritidis in poultry. However, the Danish control programme for *Salmonella* in laying hens ensures that once *Salmonella* has been detected in a flock, fresh eggs or meat from the flock will be heat treated. No concurrent increase in nalidixic acid resistance among *S.* Enteritidis isolates from domestically acquired human cases was observed.

In contrast, among *S.* Enteritidis isolates acquired abroad, the percentage of nalidixic acid resistant isolates was significantly higher than in the previous year, with a more than threefold increase from 8% in 2001 to 28% in 2002.

An increase in resistance to nalidixic acid was observed among *C. jejuni* from broilers from 0% in 1996 to 8% in 2001. However, no nalidixic acid-resistant *C. jejuni* were detected in 2002. Among *C. coli* isolates from pigs and *C. jejuni* from cattle no significant changes in nalidixic acid resistance was observed as compared to 2001. In 2002, 79% of the *C. jejuni* isolates from human infections associated with travel were resistant to ciprofloxacin and nalidixic acid whereas this percentage was significantly lower (17%) if the infection was acquired domestically. A similar, although non-significant, trend was observed for tetracycline resistance.

The prevalence of nalidixic acid resistance in human isolates of *C. jejuni, S.* Typhimurium and *S.* Enteritidis was significantly higher if the infection was acquired outside Denmark as compared to domestically acquired infections.

Most gastrointestinal infections do not require treatment; however, when treatment is required, doctors should inquire about the patient's history of travel and be aware of the high probability for resistance to nalidixic acid and ciprofloxacin in infections acquired outside of Denmark.

Resistance in indicator bacteria

Since 1996, resistance in enterococci isolated from healthy animals and foods have been reported in the DANMAP programme. From 2002, *Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli* from healthy humans were included in DANMAP, enabling farm to table comparisons for indicator bacteria. The occurrence of resistance in enterococci from humans was similar to resistance levels observed in isolates from food products. These observations seem to be consistent with the assumption that the resistance levels found in isolates from food are reflected in the occurrence of resistance in humans. However, the level of resistance in enterococci from animals and the respective food products showed large differences. This is in agreement with the assumption that meat may be contaminated with enterococci from the environment and food handlers during processing, although food products may be contaminated with enterococci from other sources. Resistance levels in *E. coli* from healthy human volunteers were comparable to resistance levels in *E. coli* from Danish foods and food animals although in a few cases differences were observed.

Ten percent of the *E. coli* isolates from healthy humans, showed the same multiple resistance patterns often found in isolates from hospitals. These results demonstrated the presence of a reservoir of antibiotic resistance genes in the general population outside hospitals, which may represent a risk for ineffective treatment, should these strains cause infections or the resistance genes be transferred to pathogenic *E. coli* strains.

Resistance in bacteria from diagnostic submissions

Generally the *E. coli* isolates from poultry have become less resistant over time and especially resistance to sulfonamide, tetracycline and nalidixic acid have decreased. Among *E. coli* isolates from cattle a significant decrease in gentamicin resistance, from 19% in 2001 to 1% in 2002 was observed. In 2001, resistance to gentamicin increased for the first time among *E. coli* from pigs and in 2002 the trend was the same. Isolates of *S. aureus* from bovine mastitis were susceptible to most of the antimicrobials tested. In 2002, 30% of the isolates were resistant to penicillin. This represented the only significant change for *S. aureus* from 2001 to 2002, and coincided with an increase in the usage of penicillins in cattle in Denmark in 2002.

For *Streptococcus pneumoniae* and *Staphylococcus aureus* from humans this report includes data covering the whole country. For *Escherichia coli* and coagulase-negative staphylococci, this report includes data from 13 of 16 counties. Data from 2002 confirm that the increase in ampicillin resistance in *E. coli* blood isolates has stopped and remains between 30 and 50%. Resistance to macrolides in *S. pneumoniae* has been increasing since 1992 and reached 5% among isolates from blood and spinal fluid in 2000. This increase seems to have stopped and the percentage of macrolide-resistant *S. pneumoniae* was 4.7% in 2002, a level which is similar to the one observed in 2001. In Denmark, the prevalence of macrolide-resistant *S. pneumoniae* started to increase following the introduction of azithromycin and showed an increase parallel to the consumption of this macrolide. Since 1999, there has been no further increase in azithromycin consumption, which could well be the cause for the interruption observed in the increase in macrolide-resistant *S. pneumoniae*.

For the past 20 years, methicillin-resistant *S. aureus* (MRSA) have represented less than 1% of *S. aureus* blood isolates and more than half of these MRSA strains have been acquired outside Denmark. This pattern has recently changed. Imported cases of infection or colonisation only represented 21% of reported MRSA in 2002, as compared to 45% in 1999. During the same period, MRSA infections acquired in Denmark have increased. Preliminary results from typing of the isolates show that an epidemic clone (EDK97-1), which was responsible for 2/3 of MRSA infections from primary health care in 2001 (see DANMAP 2001), is still present in the Danish community. This analysis should also help understand the increase in hospital MRSA cases in 2002.

Demographic data

Demographic information is presented in order to show the magnitude of animal and human populations in which antimicrobials were used during 2002.

Table1 shows the production of food animals, meat, and the population of dairy cattle. From 2001 to 2002, the number of cattle and pigs slaughtered increased by 0.2% and 4.3%, respectively, while the number of poultry slaughtered decreased by 0.2%. An estimated 41% of all beef consumed was imported, compared with 27% for poultry and 10% for pork.

Table 2 provides information on the county wise distribution of the human population and on the health care system in Denmark.

Table 1. Production of food animals and the production of meat and milk, Denmark, 1990-2002

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Year	Poultry		Cattle (slaughtered)		Dairy cows		Pigs		Farmed fish	
									Fresh water	Salt water
	1,000 heads	mio. kg	1,000 heads	mio. kg	1,000 heads	mio. kg milk	1,000 heads	mio. kg	mio. kg	mio. kg
1990	98,686	133	789	219	753	4,742	16,526	1,260		
1992	111,536	160	862	236	714	4,605	18,559	1,442	35	7
1994	120,349	185	813	210	700	4,642	20,760	1,604	35	7
1996	111,495	182	789	198	701	4,695	20,530	1,592	32	8
1998	129,294	193	733	179	669	4,655	22,873	1,770	32	7
2000	136,933	205	691	171	636	4,720	22,551	1,748	32	7
2001	140,015	213	652	169	623	4,553	23,353	1,836	31	8
2002	139,759	212	653	170	610	4,590	24,365	1,992	32	8

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

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County name	No. inhabitants (1/1/2002)	No. inh./km^2 (2002)	No. inh. /GP c) (2001)	No. bed-days d) (2001, provisional)	No. hospitals (2001)
Copenhagen Municipality a)	500,531	5,672	1,569	1,002,000 e)	4
Frederiksberg Municipality a)	91,322	10,413	1,602	-	1
Copenhagen County b)	617,336	1,174	1,629	595,000	3
Frederiksborg	370,555	275	1,512	326,000	4
Roskilde	234,820	263	1,631	218,000	2
West Zealand	298,731	100	1,540	270,000	4
Storstroem	260,498	77	1,515	275,000	5
Bornholm	44,197	75	1,300	38,000	1
Funen	472,504	136	1,486	520,000	6
South Jutland	253,166	64	1,472	209,000	4
Ribe	224,444	72	1,537	197,000	3
Vejle	351,328	117	1,612	339,000	6
Ringkoebing	274,385	57	1,550	246,000	5
Aarhus	644,666	141	1,561	681,000	8
Viborg	234,323	57	1,562	249,000	3
North Jutland	495,548	80	1,544	489,000	7
Denmark	5,368,354	125	1,552	5,654,000	66

a) Inner Copenhagen
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 use

Antimicrobial usage in animals

Distribution of antimicrobial agents
In Denmark, all antimicrobials, except those approved by the EU as feed additives, are prescription only medicines. They can reach the user only through a pharmacy or a feed mill. Approximately 80% of the total amounts of antimicrobials are sold directly to the animal owners from pharmacies on the basis of a veterinary prescription. Antimicrobial drugs for use in veterinary practise can only be purchased legally at the pharmacies and comprise approximately 15% of the usage. Finally, feed mills account for about 5% of the total sales of antimicrobials for veterinary therapy, with the majority used in aquaculture.

Comparison of usage statistics
Between 1996 and 2000, statistics on the total sales of antimicrobials for veterinary use in Denmark were compared annually, based on reports from pharmaceutical companies to the Danish Medicines Agency. These data from the top of the supply pyramid did not include information on usage in individual target animal species. For the years 1986 to 1994, good quality pharmaceutical industry estimates of total sales were made available. These data have been reported in previous DANMAP reports.

From 2001, the new Danish register of veterinary medicines, VetStat, has replaced the returns on annual sales from the pharmaceutical industry.
The VetStat data are collected close to the point of use, i.e. by pharmacies, veterinary practitioners, and by feed mills. For further detail on the VetStat system, please see Appendix 1 page 55.

Before the introduction of VetStat, statistics were presented as kilograms active compound. In VetStat, the target animal species is known and it is therefore also possible to present usage statistics as Animal Daily Dosages (ADDs), grouped acccording to the Anatomical Therapeutic Classification System (ATC and ATCvet). In Table A1 page 56 in Appendix 1, the active compound used in animals and/or in humans are listed by ATC group.

Trend in usage of therapeutics
Table 3 shows the trend from 1990 to 2002 based on reports from pharmaceutical companies until 2001 and on VetStat data from 2001. Like in previous years antimicrobials used only in companion animals were excluded. Overall, the quantity of antimicrobials used in production animals amounted to 94,300 kg active compound in 2002.

From 2000 to 2001, an increase of 17% in usage of antimicrobials in production animals was observed (DANMAP 2001). This increase exceeded the increase in animal production. The increase was mainly in antimicrobials for oral use, in particular tetracyclines, macrolides/lincosamides and penicillins with extended spectrum. Between 2001 and 2002 usage increased by 3%, which was less than the increase in animal production. The overall changes from 2001 to 2002 will be discussed below.

Usage calculated as kg active compound
Table 4 shows the total veterinary usage of antimicrobial drugs in 2002 in kg active compound by animal species and age group, including usage in companion animals. Data are based on reports from pharmacies and feed

Table 3. Trends in the estimated total usage of antimicrobials (kg active compound) for treatment of food animals, Denmark. Data 1990-1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). Data 1996-2000: Danish Medicines Agency. Data 2001-2002: VetStat

DANMAP 2002

ATC-group a)	Compound	1990	1992	1994	1996	1998	1999	2000	2001 b)	2002 b)
QJ01AA	Tetracyclines	9,300	22,000	36,500	12,900	12,100	16,200	24,000	28,300	24,300
QJ01CE	Penicillins, β–lactamase sensitive	5,000	6,700	9,400	7,200	14,300	14,700	15,100	16,000	16,900
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	6,600	7,300	8,700	9,800
QJ01EW	Sulfonamides + trimethoprim c)	3,800	7,900	9,500	4,800	7,700	6,800	7,000	9,400	10,400
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	1,000	900	850
QJ01F/QJ01XX	Macrolides, lincosamides, tiamulin	10,900	12,900	11,400	7,600	7,100	8,700	15,600	19,900	21,200
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	7,500	10,400	9,600	9,200
QJ01M/QJ01X	Others c)	6,700	6,800	4,400	600	650	350	300	900	1,600
Total		53,400	73,200	89,900	48,000	57,300	61,900	80,700	93,700	94,300

For comparability between VetStat data and previous data, see DANMAP 2000
Only veterinary drugs are included, excluding veterinary medicines obviously used in pets (small tablets, capsules, ointment, eye and ear drops)
a) Only the major contributing ATC-groups are mentioned
b) Data from VetStat 2001-2002. Aquaculture is included
c) Does not include consumption in aquaculture (sale through feed mills and sale of oxolinic acid from pharmacies) before 2001

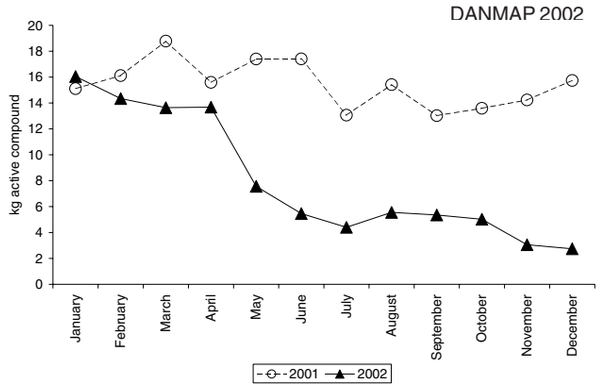

Figure 1. Total veterinary usage of fluoroquinolones in Denmark, 2001 and 2002

The total antimicrobial usage remained almost unchanged with an increase of only 866 kg from 96,202 kg in 2001 to 97,068 kg active compound in 2002. However, behind this lay considerable changes in usage of individual groups of antimicrobials. In particular, the usage of fluoroquinolones decreased significantly during spring 2002, due to the new legislation restricting their use (Figure 1). Usage of tetracyclines decreased, while usage of macrolides/lincosamides and tiamulin increased. These drugs are primarily used in pig production. The usage of cephalosporins increased by 27% from 302 kg in 2001 to 385 kg active compound in 2002, mainly due to increased usage in companion animals.

mills. In some sales to farmers, valid code for animal species was not recorded, however a valid farm ID code was available. These have separate entries in the table. Pharmacy sales for use or resale by veterinary practitioners amounted to 15,166 kg active compound. The usage in companion animals amounts to an estimated 10-15% of the total usage in veterinary practice.

In Table 4, usage in cattle is underreported because in cattle veterinarians must initiate treatment. Accordingly, usage in cattle is included in 'sales for use in practice'. Of the total 15,166 kg antimicrobial used or resold by veterinary practitioners, 7,094 kg was reported used in cattle, underestimating the usage by approximately 25%.

Table 4. Antimicrobials (kg active compound) sold from pharmacies and feedmills in Denmark in 2002 by animal species and age group, Denmark

DANMAP 2002

Therapeutic group	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macrol	Pen-β sens	Pen-other	Sulfa-TMP	Tet	Others	Total
Corresponding ATCgroups a)	QJ01B	QJ01G	QJ01DA	QJ01MA	QJ01MB	QJ01FF	QJ01FA	QJ01CE	QJ01CA, CF, CR	QJ01E	QJ01AA	QJ01X b)	
Pigs													
Breeders and suckling pigs	1	2.572	32	25	-	685	1.792	6.542	2.894	3.448	2.837	17	20.846
Weaners	1	6.087	6	9	-	825	6.613	884	2.239	1.588	10.078	158	28.488
Slaugther pigs	<1	757	5	7	-	1.129	6.112	3.551	1.602	249	8.970	3	22.386
Age not given	1	86	0,3	1	-	37	287	164	90	83	363	1	1.113
Cattle													
Cows and bulls	1	10	1	0,2	-	5	22	44	24	18	16	<1	142
Calves<12mdr	40	260	2	3	-	11	31	424	176	188	290	2	1.427
Heifers, Steers	1	3	<1	<1	-	<1	3	10	6	1	16	<1	40
Age not given	3	11	<1	<1	-	1	14	7	28	7	19	<1	91
Poultry													
Broilers	-	<1	-	1	-	<1	5	-	39	9	8	<1	62
Layers	-	<1	-	2	-	<1	5	-	7	<1	2	-	18
Rearing flocks	-	1	-	1	-	1	30	-	24	32	8	<1	96
Prod. category not given	-	2	-	2	-	-	2	<1	13	8	4	<1	31
Turkeys	-	-	-	0,1	-	<1	-	-	198	-	-	-	198
Small ruminants	<1	1	-	0,02	-	<1	1	3	9	3	2	-	18
Mink	-	167	-	1	-	45	104	<1	375	38	36	<1	766
Aquaculture	76	2	-	<1	983	0	-	1	49	2.964	3	<1	4.077
Other production animals	1	9	1	<1	-	2	4	24	18	17	4	<1	79
Horses	<1	15	0,5	<1	-	0,2	0,2	17	1	101	2	<1	138
Pet animals	0,2	102	77	5	-	8	20	21	73	80	24	11	422
Species not given													
- Farm identified	4	157	1	4	-	40	257	232	215	185	365	3	1.463
- For use in vet. practice	49	1.881	260	38	174	139	900	5.497	1.946	2.874	1.372	37	15.166
Total (pharmacy, feed mill)	178	12.126	385	97	1.157	2.928	16.203	17.421	10.026	11.894	24.420	234	97.068
Reported from vet. practice c)	29	438	72	11	10	54	511	3.630	1.023	1.350	917	6	8.051

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

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

b) Sulfaclozin (a prescription coccidiostat) is included in the Sulfadimidin/tTrimethoprim group

c) Comprises reports from practitioners of usage in production animals in relation to consultations. Originates from pharmacy sales for use in practice.

NB: incomplete data.

VetStat data show that more than 75% of all antimicrobials are used in pig production. The usage in poultry (chickens and turkeys) amounted to an estimated 610 kg active compound, or 0.6% of the total consumption in animals and only 94 kg active compound was used in broilers. Compaired to production figures from 2002 (Table 1), the usage of antimicrobials in pig production amounted to 39 mg/kg meat produced and in broilers 0.5 mg/kg meat produced. In aquaculture the usage was 105 mg/kg fish produced (Table 1). The total consumption of antimicrobials in aquaculture increased by 28% from 3,300 kg in 2001 to 4,300 kg in 2002. This increase was related to a period of exceptionally warm weather from late July to September in 2002, which caused an increased burden of disease in aquaculture.

Usage calculated as Animal Daily Dosages

The detailed information in VetStat enables standardization of drug usage, taking into account the potency of the drugs and the animals in which they are used. Animal Daily Dosages (ADDs) have been defined for each species and each therapeutic formulation. An ADD is – for each formulation – the daily dosage required to treat an animal of a certain weight. In VetStat, ADDs are calculated for each age group. Otherwise, the general principles for standardization of dosage for animals are similar to those used to calculate Defined Daily Doses (DDD) in humans.

Table 5 shows the total usage of antimicrobials calculated as ADD for all species and age groups. Tables 6 to 8 show the usage of antimicrobials in ADDs for pigs, cattle, and poultry, by ATC group and age group. In pig production, the antimicrobial consumption measured as kg animal treated increased by 3.4% from 2001 to 2002. This increase may be related to a 4% increase in pig production. A relative decline in usage has occurred in weaner pigs and a relative increase has occurred in slaughter pigs and suckling pigs. Table 7 shows the usage of antimicrobials in cattle in ADDs.

The usage of antimicrobials in poultry is shown in Tables 5 and 8. Pharmacy data show that usage in poultry increased by 50% from 2001 to 2002. A similar increase

Table 5. Usage of antimicrobials measured as Animal Daily Dosages (ADDs) and kg antimicrobial (kg active compound), based on sales from pharmacies and feed mills to specified animal species and age groups, Denmark

DANMAP 2002

Animal species	Age group / production type	Standard animal a) (kg)	Pharmacies and feed mills			Veterinary practice b)
			Kg antimicrobial	ADD (1000s)		ADD (1000s)
			2002	2001	2002	2002
Pigs	Breeders + suckling pigs	200	20,846	6,885	7,540	80
	Weaners	15	28,488	161,733	159,300	1,300
	Slaugther pigs	50	22,386	37,700	39,250	470
	Age not given	50	1,113	1,434	1,898	-
Cattle	Cows and bulls	600	142	31	39	1,700
	Intramamm/cow c)	600	346	1,147	1,145	-
	Calves<12mo.	100	1,427	953	1,030	410
	Heifers, steers >12mo.	300	40	9	11	70
	Age not given	600	91	7	10	-
Small ruminants	>12 mo.	50	12	12	14	1
	<12 mo.	20	2	4	6	5
	Age not given	50	4	4	5	-
Poultry	Broilers	1	62	4,600	3,100	2,345
	Layers	1	18	1,590	835	234
	Rearing flocks	1	96	3,570	2,820	1,247
	"Age" not given	1	31	935	1,270	-
	Turkeys	1	198	6,390	13,000	10,650
Horses	Age not given	500	138	9	9	-
Mink (ADD)	Age not given	1	766	36,700	40,550	2,800
Aquaculture	Age not given	1	4,251	nc d)	nc	e)
Other prod. animals	Age not given	(ADD) f) 1	79	nc 5,670	2 4,378	34
Pet animals	Age not given	1	422	12,400	11,780	-
Species not given g)			16,109	nc	nc	-
Total			97,068	-	-	-

a) For some species age group is not given. Standard weights are are then set as 1 kg animal and usage is given as 'kg animals treated'
b) Data incomplete, see tex
c) Species not given (used in practice). Calculated as ADDs for cows because the intrammammaries are almost entirely used in cows
d) nc: not calculated
e) 174 kg quinolone used in pratice is included in the data from pharmacies in 2001 and 2002
f) ADD for intramammaries, independant of animal weight
g) For use in practice (15,166 kg minus intramammaries, 346 kg) or for production animals when species not given at the pharmacies

Table 6. Usage of antimicrobials in pigs measured as Animal Daily Dosages (ADDs) in 1,000s in 2001 and 2002, Denmark

DANMAP 2002

ATC group	Compound	Pharmacies and feed mills								Veterinary practice a)		
		Breeders/suckling pigs 200 kg b)		Weaners 15 kg b)		Slaugther pigs 50 kg b)		Age not given 50 kg b)		Breed/suckl. 200 kg b)	Weaners 15 kg b)	Slaugth. 50 kg b)
		2001	2002	2001	2002	2001	2002	2001	2002	2002		
QJ01A	Tetracyclines	887	879	37,207	32,136	9,151	8,723	356	417	16	326	53
QJ01B	Amphenicols	0.2	0.2	0.1	3	0.03	0.4	0.2	1	0.02	0.03	1
QJ01CE	Penicillins, β–lact. sensit.	1,569	1,767	2,241	2,498	4,129	4,534	111	204	21	65	128
QJ01CA/CR	Penicillins, other	829	1,026	8,572	9,955	1,824	2,145	105	122	5	89	47
QJ01DA	Cephalosporins	38	60	60	146	16	36	1	2	0.4	1	1
QJ01E/QP51	Sulfadim./trimeth.	807	968	3,439	3,986	172	205	44	76	11	42	16
QJ01FA	Macrolides	818	812	50,865	46,449	11,435	11,593	369	532	6	103	110
QJ01FF	Lincosamides	440	564	14,042	17,501	3,243	3,815	105	154	6	89	34
QJ01G	Aminogl. (system.)	80	44	8,114	4,643	49	57	27	28	-	8	1
QA07AA*	Aminogl. (local GI)	215	210	19,463	19,297	215	167	55	50	2	188	15
QA07AA10	Colistin (local GI)	1	17	50	2,109	1	14	-	5	-	1	1
QJ01MA	Fluoroquinolones	92	49	526	182	125	67	14	3	3	9	3
QJ01R	Combinations	596	642	1,930	2,151	295	349	22	25	4	17	4
QJ01X	Other antibacterials	506	495	15,203	18,232	7,038	7,544	225	278	10	352	59
QJ51	Intramammaries	8	6	1	1	1	1	-	-	1	0.4	0.5
QG01AA	Gynecologic (local)	0.1	0.1	-	-	0.03	-	-	-	0.01	-	-
Total		6,887	7,539	161,712	159,289	37,694	39,250	1,434	1,898	86	1,290	473

a) Reporting from veterinary practice is incomplete, lacking an estimated 50% of the drugs used by the veterinarian
b) Animal standard weight

Table 7. Usage of antimicrobials in cattle measured as Animal Daily Dosages (ADDs) in 1,000s in 2001 and 2002, Denmark

DANMAP 2002

ATC group	Compound	Pharmacies and feed mills								Veterinary practice		
		Cows, bulls 600 kg a)		Calves <12 mo. 100 kg a)		Heifers, steers 300 kg a)		Age not given 600 kg a)		Cows,bulls 600 kg a)	Calves 100 kg a)	Heifers/steers 300 kg a)
		2001	2002	2001	2002	2001	2002	2001	2002	2002		
QJ01A	Tetracyclines	3	3	307	348	4	6	3	2	133	141	15
QJ01B	Amphenicols	0.03	0.1	16	20	0.1	0.2	0.2	0.2	0.3	12	0.2
QJ01CE	Penicillins, β–lact. sensit	4	5	245	203	2	2	0.5	0.5	382	63	24
QJ01CA/CR	Penicllins, other	2	3	70	121	1	1	0.4	3	77	53	5
QJ01DA	Cephalosporins	0.6	0.9	7	15	0.3	0.4	0.1	0.1	32	11	4
QJ01E	Sulfadimidin/trimeth.	1.2	1.4	63	78	0.1	0.3	0.2	0.4	70	35	3
QJ01FA	Macrolides	2	3	36	38	0.3	0.4	0.5	0.8	78	16	6
QJ01FF	Lincosamides	0.4	0.6	16	21	0.04	0.1	0.6	0.3	2	7	0.2
QJ01G	Aminoglyc. (system.)	0.02	0.01	2	2	0.01	0.03	0.02	0.02	0.01	1	-
QA07AA	Aminoglyc. (local GI)	0.3	0.5	52	48	0.03	0.1	0.3	0.7	0.7	24	0.2
QA07AA10	Colistin (local GI)	0.01	0.01	0.1	3	-	0.01	-	-	-	-	-
QJ01MA	Fluoroquinolones	0.2	0.1	25	10	0.1	0.03	0.1	0.1	0.6	8	0.3
QJ01R	Combinations	0.4	0.7	116	122	0.5	0.6	0.2	0.4	17	37	2
QJ01X	Other antibacterials	0.1	0.4	0.2	0.6	0.5	0.05	1.0	0.9	0.1	0.03	-
QJ51	Intramammaries	16	20	1	0.8	0.2	0.1	-	-	804	5	12
QG01AA	Gynecologic (local)	0.1	0.3	-	-	-	-	-	-	83	0.1	3
Total		30	39	954	1,031	9	11	7	10	1,680	412	74

a) Animal standard weight

Table 8. Usage of antimicrobials in poultry measured as Animal Daily Dosages (ADDs) in 1,000s in 2001 and 2002, Denmark

DANMAP 2002

ATC group	Compound	Pharmacies and feed mills								Veterinary practice		
		Broilers 1 kg a)		Layers 1 kg a)		Rearing 1 kg a)		Prod. type not given 1 kg a)		Broilers 1 kg a)	Layers 1 kg a)	Rearing 1 kg a)
		2001	2002	2001	2002	2001	2002	2001	2002	2002		
QJ01A	Tetracyclines	35	160	15	35	168	137	23	81	-	1	45
QJ01CA	Penicillin (amoxicillin)	4,008	2,650	1,317	504	2,125	1,650	475	867	2,075	50	1,054
QJ01E/QP51	Sulfadim./trimeth.	80	146	40	3	636	530	111	125	-	63	68
QJ01FA	Macrolides	48	63	59	70	302	390	29	32	-	-	-
QJ01FF	Lincosamides	-	2	2	8	87	40	3	-	-	-	-
QJ01MA	Fluorqouinolones	430	72	160	210	242	60	287	160	270	120	80
QJ01X	Other antibacterials	-	3	-	5	8	8	8	-	-	-	-
Total		4,601	3,096	1,593	835	3,568	2,815	935	1,265	2,345	234	1,247

a) Animal standard weight

Table 9. Usage of antimicrobial growth promoters (kg active compound), Denmark

DANMAP 2002

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

a) n = not monitored, assumend to be zero
b) Sold to an exporting feed mill company and a farm near the border to Poland/Germany (pigs treated are presumed exported for slaughter)

Table 10. Usage of coccidiostats in poultry (kg active compound), Denmark

DANMAP 2002

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

a) Based on VetStat data

occurred in poultry practice (data not shown). The increase was related to an increased usage in the turkey production and was due to disease outbreaks. While the poultry production declined 0.2%, the antimicrobial usage in broilers, rearing hens and layers declined by 33%, 24%, and 46%, respectively, from 2001 to 2002 (Table 8). Whether this is a true decrease can not be determined due to lack of data for use in poultry practice in 2001.

Antimicrobial growth promoters

Antimicrobials for growth promotion include only those agents approved by the EU as feed additives, currently avilamycin, flavomycin and the ionophores salinomycin and monensin. Following the official ban on the growth promoter virginiamycin in January 1998, the Danish food animal industries decided to voluntarily discontinue all further use of antimicrobial growth promoters (AGPs). This became effective in broilers, slaughter pigs and cattle in February and March 1998. The use of AGPs was phased out in weaner pigs during 1999. In 2000, use of AGPs was zero. In 2001, VetStat showed the use of very small quantities of flavomycin (11 kg) and avilamycin (3 kg), both among the four AGPs remaining approved by the EU. AGPs sold in 2002 included only small amounts of flavomycin used in pigs (Table 9). These additives have been used in a few farms, exporting pigs for slaughter.

Coccidiostats

Antimicrobials used as coccidiostats in poultry feed must have EU approval as feed additives. The distribution system for AGPs and coccidiostats is well defined and different from that used for prescription-only medicines.

In contrast to previous years where usage information was provided by the Danish Plant Directorate, the data

for 2001 and 2002 were obtained using VetStat. Table 10 shows usage of coccidiostats in poultry production. Almost all coccidiostats used belong to the ionophore group of compounds.
From 2001 to 2002, the consumption of coccidostats decreased by 3,700 kg, while the poultry production in Denmark declined by 0.2%.

Antimicrobial residues
In the previous report (DANMAP 2001, page 16) it was concluded that the frequency of violations of residue limits for slaughter pigs had been found at the extremely low level of 0.02% for a period of 15 years.
For that reason, the frequency of sampling was reduced by 85%. In 2002, no residues were found among 3,835 targeted samples of slaughter animals, eggs and milk. results.

However, analyses of samples from high risk animals did reveal antimicrobial residues especially in sows. Accordingly, future sampling strategy will be even more targeted towards high risk animals.

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.foedevaredirektoratet.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)

Human consumption

Overall

In 2002, overall consumption of antibacterials for systemic use (ATC Group J01, 2002 definition) in humans in Denmark amounted to 28.9 million DDDs or 14.8 DDD/1,000 inhabitant-days. To allow comparison with consumption in food animals, we also present data as kilograms of active compounds (Table 11). In 2002, approximately 44 tonnes of antibacterials were used in humans in Denmark, which represents an increase as compared to 2001.

Primary health care sector

Table 12 presents the consumption of antibacterials for systemic use in primary health care from 1997 to 2002. The data were calculated using the 2002 update of the ATC classification, which includes nitrofurantoin and methenamine. As for previous years, beta-lactamase sensitive penicillins (mostly phenoxymethylpenicillin) represented 38% of total antimicrobial use in primary health care in 2002. Other frequently used antimicrobials were penicillins with extended spectrum (mostly amoxicillin, pivampicillin and pivmecillinam), which accounted for 19% and macrolides which accounted for 16% of total consumption. Detailed data on the consumption of various macrolides is presented in Figure 2. Finally, in 2002, fluoroquinolones, combinations of penicillins including beta-lactamase inhibitors (essentially amoxicillin + clavulanate) and cephalosporins only represented 1.3%, 0.5% and 0.2% of the total consumption in primary health care, respectively.

Total consumption in primary health care showed a 3% increase between 2001 and 2002. Beta-lactamase sensitive penicillins and beta-lactamase resistant penicillins were responsible for more than 50% of this increase. The reason for this increase in antimicrobial use in primary health care remains unexplained; however, it presented a different pattern from the one observed in 2000-2001, which concerned antimicrobials often used to treat respiratory tract infections, i.e. beta-lactamase sensitive penicillins, penicillins with extended spectrum and macrolides (DANMAP 2001).

Table 11. Consumption of antibacterials for systemic use in humans (kg active compound), Denmark. These data must only be used for comparison with consumption in food animals. For monitoring in human primary health care and hospitals, the correct way of expressing consumption is to use DDDs per population-days (see Tables 12 and 13). Consumption in kg active compound has been recalculated from original data expressed as a number of DDDs and includes both primary health care and hospitals. These data therefore represent an estimate of consumption expressed as kg active compound.

DANMAP 2002

ATC group a)	Therapeutic group	Year						
		1997	1998	1999	2000	2001	2002	(low est.-high est.) b)
J01AA	Tetracyclines	1,692	1,692	1,590	1,701	1,705	1,759	
J01B	Amphenicols	1	1	0	1	0	0	
J01CA	Penicillins with extended spectrum	5,513	5,467	5,181	5,135	5,371	5,340	
J01CE	Beta-lactamase sensitive penicillins	18,813	19,947	18,790	19,782	20,715	21,256	
J01CF	Beta-lactamase resistant penicillins	1,913	2,115	2,416	2,654	3,225	3,736	
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	48	55	51	51	144	245	
J01D	Cephalosporins and related substances	660	657	685	727	785	853	(399 - 1,308)
J01EA	Trimethoprim and derivatives	245	256	258	263	280	293	
J01EB	Short-acting sulfonamides	3,498	3,493	3,289	3,148	3,111	3,091	
J01EE	Comb. of sulfonamides and trimethoprim, incl. derivatives	337	322	279	285	283	282	
J01FA	Macrolides	4,227	4,536	4,147	4,040	4,089	4,150	(3,018 - 5,281)
J01FF	Lincosamides	28	38	33	33	42	46	(37 - 56)
J01G	Aminoglycosides	32	31	32	32	28	31	
J01MA	Fluoroquinolones	320	343	321	290	335	381	(265 - 497)
J01MB	Other quinolones	15	17	16	0	0	0	
J01XA	Glycopeptides	25	27	32	37	36	42	
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70	59	59	
J01XD	Imidazoles	128	127	140	154	168	179	
J01XE	Nitrofuran derivatives	137	141	141	149	152	163	
J01XX	Other antibacterials (methenamine, linezolid)	2,233	2,132	1,956	1,792	1,637	1,662	(1,338 - 1,986)
J01	Antibacterials for systemic use (Total) c)	39,938	41,469	39,436	40,344	42,163	43,570	(41,604 - 45,657)

a) From the 2002 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) When 2 different DDDs existed for different presentations, e.g. oral and parenteral, of an antimicrobial, i.e. for cefuroxime, erythromycin, clindamycin ciprofloxacin and methenamine, an average DDD was used. For 2002, extreme values, i.e. estimates using the lowest and the highest DDD, are given in parentheses
c) Does not include polymyxins

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

DANMAP 2002

ATC group a)	Therapeutic group	Year					
		1997	1998	1999	2000	2001	2002
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	0.99	1.04
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	2.47	2.51
J01CE	Beta-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	4.91	5.00
J01CF	Beta-lactamase resistant penicillins	0.34	0.40	0.48	0.52	0.65	0.77
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.02	0.03	0.02	0.02	0.03	0.04
J01DA	Cephalosporins and related substances	0.02	0.03	0.02	0.02	0.03	0.03
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	0.35	0.36
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	0.36	0.36
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.08	0.04	0.03	0.03	0.04	0.03
J01FA	Macrolides	2.03	2.28	2.17	2.02	2.10	2.15
J01FF	Lincosamides	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
J01XB	Polymyxins	0.03	0.02	0.03	0.03	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.02	0.02	0.02	0.02	0.01	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	0.39	0.41
J01XX	Other antibacterials (methenamine, linezolid)	0.46	0.43	0.40	0.36	0.33	0.34
J01	Antibacterials for systemic use (total)	12.24	12.76	12.14	12.25	12.85	13.26

a) From the 2002 edition of the Anatomical Therapeutic Chemical (ATC) classification system

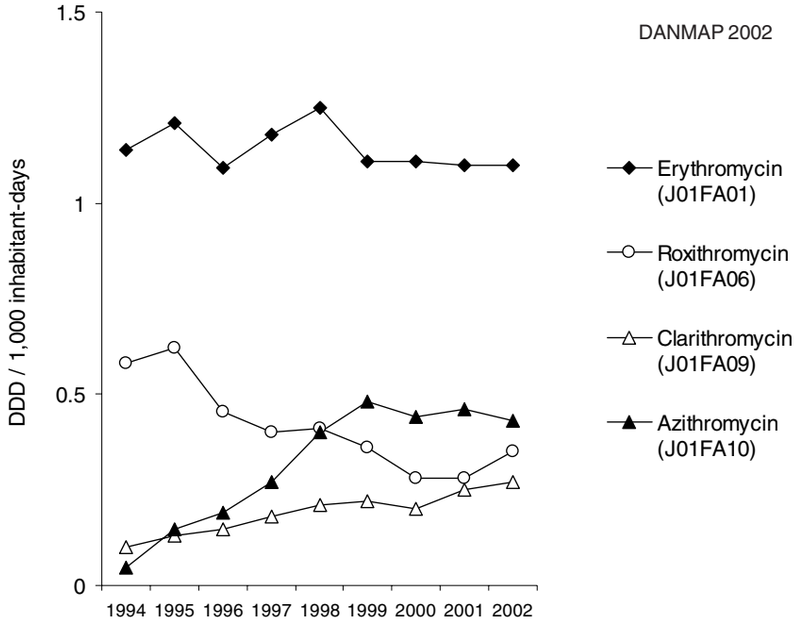

Figure 2. Consumption of macrolides (J01FA) in primary health care, Denmark, 1994-2002

Hospitals

Table 13 presents the consumption of antibacterials for systemic use in hospitals from 1997 to 2002. Total consumption in hospitals was 490 DDD/1,000 bed-days in 2001 and estimated to be approximately 516 DDD/1,000 bed-days in 2002. The numerator includes small amounts of antibacterials given to outpatients, whereas the denominator (number of bed-days) does not include outpatient care. Therefore, hospital consumption data in this report are likely to be slightly overestimated. Nevertheless, comparison with those few data available from other countries shows that antimicrobial consumption in Danish hospitals is low. However, as seen in Table 13**,** there has been a steady increase in antimicrobial consumption in hospitals since 1997. This increase was mainly due to a 17% increase in the number of DDDs of antimicrobials registered by hospital pharmacies (numerator) from approximately 2.3 million DDDs in 1997 to 2.8 million DDDs in 2001, while there was only a 6% decrease in the total number of hospital bed-days registered in Denmark (denominator) between 1997 and 2001. However, the average length of stay in Danish hospitals decreased from 5.7 days in 1997 to 5.2 days in 2001. An explanation for the increase in total hospital antimicrobial consumption could therefore be that, as a result of earlier discharge, bed-days registered by hospitals may now originate from sicker patients. It could also be that to expedite patient discharge, patients now receive more intensive treatment.

In 2002, penicillins represented almost 57% of hospital antimicrobial use in Denmark. Cephalosporins (mainly cefuroxime), fluoroquinolones (mainly ciprofloxacin) and carbapenems only represented 12.5%, 6.8% and 1.1% of total hospital use, respectively. Tetracyclines, combinations of penicillins incl. beta-lactamase inhibitors and glycopeptides each represented less than 1% of total hospital use. Although the prescription of antimicrobials in Danish hospitals can still be described as conservative, there has been an increase in the use of specific classes including cephalosporins, fluoroquinolones and combinations of penicillins incl. beta-lactamase inhibitors (Table 13). Use of these three classes represented 30% of the increase in total antimicrobial use in hospitals between 1997 and 2001. This slow but steady shift towards more use of so-called “broad-spectrum” antimicrobials in Danish hospitals is of concern and deserves close surveillance.

Table 13. Consumption of antibacterials for systemic use in hospitals (DDD/1,000 bed-days), Denmark.
This represented more than 98% of the total DDDs used in hospitals in Denmark in 2002. Psychiatric hospitals, private hospitals and one rehabilitation center were excluded. DANMAP 2002

ATC group a)	Therapeutic group	Year					
		1997	1998	1999	2000	2001 b)	2002 c)
J01AA	Tetracyclines	3.3	3.1	2.7	2.9	2.8	3.2
J01CA	Penicillins with extended spectrum	108.3	109.2	108.1	114.0	115.3	113.6
J01CE	Beta-lactamase sensitive penicillins	77.2	85.7	91.7	99.7	106.2	113.9
J01CF	Beta-lactamase resistant penicillins	42.4	44.3	46.8	53.1	60.0	62.5
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.3	0.4	0.4	0.9	1.7	3.1
J01DA	Cephalosporins	45.3	46.4	49.9	54.4	59.2	64.5
J01DF	Monobactams	0.6	0.1	0.1	0.2	0.1	0.0
J01DH	Carbapenems	3.6	2.4	3.2	3.9	4.2	5.8
J01EA	Trimethoprim and derivatives	4.0	4.2	3.6	3.7	4.3	4.1
J01EB	Short-acting sulfonamides	12.4	12.7	12.3	12.2	12.4	12.3
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	4.4	12.9	13.6	14.0	13.5	14.7
J01FA	Macrolides	31.0	34.0	32.1	32.4	32.6	32.0
J01FF	Lincosamides	1.3	1.8	1.4	1.6	1.7	1.9
J01GB	Aminoglycosides	19.5	19.4	20.2	21.3	18.5	17.6
J01MA	Fluoroquinolones	14.2	15.1	18.4	23.0	28.1	34.9
J01XA	Glycopeptides	2.1	2.3	2.8	3.3	3.2	3.8
J01XB	Polymyxins	0.4	0.2	0.3	0.4	0.3	0.3
J01XC	Steroid antibacterials (fusidic acid)	2.4	2.4	2.6	2.3	2.0	1.9
J01XD	Imidazoles	13.9	14.0	15.8	17.8	19.7	21.2
J01XE	Nitrofuran derivatives	3.6	3.3	2.9	2.8	2.9	2.8
J01XX	Other antibacterials (methenamine, linezolid)	1.7	1.8	1.5	1.4	1.3	1.6
J01	Antibacterials for systemic use (Total)	391.9	415.9	430.4	465.4	490.1	515.9

a) From the 2002 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) Provisional
c) Estimated using a provisional number of DDDs and an estimate of the number of bed-days in 2002 based on past trends

Resistance in zoonotic bacteria

Salmonella

Table 14 shows the *Salmonella* serotype distribution of isolates from food animals, food and humans in 2002. The phage type distributions of *Salmonella* Typhimurium and *Salmonella* Enteritidis are presented in Tables 15 and 16.

Salmonella from food animals

Salmonella isolates from pigs and poultry (broilers and layers) were mainly from subclinical infections, while the majority of isolates from cattle were from clinical cases of salmonellosis. Only one isolate of each serotype per farm was included in this report. In 2002, the analyses concerning isolates from pigs were based on all available *Salmonella* isolates (one isolate per serotype per farm), whereas in previous years only a representative subsample was included.

Tables 17 and 18 show the MIC distribution and the occurrence of resistance in *S.* Enteritidis from poultry and in *S.* Typhimurium from poultry, cattle and pigs in 2002. Trends in resistance to some selected antimicrobials are shown in Figure 3.
No significant changes in resistance among *S.* Enteritidis isolates from poultry were observed, except for resistance to nalidixic acid, which increased from 0% in 2001 to 23% in 2002. Usage of fluoroquinolones in food animals decreased markedly in 2002 and the increase in resistance was most likely a result of clonal spread caused by trade with day-old chickens carrying nalidixic acid resistant *S.* Enteritidis. A similar event occurred in 1999, where 8 farms were infected with strains resistant to nalidixic acid, most likely through trade contacts to an infected hatchery (see DANMAP 1999, p. 20 for details). These are examples of how the association between usage of antimicrobials and occurrence of resistance may be confounded by other factors, such as transmission of resistant bacterial strains between premises.

Among *S.* Typhimurium isolates from cattle, belonging to phage types other than DT104/104a/104b and DTU302, resistance to sulfonamides and streptomycin decreased significantly. In isolates from pigs resistance to ampicillin and streptomycin increased significantly. Among isolates from poultry no significant changes were observed from 2001 to 2002.

Table 14. Distribution (%) of Salmonella *serotypes isolated from animals, foods and humans, Denmark*

DANMAP 2002

Serotypes	Poultry a)	Broiler meat	Cattle a)	Beef	Pigs a)	Pork	Humans
Agona	2	1			<1		2
Bovismor-bificans	1		<1		<1		2
Derby	3	1		11	14	12	<1
Dublin			60	47	<1	6	2
Enteritidis	30	14	2	2	<1		54
Hadar		4		4			1
Infantis	13	7		13	7	10	1
Newport		3	<1				1
Stanley					<1		2
Typhi-murium	15	3	28	11	67	39	18
Virchow		8			<1		2
incl. not typable	36	60	8	13	10	33	16
Number of isolates	88	192	109	47	1,096	146	2,072

a) Only one isolate per serotype per farm

Table 15. Distribution (%) of Salmonella *Typhimurium phage types from animals, food and humans among the isolates selected for susceptibility testing, Denmark*

DANMAP 2002

Phage type	Poultry	Cattle	Pigs	Pork	Humans a)
1					1
3				2	4
12	8	55	36	5	10
17	8	3	7	2	1
41	31		<1		1
66	15		5	2	2
104/104a/104b	8	10	7	32	14
110/110b	15		2		1
120		3	9	9	15
135			1		3
170		3	9	12	4
193		3	5	4	5
U302			<1	2	5
Others incl. not typable	15	23	18	30	34
Number of isolates	13	31	736	57	367

a) Not all isolates selected for susceptibility testing were phage typed

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

DANMAP 2002

Phage type	Poultry	Broiler meat	Humans a)
1		33	6
4	12	19	22
6	4	7	11
6a		19	1
8	46		40
14b			5
21/21b	4	11	6
34			0
Others incl. not typable	35	11	9
Number of isolates	26	27	501

a) Not all isolates selected for susceptibility testing were phage typed

Table 17. Distribution of MICs and occurrence of resistance among Salmonella *Enteritidis from poultry (n=26), Denmark*

DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512 1024
Tetracycline	0	[0.0-13.2]	96.2 3.8
Chloramphenicol	0	[0.0-13.2]	80.8 15.4 3.8
Florfenicol	0	[0.0-13.2]	3.8 88.5 7.7
Ampicillin	4	[0.1-19.6]	69.2 26.9 3.8 a)
Ceftiofur	0	[0.0-13.2]	69.2 23.1 7.7
Cephalothin	0	[0.0-13.2]	61.5 23.1 15.4
Sulfamethoxazole	0	[0.0-13.2]	92.3 7.7
Trimethoprim	0	[0.0-13.2]	100
Apramycin	0	[0.0-13.2]	100
Gentamicin	0	[0.0-13.2]	100
Neomycin	0	[0.0-13.2]	100
Spectinomycin	0	[0.0-13.2]	34.6 65.4
Streptomycin	0	[0.0-13.2]	92.3 7.7
Ciprofloxacin	23	[0.0-13.2]	73.1 3.8 7.7 7.7 7.7
Nalidixic acid	23	[9.0-43.7]	76.9 23.1
Colistin	0	[0.0-13.2]	100

Vertical lines indicate breakpoints for resistance

a) 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

Salmonella from food

In 2002, 389 *Salmonella* isolates obtained from broiler meat, beef and pork sampled at wholesale and retail outlets were examined. The most prevalent serotypes are presented in Table 14. Fifty-seven *S.* Typhimurium isolates were obtained from Danish and imported pork. The results of the susceptibility testing are shown in Table 19. A total of 18 isolates belonged to phage types DT104/104b, some of which belonged to the classical penta-resistant traits.

Salmonella from humans

In 2002, 2,072 cases of human salmonellosis were reported to the Statens Serum Institut in Denmark. This represents a decrease in incidence from 43.3 cases per 100,000 inhabitants in 2001 to 38.5 cases per 100,000 inhabitants in 2002. Since 1998, a decrease was observed in the number of cases of human salmonellosis. This decrease was probably due to successful implementation of measures to contain *Salmonella* in Danish pigs and poultry, as well as promotion of better food hygiene in households. The proportion of reported *Salmonella* infections that were acquired abroad was 14%.

The distribution of *Salmonella* serotypes is presented in Table 14. Susceptibility testing was performed on 521 (47%) *S.* Enteritidis isolates. The occurrence of resistance was generally low, in domestically acquired isolates as well as in those acquired abroad (Table 20). In 2002, there were no significant changes in resistance levels in domestically acquired isolates compared with 2001. In contrast, among isolates acquired abroad, the percentage of nalidixic acid resistant isolates was significantly higher than in the previous year, with a more than threefold increase from 8% (95% CI, 3.3-16.1) in 2001 to 28% (95% CI, 17.9-39.6) in 2002.

Three-hundred and eighty (99%) of the isolates from *S.* Typhimurium infections in humans were subjected to susceptibility testing. As observed in previous years, resistance to ampicillin and sulfonamide in *S.* Typhimurium continued to increase in isolates from domestically acquired infections (Table 21, Figure 3), with a significant increase in 2002 as compared to 2001. However, resistance to trimethoprim decreased significantly. The proportion of DT104 and related phage types (DT104b and DTU302) among the *S.* Typhimurium isolates was similar in 2002 and 2001 (16% and 14%, respectively). For several antimicrobials resistance was significantly higher in isolates from infections acquired abroad compared to isolates from domestically acquired infections (Table 21). No significant changes were observed in levels of resistance among *S.* Typhimurium isolates acquired abroad.

Farm to table

Table 22 presents the occurrence of resistance among *S.* Enteritidis from Danish poultry, imported broiler meat and isolates from human cases acquired domestically and abroad. Comparison of resistance among *S.* Enteritidis isolates from poultry and humans showed that the levels of resistance were similar, except for resistance to nalidixic acid. The sudden increase in resistance to nalidixic acid among *S.* Enteritidis isolates from poultry in 2002 occurred due to the isolation of nalidixic acid resistant *S.* Enteritidis from six flocks of

laying hens. However, the Danish action plan to contain *Salmonella* in laying hens ensures that once *Salmonella* has been detected in a flock, fresh eggs or meat from the flock will be heat treated. No concurrent increase in nalidixic acid resistance among *S.* Enteritidis isolates from humans was observed.

In 2002, 28% of *S.* Typhimurium from Danish pork belonged to phage types DT104/104a/104b. This was a significantly higher proportion than in pigs where only 7% of the isolates belonged to these phage types. In contrast, 36% of *S.* Typhimurium phage types from pigs were DT12 and in Danish pork the prevalence was only 8%. The reason for the high

Table 18. Distribution of MICs and occurrence of resistance among Salmonella *Typhimurium from poultry (n=13), cattle (n=31) and pigs (n=736), Denmark*

DANMAP 2002

Compound	Animal species	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Poultry	15	[1.9-45.5]							84.6				7.7	7.7a)					
	Cattle	23	[9.6-41.1]							74.2	3.2			9.7	12.9					
	Pigs	33	[30.0-37.0]							60.7	5.6	0.3	1.5	4.6	27.3					
Chloramphenicol	Poultry	8	[0.2-36.0]							7.7	53.8	30.8				7.7				
	Cattle	10	[2.0-25.8]							12.9	67.7	9.7				9.7				
	Pigs	7	[5.3-9.2]							8.3	61.4	21.3	1.9	0.3	1.2	5.6				
Florfenicol	Poultry	8	[0.2-36.0]							23.1	69.2			7.7						
	Cattle	10	[2.0-25.8]							16.1	67.7	6.5		9.7						
	Pigs	3	[2.2-5.0]							8.4	72.7	12.9	2.6	2.9	0.1	0.4				
Ampicillin	Poultry	15	[1.9-45.5]						69.2	15.4					15.4					
	Cattle	19	[7.5-37.5]						77.4	3.2					19.4					
	Pigs	18	[15.1-20.8]						70.0	11.3	1.0				17.8					
Ceftiofur	Poultry	0	[0.0-24.7]					76.9	23.1											
	Cattle	0	[0.0-11.2]					96.8	3.2											
	Pigs	0	[0.0-0.5]					77.2	20.5	2.3										
Cephalothin	Poultry	0	[0.0-24.7]							69.2	30.8									
	Cattle	0	[0.0-11.2]							79.3	20.7									
	Pigs	<1	[0.3-1.8]							61.7	24.0	10.7	2.7	0.7	0.1					
Sulfamethoxazole	Poultry	15	[1.9-45.5]												84.6					15.4
	Cattle	19	[7.5-37.5]												77.4	3.2			6.5	12.9
	Pigs	32	[29.1-36.0]												65.9	1.5	0.1		0.3	32.2
Trimethoprim	Poultry	8	[0.2-36.0]								92.3				7.7					
	Cattle	0	[0.0-11.2]								100									
	Pigs	8	[6.3-10.4]								91.8				8.2					
Apramycin	Poultry	0	[0.0-24.7]								92.3	7.7								
	Cattle	0	[0.0-11.2]								100									
	Pigs	1	[0.5-2.1]								97.6	1.4				1.1				
Gentamicin	Poultry	0	[0.0-24.7]						100											
	Cattle	0	[0.0-11.2]						100											
	Pigs	1	[0.5-2.1]						98.8			0.1	0.3	0.7	0.1					
Neomycin	Poultry	0	[0.0-24.7]							100										
	Cattle	0	[0.0-11.2]							100										
	Pigs	7	[5.6-9.5]							91.7	1.0			0.5	6.8					
Spectinomycin	Poultry	15	[1.9-45.5]										7.7	69.2	7.7		15.4			
	Cattle	10	[2.0-25.8]										6.5	83.9			9.7			
	Pigs	14	[11.7-16.9]										16.3	68.3	1.2	2.0	12.1			
Streptomycin	Poultry	15	[1.9-45.5]									61.5	23.1		7.7	7.7				
	Cattle	23	[9.6-41.1]								22.6	54.8			9.7	12.9				
	Pigs	33	[29.2-36.1]								11.0	50.8	5.6	3.0	5.0	24.6				
Ciprofloxacin	Poultry	0	[0.0-24.7]	100																
	Cattle	0	[0.0-11.2]	100																
	Pigs	<1	[0.0-0.5]	94.4	4.9	0.3	0.4													
Nalidixic acid	Poultry	0	[0.0-24.7]									100								
	Cattle	0	[0.0-11.2]									100								
	Pigs	<1	[0.2-1.4]									98.0	1.5			0.1	0.4			
Colistin	Poultry	0	[0.0-24.7]								100									
	Cattle	0	[0.0-11.2]								100									
	Pigs	0	[0.0-0.5]								100									

Vertical lines indicate breakpoints for resistance.

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

DANMAP 2002

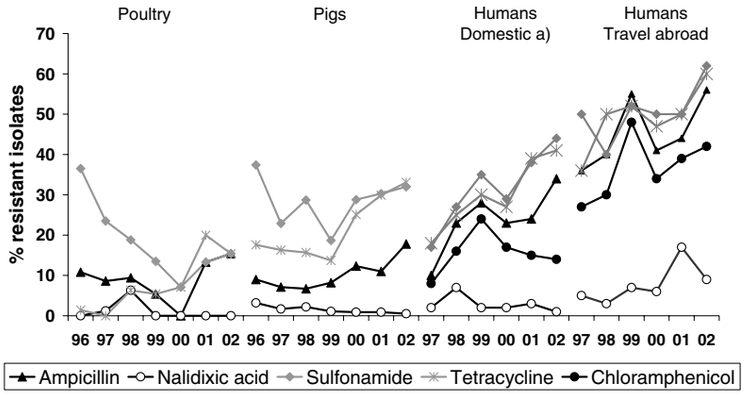

Figure 3. Trends in resistance to some selected antimicrobials among Salmonella *Typhimurium isolated from poultry and pigs and from human cases, Denmark*

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

Table 19. Distribution of MICs and occurrence of resistance in Salmonella *Typhimurium from pork (n=57), Denmark*

DANMAP 2002

Compound	% Resistant	[95% Confidence interval]	Distribution (%) of MICs																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	56	[42.4-69.3]							40.4	3.5	0.0	5.3	12.3	38.6 a)					
Chloramphen	33	[21.4-47.1]								54.4	10.5	1.8	0.0	5.3	28.1				
Florfenicol	26	[15.5-39.7]							8.8	52.6	7.0	5.3	22.8	1.8	1.8				
Ampicillin	44	[30.7-57.6]						42.1	12.3	1.8	0.0	0.0	1.8	42.1					
Ceftiofur	0	[0.0-6.3]					80.7	17.5	1.8										
Sulfamethoxa	60	[45.8-72.4]												40.4	0.0	0.0		8.8	50.9
Trimethoprim	12	[5.1-23.7]								87.7	0.0	0.0	0.0	12.3					
Apramycin	5	[1.1-14.6]								94.7	0.0	0.0	0.0	0.0	5.3				
Gentamicin	5	[1.1-14.6]						94.7	0.0	0.0	0.0	1.8	3.5						
Neomycin	4	[0.4-12.1]							94.7	1.8	0.0	0.0	0.0	3.5					
Spectinomyci	35	[22.9-48.9]										17.5	47.4	0.0	3.5	31.6			
Streptomycin	56	[42.4-69.3]								7.0	31.6	5.3	7.0	17.5	31.6				
Ciprofloxacin	0	[0.0-6.7]	93.0	1.8		3.5	0.0	1.8											
Nalidixic acid	5	[1.1-14.6]								7.0	86.0	1.8	0.0	0.0	0.0	5.3			
Colistin	0	[0.0-6.3]								100									

Vertical lines indicate breakpoints for resistance

a) 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 20. Occurrence of resistance (%) among Salmonella *Enteritidis isolated from humans by origin of infection, Denmark*

DANMAP 2002

Compound	Travel abroad		Domestic a)	
	% Resistant	[95% Confidence interval]	% Resistant	[95% Confidence interval]
Tetracycline	3	[0.3-9.7]	1	[0.4-2.6]
Chloramphenicol	0	[0.0-5.0]	0	[0.0-1.2]
Ampicillin	4	[0.9-11.7]	2	[0.9-3.8]
Ceftiofur	0	[0.0-5.0]	0	[0.0-0.8]
Sulfonamide	3	[0.3-9.7]	1	[0.4-2.6]
Trimethoprim	1	[0.0-7.5]	1	[0.1-1.9]
Apramycin	0	[0.0-5.0]	0	[0.0-0.8]
Gentamicin	1	[0.0-7.5]	0	[0.0-0.8]
Spectinomycin	0	[0.0-5.0]	0	[0.0-1.2]
Streptomycin	0	[0.0-5.0]	0	[0.1-1.6]
Nalidixic acid	28	[17.9-39.6]	4	[2.6-6.5]
Colistin	0	[0.0-5.0]	0	[0.0-0.8]
Number of isolates	72		449	

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. Occurrence of resistance (%) among Salmonella *Typhimurium isolated from humans by origin of infection, Denmark*

DANMAP 2002

Compound	Travel abroad		Domestic a)	
	% Resistant	[95% Confidence interval]	% Resistant	[95% Confidence interval]
Tetracycline	60	[45.9-73.0]	41	[35.2-46.2]
Chloramphenicol	42	[28.7-55.9]	14	[10.0-17.8]
Ampicillin	56	[42.3-69.7]	34	[28.4-38.9]
Ceftiofur	0	[0.0-6.5]	0	[0.0-1.7]
Sulfonamide	62	[47.7-74.6]	44	[38.5-49.6]
Trimethoprim	18	[9.1-30.9]	6	[4.0-9.1]
Apramycin	7	[2.0-17.6]	0	[0.0-1.7]
Gentamicin	7	[2.0-17.6]	1	[0.3-3.1]
Spectinomycin	40	[27.0-54.1]	15	[11.1-19.1]
Streptomycin	51	[37.1-64.6]	38	[32.3-43.1]
Nalidixic acid	9	[3.0-20.0]	1	[0.2-2.7]
Colistin	0	[0.0-6.5]	0	[0.0-1.1]
Number of isolates	55		325	

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

Table 22. Comparison of resistance (%) among Salmonella *Enteritidis from food animals, imported food and human cases acquired domestically or associated with travel abroad, Denmark.*

DANMAP 2002

Compound	Poultry	Broiler meat	Humans	
	Danish	Imported	Domestic a)	Travel abroad
	%	%	%	%
Tetracyclines	0	0	1	3
Chloramphenicol	0	0	0	0
Ampicillin	4	0	2	4
Ceftiofur	0	0	0	0
Sulfonamide	0	0	1	3
Trimethoprim	0	0	1	1
Apramycin	0	5	0	0
Gentamicin	0	5	0	1
Spectinomycin	0	0	0	0
Streptomycin	0	11	0	0
Nalidixic acid	23	16	4	28
Colistin	0	0	0	0
Number of isolates	26	19	449	72

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

prevalence of phage type DT104/104a/104b and the low prevalence of DT12 among isolates from routine inspection of retail outlets is unknown. The occurrence of resistance among *S.* Typhimurium isolates from food animals, pork and domestically acquired human cases is presented in Table 23. The occurrence of resistance in *S.* Typhimurium from imported pork seems high compared to Danish pork. However, the low number of isolates makes it difficult to demonstrate significant differences. The frequency of pentaresistant *S.* Typhimurium DT104 in the samples has a strong influence on the resistance levels. Therefore comparison of resistance levels were made for *S.* Typhimurium phage types other than DT104 and related phage types (DT104a, DT104b, DTU302) from food animals, pork and domestically acquired human cases. These results are presented in Table 24. In general, the levels of resistance observed in isolates from food animals, Danish pork and humans were similar.

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

DANMAP 2002

Compound	Poultry	Cattle	Pigs	Pork a)		Humans	
	Danish	Danish	Danish	Danish	Imported	Domestic b)	Travel abroad
	%	%	%	%	%	%	%
Tetracycline	15	23	33	44	72	41	60
Chloramphenicol	8	10	7	22	61	14	42
Ampicillin	15	19	18	31	72	34	56
Ceftiofur	0	0	0	0	0	0	0
Sulfonamide	15	19	32	53	67	44	62
Trimethoprim	8	0	8	11	17	6	18
Apramycin	0	0	1	0	17	0	7
Gentamicin	0	0	1	0	17	1	7
Spectinomycin	15	10	14	22	61	15	40
Streptomycin	15	23	33	44	67	38	51
Nalidixic acid	0	0	<1	3	11	1	9
Colistin	0	0	0	0	0	0	0
Number of isolates	13	31	736	36	18	325	55

a) 3 isolates are not included as the country of origin is unknown
b) Includes cases where origin of infection is not documented and may therefore include some isolates acquired abroad but not documented as such

The increase in tetracycline resistance in domestically acquired *S.* Typhimurium isolates from humans, observed in recent years, continued in 2002, and may be associated with the concurrent increase in tetracycline resistance among isolates from pigs (Figure 3). Likewise, resistance to sulfonamides and ampicillin among domestically acquired *S.* Typhimurium isolates from humans increased in 2002 and may be related to a concurrent increase (since 1999) in sulfonamide resistance, and to the increase (since 2001) in resistance to ampicillin among isolates from pigs (Figure 3).

Table 24. Comparison of resistance (%) among Salmonella *Typhimurium other than DT104, DT104a, DT104b and DTU302 from food animals, pork and human cases acquired domestically, Denmark* DANMAP 2002

Compound	Poultry	Cattle	Pigs	Pork	Humans
	Danish %	Danish %	Danish %	Danish %	Domestic a) %
Tetracyclines	8	14	31	31	35
Chloramphenicol	0	0	4	0	3
Ampicillin	8	11	14	8	25
Ceftiofur	0	0	0	0	0
Sulfonamide	8	11	30	38	37
Trimethoprim	8	0	8	12	7
Apramycin	0	0	1	0	0
Gentamicin	0	0	1	0	1
Spectinomycin	8	0	11	4	3
Streptomycin	8	14	30	31	30
Nalidixic acid	0	0	<1	4	0
Colistin	0	0	0	0	0
Number of isolates	12	28	684	26	263

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

Text box 1

Quinolone resistance and breakpoint

In *Enterobacteriaceae* resistance to quinolones is most commonly acquired by mutations in two steps. One mutation in the *gyrA* gene mediates full resistance to first generation quinolones such as nalidixic acid and decreased susceptibility to fluoroquinolones, such as ciprofloxacin and enrofloxacin. A second mutation in either *gyrA* or *gyrB* genes mediates full resistance to fluoroquinolones. The current National Committee for Clinical Laboratory standards (NCCLS) breakpoint for resistance to the fluoroquinolone ciprofloxacin is ≥ 4 μg/ml, while the break point for resistance to its veterinary equivalent enrofloxacin is ≥ 2 μg/ml. Even though NCCLS does not provide specific breakpoints for bacteria associated with gastro-intestinal infections, the current breakpoints are widely used by clinicians, veterinarians, microbiologists, and others involved with the issues around selection for fluoroquinolone resistance.

Recently, clinical reports have shown that isolates with a single mutation in *gyrA* to some extent are refractory to the bactericidal effect of fluoroquinolones. There is now compelling evidence that the treatment efficacy of fluoroquinolones is reduced in humans infected with strains of *Salmonella enterica* with what is regarded as decreased susceptibility to fluoroquinolones (MIC values ≥ 0.125 μg/ml). Thus, the use of the NCCLS breakpoint at ≥ 4 μg/ml for ciprofloxacin may have the effect of obscuring the true occurrence of resistance to quinolones among *Salmonella*. In several cases decreased susceptibility to fluoroquinolones has only been detected on the basis of resistance to nalidixic acid after the patient has failed to respond to treatment with ciprofloxacin.

Thus, to reduce the risk for humans, we recommend that for *Salmonella* a breakpoint of ≥ 0.125 μg/ml for fluoroquinolones should be used when evaluating both laboratory sensitivity tests and for the surveillance of resistance to this important group of antimicrobials in both human and veterinary medicine. The most optimal solution may be to use testing for susceptibility to nalidixic acid as a screening tool, and then perform MIC against ciprofloxacin on all nalidixic acid resistant isolates.

In this DANMAP report a break point of ≥ 0.125 μg/ml for fluoroquinolones is used for *Salmonella*. There is neither data on treatment effect of fluoroquinolones against *Escherichia coli* with decreased susceptibility to fluoroquinolones, nor any reports on treatment failures. The NCCLS breakpoint is still used for *E. coli* isolates from animals and foods, whereas for isolates from humans a break point of ≥ 0.125 μg/ml is used.

Aarestrup, F. M., C. Wiuff, K. Mølbak, and E. J. Threlfall. 2003. Is it time to change fluoroquinolone breakpoints for *Salmonella* spp.? Antimicrob. Agents Chemother. **47**: 827-9.

Campylobacter

With 4,379 reported cases, *Campylobacter* was the most common bacterial cause of diarrheal illness in Denmark in 2002. This corresponds to 81.6 cases per 100,000 inhabitants. An estimated 90-95% of these cases was caused by *Campylobacter jejuni. Campylobacter coli* was the second most common species. In poultry and cattle *C. jejuni* was the predominant species, while *C. coli* was the predominant species in pigs. Approximately 80% of all reported human cases of campylobacteriosis in 2002 were acquired in Denmark.

Campylobacter from food animals

Table 25 presents the MIC distributions and occurrence of antimicrobial resistance among *C. jejuni* from broilers and cattle in 2002 and Table 26 presents data for *C. coli* from pigs in 2002. Trends in resistance to selected antimicrobials among *C. jejuni* and *C. coli* from 1996 to 2002 are presented in Figures 4 and 5, respectively. An increase in resistance to nalidixic acid was observed among *C. jejuni* from broilers from 0% in 1996 to 8% in 2001. However, no nalidixic acid resistant *C. jejuni* were detected in 2002. In 2002, the consumption of fluoroquinolones decreased, following restrictions imposed by the Danish Veterinary and Food Administration to reduce fluoroquinolone consumption.

Erythromycin resistance among *C. coli* from pigs was substantially reduced after withdrawal of the antimicrobial growth promoter tylosin. However as macrolides are widely used for treatment of infections in pigs (Table 6) erythromycin resistance has remained above 30%.

Campylobacter from food

Ninety-nine *C. jejuni* isolates obtained from poultry meat samples collected at retail outlets were subjected to susceptibility testing. The results are presented in Table 27. Resistance to tetracycline, nalidixic acid and ciprofloxacin was detected. The occurrence of resistance to tetracycline was significantly higher in isolates from imported broiler meat than in broiler meat of Danish origin.

Campylobacter in humans

In 2002, susceptibility testing and serotyping was performed for 120 (3%) of all isolates submitted to the Department of Gastrointestinal and Parasitic Infections at the Statens Serum Institut. One hundred and twelve (93%) of the isolates were *C. jejuni.* Table 28 presents the occurrence of resistance among *C. jejuni* isolates from humans. Due to the low number of *C. coli* isolates, resistance data from this species is not presented.

Table 25. Distribution of MICs and occurrence of resistance among Campylobacter jejuni *from broilers (n=53 isolates) and cattle (n=53 isolates), Denmark* DANMAP 2002

Compound	Animal species	% Resistant	[95% Confidence interval]	Distribution (%) of MICs														
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Tetracycline	Broilers	2	[0.05-10.1]					98.1							1.9 a)			
	Cattle	6	[1.2-15.7]					94.3					1.9		3.8			
Chloramphenicol	Broilers	0	[0.0-6.7]						15.1	77.4	7.5							
	Cattle	0	[0.0-6.7]						1.9	88.7	9.4							
Ampicillin	Broilers	8	[2.1-18.2]						11.3	18.9	45.3	15.1	1.9	5.7	1.9			
	Cattle	11	[4.3-23.0]						3.8	18.9	47.2	17.0	1.9	3.8	7.5			
Sulfamethoxazole	Broilers	0	[0.0-6.7]									22.6	26.4	17.0	22.6	7.5	3.8	
	Cattle	0	[0.0-6.7]									7.5	45.3	20.8	3.8	11.3	11.3	
Erythromycin	Broilers	0	[0.0-6.7]				5.7	41.5	49.1	3.8								
	Cattle	0	[0.0-6.7]				13.2	43.4	30.2	13.2								
Gentamicin	Broilers	0	[0.0-6.7]					54.7	43.4	1.9								
	Cattle	0	[0.0-6.7]					54.7	35.8	9.4								
Neomycin	Broilers	0	[0.0-6.7]						47.2	37.7	15.1							
	Cattle	2	[0.05-10.1]						32.1	34.0	32.1					1.9		
Streptomycin	Broilers	0	[0.0-6.7]						39.6	52.8	7.5							
	Cattle	2	[0.05-10.1]						34.0	37.7	22.6	3.8				1.9		
Ciprofloxacin	Broilers	0	[0.0-6.7]	1.9	9.4	54.7	34.0											
	Cattle	11	[4.3-23.0]		13.2	45.3	30.2					1.9		7.5	1.9			
Nalidixic acid	Broilers	0	[0.0-6.7]									17.0	83.0					
	Cattle	11	[4.3-23.0]									9.4	71.7	7.5		3.8	3.8	3.8
Colistin	Broilers	0	[0.0-6.7]						7.5	13.2	30.2	28.3	20.8					
	Cattle	0	[0.0-6.7]					1.9	7.5	15.1	20.8	20.8	26.4	7.5				

Vertical lines indicate breakpoints for resistance

a) 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 26. Distribution of MICs and occurrence of resistance among Campylobacter coli *from pigs (n=92), Denmark*

DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs															
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline	1	[0.03-5.9]					82.6	13.0	2.2	1.1			1.1					
Chloramphenicol	0	[0.0-3.9]							5.4	65.2	26.1	3.3						
Ampicillin	1	[0.03-5.9]							3.3	21.7	53.3	20.7	1.1					
Sulfamethoxazole	8	[3.1-15.1]									9.8	21.7	19.6	15.2	17.4	8.7	6.5	1.1a)
Erythromycin	32	[22.2-42.0]				1.1	7.6	2.2	21.7	31.5	4.3		1.1	30.4				
Gentamicin	0	[0.0-3.9]					8.7	58.7	31.5	1.1								
Neomycin	1	[0.03-5.9]							26.1	45.7	27.2			1.1				
Streptomycin	52	[41.5-62.7]							4.3	32.6	10.9	1.1		1.1	50.0			
Ciprofloxacin	8	[3.1-15.1]		1.1	30.4	40.2	18.5	2.2			1.1	4.3	2.2					
Nalidixic acid	8	[3.1-15.1]								4.3	56.5	29.3	2.2	2.2	5.4			
Colistin	1	[0.03-5.9]					6.5	32.6	21.7	21.7	6.5	4.3	5.4	1.1				

Vertical lines indicate breakpoints for resistance

a) 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

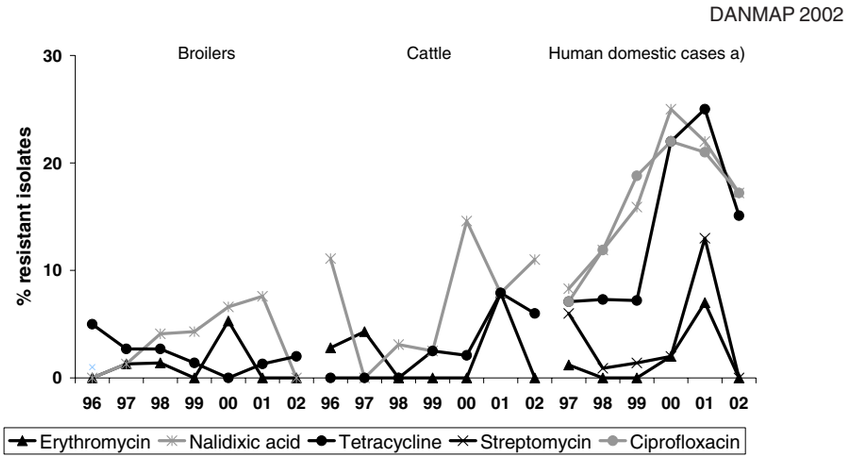

Figure 4. Trends in resistance to selected antimicrobials among Campylobacter jejuni *from broilers, cattle and human domestic cases, Denmark*

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

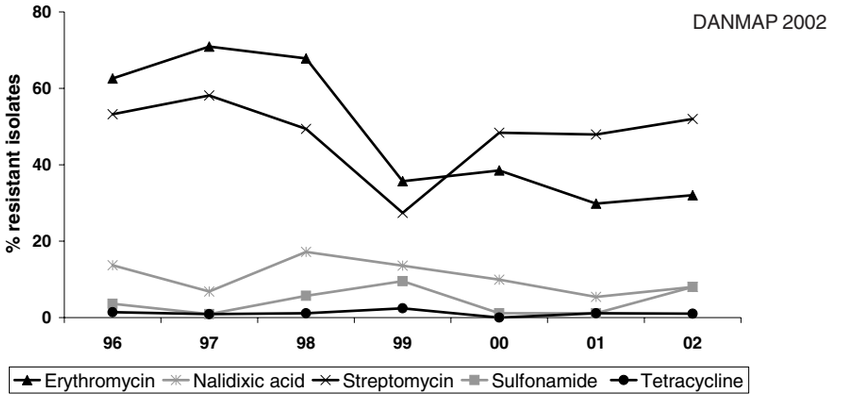

Figure 5. Trends in resistance to selected antimicrobials among Campylobacter coli *from pigs, Denmark*

Trends in resistance to selected antimicrobials among *C. jejuni* in domestically acquired cases are shown in Figure 4. A significant decrease in tetracycline, erythromycin and streptomycin resistance was observed, as compared to 2001.

As previously reported in DANMAP, resistance in *C. jejuni* was generally higher in isolates from infections associated with travel than from infections acquired in Denmark. In 2002, resistance to ciprofloxacin and nalidixic acid were significantly higher in *C. jejuni* from infections associated with travel and a similar, although non-significant, trend was observed for tetracycline resistance (Tables 28 and 29). These results should be taken into account when prescribing antimicrobial treatment to Danish patients with *Campylobacter* infections. Most *Campylobacter* infections do not require treatment; however, when treatment is required, doctors should inquire about the patient's history of foreign travel and be aware of the high probability for resistance to nalidixic acid and ciprofloxacin in *Campylobacter* infections acquired outside Denmark.

Table 27. Susceptibility and occurrence of resistance among Campylobacter jejuni *from broiler meat (n=99), Denmark*

DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs															
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline a)	7	[3.0-14.3]					69.1	14.4	5.2	2.1	2.1	1.0		6.2 b)				
Chloramphenicol	0	[0.0-3.7]						10.2	49.0	28.6	5.1	7.1						
Erythromycin	0	[0.0-3.7]					10.3	45.4	35.1	9.3								
Gentamicin	0	[0.0-3.7]					100											
Streptomycin	0	[0.0-3.7]						99.0		1.0								
Ciprofloxacin	6	[2.3-12.7]	2.0	6.1	52.5	20.2	9.1	2.0	2.0		2.0	1.0	3.0					
Nalidixic acid	6	[2.3-12.7]						5.1	18.2	49.5	12.1	7.1	2.0	1.0	1.0	4.0		

Vertical lines indicate breakpoints for resistance

a) 97 isolates from broiler meat were tested against tetracycline

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

Table 28. Occurrence of resistance (%) among Campylobacter jejuni *isolated from humans by origin of infection, Denmark*

DANMAP 2002

Compound	Travel abroad		Domestic a)	
	% Resistant	[95% Confidence interval]	% Resistant	[95% Confidence interval]
Tetracycline	42	[20.3-66.5]	15	[8.5-24.0]
Chloramphenicol	0	[0.0-17.7]	2	[0.3-7.6]
Gentamicin	0	[0.0-17.7]	0	[0.0-3.9]
Erythromycin	5	[0.1-26.0]	0	[0.0-3.9]
Streptomycin	5	[0.1-26.0]	0	[0.0-3.9]
Ciprofloxacin	79	[54.4-94.0]	17	[10.2-26.4]
Nalidixic acid	79	[54.4-94.0]	17	[10.2-26.4]
Number of isolates	19		93	

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

Table 29. Resistance (%) among Campylobacter jejuni *from Danish food animals, food of Danish origin, imported food and from human cases acquired domestically or associated with travel, Denmark*

DANMAP 2002

Compound	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestic a)	Travel associated
	%	%	%	%	%	%
Tetracycline	6	2	1 b)	21	15	42
Chloramphenicol	0	0	0	0	2	0
Erythromycin	0	0	0	0	0	5
Gentamicin	0	0	0	0	0	0
Streptomycin	2	0	0	0	0	5
Ciprofloxacin	11	0	3	14	17	79
Nalidixic acid	11	0	3	14	17	79
Number of isolates	53	53	70	29	93	19

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

b) only 68 isolates from Danish broiler meat were tested against tetracycline

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 is presented in Table 29. In 2002, resistance levels in food animals, food and domestic human cases were similar (Table 29). However, the occurrence of resistance to tetracycline, ciprofloxacin and nalidixic acid was significantly higher in isolates from domestic human cases compared to isolates from Danish broiler meat. For isolates from Danish broilers and domestic human cases differences were significant for ciprofloxacin and nalidixic acid. This may indicate that sources other than Danish broiler meat contribute to *C. jejuni* infections in humans. Twenty-seven percent of poultry meat consumed in Denmark is imported.

In 2002, a decrease was detected in resistance to tetracycline among isolates from domestic human cases (Figure 4). However, this was not reflected among *C. jejuni* from broilers and cattle. In 2002, resistance to erythromycin was detected neither in *C. jejuni* isolates from cattle and broilers, nor in isolates from domestic human infections (Figure 4).

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 30 and 31. Trends in resistance among *E. faecium* isolates from broilers and pigs, to antimicrobial growth promoters and tetracycline are presented in Figures 6 to12.

Table 30. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broilers (n=102), cattle (n=15) and pigs (n=194), Denmark*

DANMAP 2002

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	7	[2.8-13.6]			93.1					3.9	2.9 a)						
	Cattle	0	[0.0-21.8]			100												
	Pigs	53	[45.3-59.8]			44.8	1.5	0.5	0.5	1.5	12.4	38.7						
Chloramphenicol	Broilers	<1	[0.02-5.3]				2.0	19.6	73.5	3.9			1.0					
	Cattle	0	[0.0-21.8]					53.3	46.7									
	Pigs	1	[0.1-3.7]				1.0	43.3	52.6	2.1	0.5	0.5						
Florfenicol	Broilers	<1	[0.02-5.3]				24.5	73.5	1.0			1.0						
	Cattle	0	[0.0-21.8]				66.7	33.3										
	Pigs	0	[0.0-1.9]				44.8	55.2										
Penicillin	Broilers	61	[50.6-70.3]				22.5	10.8	5.9	9.8	40.2	10.8						
	Cattle	0	[0.0-21.8]				33.3	46.7	20.0									
	Pigs	38	[30.8-44.9]				28.4	20.6	13.4	32.0	5.7							
Erythromycin	Broilers	22	[14.0-30.8]			69.6	5.9	2.9	3.9	4.9	2.0	10.8						
	Cattle	13	[1.7-40.5]			20.0	13.3	53.3	13.3									
	Pigs	30	[24.0-37.4]			14.4	39.2	16.0	1.5		1.0	27.8						
Gentamicin	Broilers	<1	[0.02-5.3]										99.0					1.0
	Cattle	0	[0.0-21.8]										100					
	Pigs	<1	[0.01-2.8]										99.5				0.5	
Kanamycin	Broilers	4	[1.1-9.7]										12.7	52.9	27.5	2.9	2.0	2.0
	Cattle	0	[0.0-21.8]										60.0	20.0	20.0			
	Pigs	21	[15.2-27.0]										46.9	20.6	8.8	3.1	0.5	20.1
Streptomycin	Broilers	5	[1.6-11.1]										95.1					4.9
	Cattle	0	[0.0-21.8]										100					
	Pigs	24	[17.9-30.3]										67.5	1.5	4.6	2.6	11.3	12.4
Teicoplanin	Broilers	5	[1.6-11.1]		92.2		2.9				4.9							
	Cattle	0	[0.0-21.8]		100													
	Pigs	2	[0.6-5.2]		97.4	0.5				1.0	1.0							
Vancomycin	Broilers	5	[1.6-11.1]				90.2	3.9	1.0			4.9						
	Cattle	0	[0.0-21.8]				100											
	Pigs	2	[0.6-5.2]				93.8	2.1	2.1			2.1						
Quinupristin/dalfopristin	Broilers	28	[19.9-38.2]		20.6	10.8	40.2	6.9	18.6	2.0		1.0						
	Cattle	0	[0.0-21.8]		26.7	20.0	53.3											
	Pigs	13	[8.5-18.4]		23.7	7.7	55.7	12.4	0.5									
Avilamycin	Broilers	10	[4.8-17.3]			7.8	27.5	22.5	32.4	4.9	3.9	1.0						
	Cattle	0	[0.0-21.8]			6.7	80.0	6.7	6.7									
	Pigs	0	[0.0-1.9]			16.5	75.3	8.2										
Salinomycin	Broilers	0	[0.0-3.6]			9.8	1.0	9.8	79.4									
	Cattle	0	[0.0-21.8]			100												
	Pigs	0	[0.0-1.9]			95.4	4.6											
Linezolid	Broilers	0	[0.0-3.6]				10.8	82.4	6.9									
	Cattle	0	[0.0-21.8]				6.7	93.3										
	Pigs	0	[0.0-1.9]		2.6		18.0	79.4										

Vertical lines indicate breakpoints for resistance

a) 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

Among *E. faecium* and *E. faecalis* isolates from broilers, no significant changes of proportions of resistant isolates were observed from 2001 to 2002. Resistance to penicillin among *E. faecium* isolates from broilers has remained at a relatively high level (50-60%) since 1999.

After the withdrawal of antimicrobial growth promoters from broiler production in Denmark, the occurrence of resistance to glycopeptides and avilamycin decreased markedly among *E. faecium* isolates from broilers, and has remained at a low level in 2002 (Figures 7 and 8). Resistance to steptogramins decreased until 1999 (Figure 6). Thereafter the resistance level remained unchanged. Most of the isolates resistant to quinupristin/dalfopristin are also resistant to penicillin. Therefore consumption of penicillins in broilers may maintain quinupristin/dalfopristin resistance.
Among *E. faecium* and *E. faecalis* isolates from pigs, no significant changes in the proportions of resistant isolates were observed from 2001 to 2002. Erythromycin and tetracycline resistance was common among *E. faecium* isolates from pigs (Figure 12). This may be linked to the high consumption of macrolides and tetracycline for treatment of disease in pig production (Table 6).

Among *E. faecium* and *E. faecalis* isolates from cattle, no significant changes in the proportions of resistant isolates were observed from 2001 to 2002.

Enterococci from food

Isolation of enterococci from samples of broiler meat, beef and pork from retail outlets yielded 171 isolates of *E. faecium* and 144 isolates of *E. faecalis*. Twenty-nine percent of these isolates originated from imported foods.

The MIC distribution and occurrence of resistance among enterococci from foodstuffs are shown in Tables 32 and 33. Trends in resistance presented in Figures 6 to 8 showed that after the withdrawal of growth promoters a decrease in resistance to glycopeptides, avilamycin and streptogramins was observed among *E. faecium* from broiler meat. In pork the occurrence of resistance has remained at a low level from 1996 to 2002 (Figures 9-11).

Twelve isolates of *E. faecium* from broiler meat exhibited penicillin resistance. Vancomycin resistance was observed in one isolate from imported broiler meat.

Table 31. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broilers (n=69) and pigs (n=238), Denmark*

DANMAP 2002

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	45	[32.9-57.4]			55.1				2.9	17.4	24.6 a)						
	Pigs	83	[77.8-87.7]			16.4		0.4		8.0	20.6	54.6						
Chloramphenicol	Broilers	0	[0.0-5.2]				1.4	20.3	78.3									
	Pigs	2	[0.7-4.8]				1.3	10.1	83.6	2.9		2.1						
Florfenicol	Broilers	0	[0.0-5.2]				55.1	44.9										
	Pigs	0	[0.0-1.5]				28.2	71.8										
Penicillin	Broilers	0	[0.0-5.2]				24.6	69.6	5.8									
	Pigs	<1	[0.01-2.3]				17.6	81.1	0.8	0.4								
Erythromycin	Broilers	25	[15.1-36.5]			46.4	27.5	1.4	4.3	8.7	1.4	10.1						
	Pigs	35	[29.2-41.7]			53.4	10.5	0.8	0.4		0.4	34.5						
Gentamicin	Broilers	0	[0.0-5.2]										100					
	Pigs	6	[3.6-10.2]										93.3		0.4	1.7	1.3	3.4
Kanamycin	Broilers	0	[0.0-5.2]										94.2	4.3		1.4		
	Pigs	16	[11.9-21.7]										83.2			0.4	0.8	15.5
Streptomycin	Broilers	4	[0.9-12.2]										79.7	14.5	1.4		1.4	2.9
	Pigs	30	[24.5-36.5]										61.3	8.4			1.7	28.6
Teicoplanin	Broilers	0	[0.0-5.2]		100													
	Pigs	0	[0.0-1.5]		100													
Vancomycin	Broilers	0	[0.0-5.2]				88.4	11.6										
	Pigs	0	[0.0-1.5]				97.5	2.5										
Avilamycin	Broilers	0	[0.0-5.2]			8.7	82.6	8.7										
	Pigs	0	[0.0-1.5]			16.4	80.3	3.4										
Flavomycin	Broilers	6	[1.6-14.2]		7.2	29.0	47.8	10.1				5.8						
	Pigs	<1	[0.01-2.3]		34.0	56.3	8.8	0.4				0.4						
Salinomycin	Broilers	0	[0.0-5.2]			65.2	1.4	31.9	1.4									
	Pigs	0	[0.0-1.5]			100												
Linezolid	Broilers	0	[0.0-5.2]		2.9	27.5	69.6											
	Pigs b)	0	[0.0-1.6]	0.4	2.6	17.1	79.9											

Vertical lines indicate breakpoints for resistance
a) 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
b) Only 234 isolates tested

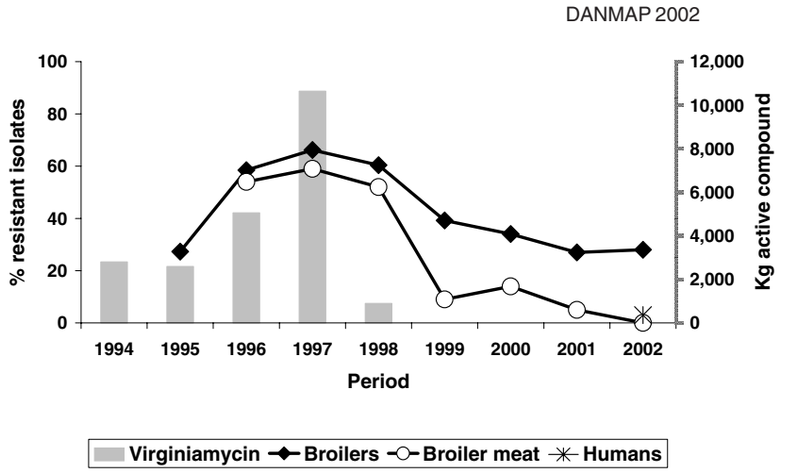

Figure 6. Trends in streptogramin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

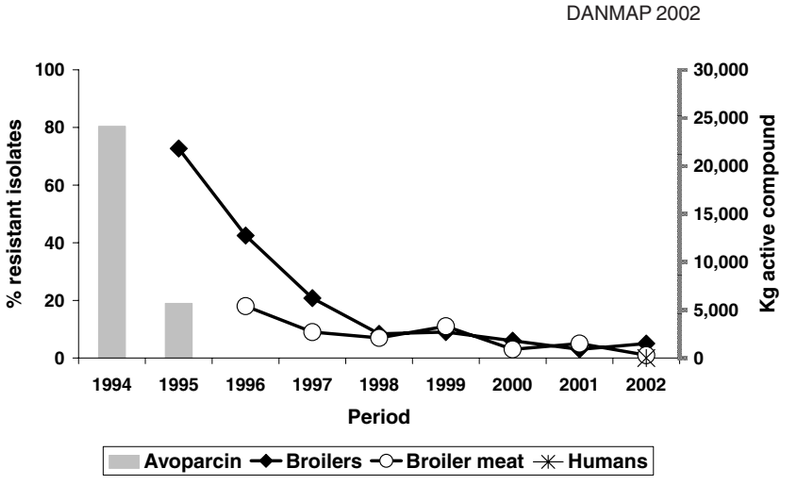

Figure 7. Trends in glycopeptide resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avoparcin in animals, Denmark*

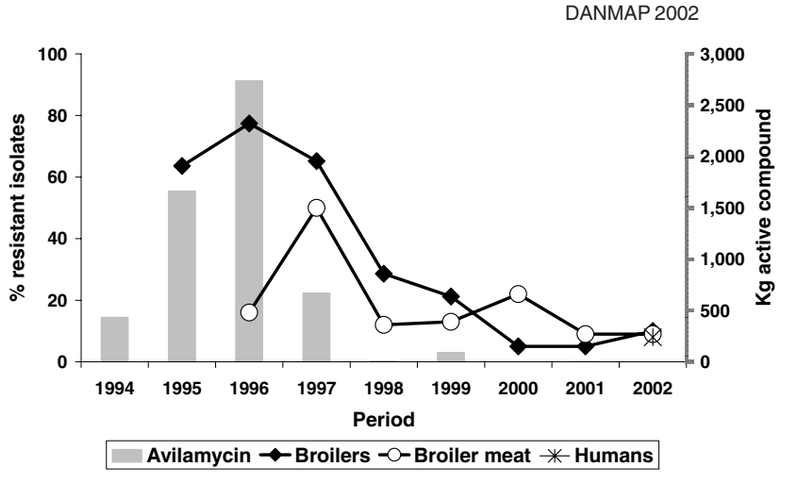

Figure 8. Trends in avilamycin resistance among Enterococcus faecium *from broilers, broiler meat and healthy humans in the community and the consumption of the growth promoter avilamycin in animals, Denmark*

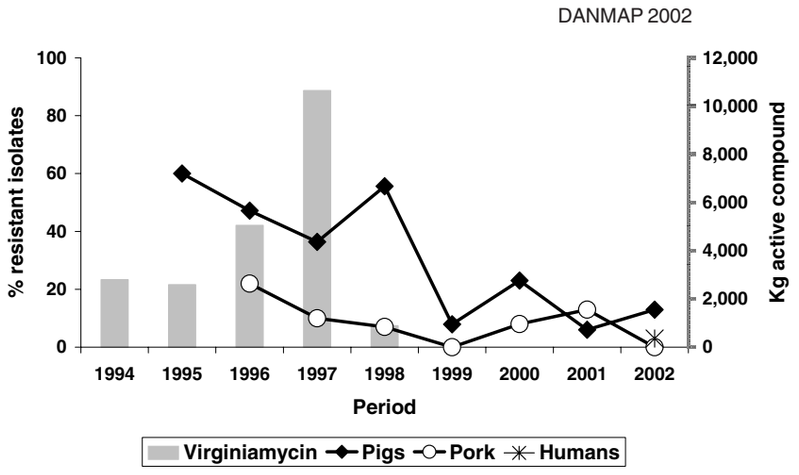

Figure 9. Trends in streptogramin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of the growth promoter virginiamycin in animals, Denmark*

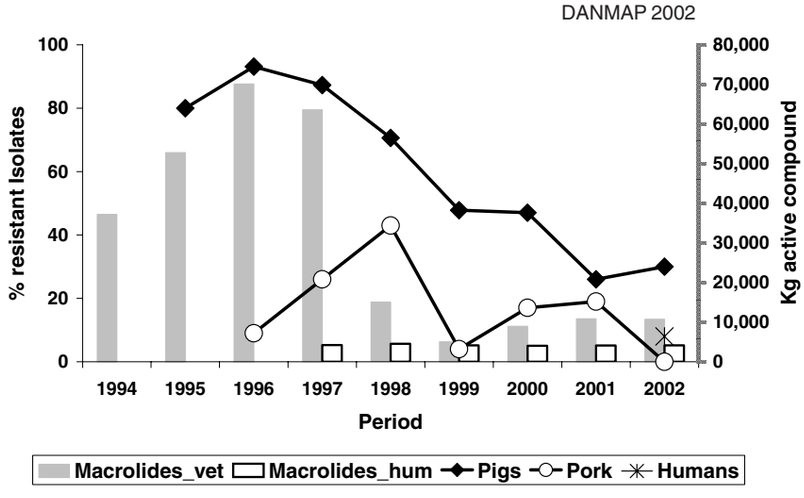

Figure 10. Trends in erythromycin resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of macrolides, both as growth promoters in animals and therapeutics in animals and humans, Denmark*

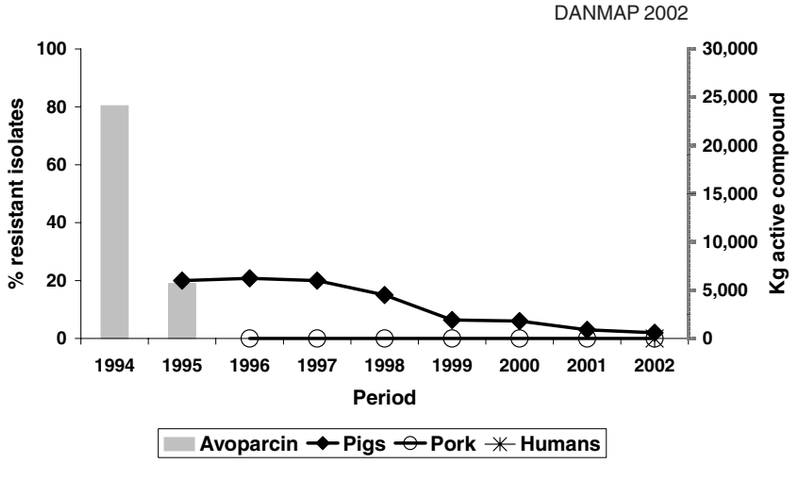

Figure 11. Trends in glycopeptide resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of the growth promoter avoparcin in animals, Denmark*

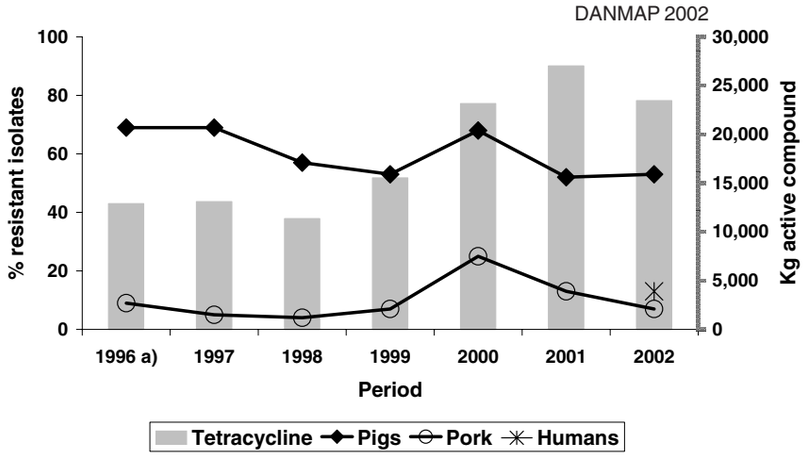

Figure 12. Trends in tetracycline resistance among Enterococcus faecium *from pigs, pork and healthy humans in the community and the consumption of tetracycline in pigs, Denmark*

a) From 1996 to 2000, 98% of the total oral and 85% of the total parenteral consumption of tetracycline was used in pigs

Table 32. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from broiler meat (n=79), beef (n=64) and pork (n=28), Denmark* DANMAP 2002

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	28	[18.3-39.1]			70.9	1.3			1.3	1.3	25.3 a)						
	Pork	7	[0.9-23.5]			89.3			3.6		3.6	3.6						
	Beef	3	[0.4-10.8]			95.2		1.6				3.2						
Chloramphenicol	Broiler meat	1	[0.0-6.9]				54.4	29.1	10.1	5.1	1.3							
	Pork	0	[0.0-12.3]				64.3	32.1	3.6									
	Beef	0	[0.0-5.6]				41.3	41.3	14.3	3.2								
Florfenicol	Broiler meat	0	[0.0-4.6]				96.2	3.8										
	Pork	0	[0.0-12.3]				96.4	3.6										
	Beef	0	[0.0-5.6]				96.8	3.2										
Penicillin	Broiler meat	15	[8.1-25.0]				70.9	5.1	8.9	8.9	5.1			1.3				
	Pork	0	[0.0-12.3]				96.4		3.6									
	Beef	0	[0.0-5.6]				93.7	6.3										
Erythromycin	Broiler meat	13	[6.2-22.0]			70.9	8.9	7.6	1.3	2.5	1.3	7.6						
	Pork	0	[0.0-12.3]			78.6	7.1	14.3										
	Beef	5	[1.0-13.1]			71.4	11.1	12.7	1.6			3.2						
Gentamicin	Broiler meat	3	[0.3-8.8]										94.9		2.5	2.5		
	Pork	0	[0.0-12.3]										100					
	Beef	0	[0.0-5.6]										100					
Kanamycin	Broiler meat	4	[0.8-10.7]										64.6	20.3	7.6	3.8	3.8	
	Pork	0	[0.0-12.3]										85.7	14.3				
	Beef	2	[0.0-8.4]										73	17.5	6.3	1.6	1.6	
Streptomycin	Broiler meat	3	[0.3-8.8]										96.2		1.3		2.5	
	Pork	0	[0.0-12.3]										100					
	Beef	2	[0.0-8.4]										98.4				1.6	
Teicoplanin	Broiler meat	1	[0.0-6.9]		98.7						1.3							
	Pork	0	[0.0-12.3]		100													
	Beef	0	[0.0-5.6]		100													
Vancomycin	Broiler meat	1	[0.0-6.9]				98.7					1.3						
	Pork	0	[0.0-12.3]				100											
	Beef	0	[0.0-5.6]				100											
Quinupristin/dalfopristin	Broiler meat	0	[0.0-4.6]		53.2	31.6	15.2											
	Pork	0	[0.0-12.3]		28.6	42.9	28.6											
	Beef	0	[0.0-5.6]		42.9	41.3	15.9											
Avilamycin	Broiler meat	9	[3.6-17.4]			51.9	31.6	7.6		1.3		7.6						
	Pork	0	[0.0-12.3]			57.1	42.9											
	Beef	0	[0.0-5.6]			55.6	31.7	12.7										
Salinomycin	Broiler meat	0	[0.0-4.6]			36.7	29.1	29.1	5.1									
	Pork	0	[0.0-12.3]			96.4	3.6											
	Beef	0	[0.0-5.6]			96.8	3.2											
Linezolid	Broiler meat	0	[0.0-4.6]	2.6	32.1	41.0	23.1	1.3										
	Pork	0	[0.0-12.3]	4.6	32.0	32.0	20.0	12.0										
	Beef	0	[0.0-5.6]	1.6	16.4	55.7	24.6	1.6										

Vertical lines indicate breakpoints for resistance

a) 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

The trend in resistance indicates that tetracycline resistance has decreased in both broiler meat and pork of Danish origin. Resistance levels in Danish and imported beef are at the same level. In Danish and imported broiler meat the resistance levels are at the same level for most antimicrobials except for tetracycline, for which the resistance level is significantly higher in imported broiler meat.

Enterococci from healthy human volunteers in the community

From the middle of March 2002 to December 2002 stool samples from 109 persons were collected. Forty *E. faecium* isolates and 50 *E. faecalis* isolates were obtained. Please see Appendix 1 page 57 and Textbox 3 for details on specimen collection. The MIC distribution and occurrence of resistance among enterococci from healthy humans are shown in Tables 34 and 35.

Resistance to tetracycline (13%) and penicillin (13%) were most common among *E. faecium* isolates. Resistance to tetracycline (36%) and erythromycin (20%) were most common among *E. faecalis* isolates. None of the isolates of enterococci were resistant to vancomycin, teicoplanin or gentamicin. Vancomycin resistant enterococci (VRE) were not detected by enrichment of stool samples followed by planting on media containing vancomycin.

Enterococci from farm to table

A comparison of resistance among enterococci from Danish food animals, foods of Danish and imported origin and healthy humans in the community are presented in Tables 36 and 37.

Like in previous years the most pronounced differences between levels of resistance in food animals and food

Table 33. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from broiler meat (n=56), beef (n=46) and pork (n=42), Denmark*

DANMAP 2002

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	52	[38.0-65.3]			46.4	1.8			1.8	8.9	41.1 a)						
	Pork	33	[18.1-48.1]			66.7					2.4	31.0						
	Beef	24	[12.6-38.8]			76.1					2.2	21.7						
Chloramphenicol	Broiler meat	0	[0.0-6.4]				44.6	41.1	12.5	1.8								
	Pork	2	[0.1-12.6]				40.5	38.1	19.0			2.4						
	Beef	0	[0.0-7.7]				37.0	45.7	17.4									
Florfenicol	Broiler meat	0	[0.0-3.7]				89.3	10.7										
	Pork	0	[0.0-8.6]				88.1	11.9										
	Beef	0	[0.0-7,7]				95.7	4.3										
Penicillin	Broiler meat	0	[0.0-6.4]				82.1	16.1	1.8									
	Pork	0	[0.0-8.6]				85.7	14.3										
	Beef	0	[0.0-7.7]				87	13										
Erythromycin	Broiler meat	23	[13.0-36.4]			75.0	1.8		1.8		3.6	17.9						
	Pork	10	[2.7-22.6]			83.3	7.1					9.5						
	Beef	0	[0.0-7.7]			76.1	23.9											
Gentamicin	Broiler meat	2	[0.0-9.6]										98.2			1.8		
	Pork	5	[0.1-12.9]										95.2					4.8
	Beef	0	[0.0-7.7]										100					
Kanamycin	Broiler meat	2	[0.0-9.6]										96.4			1.8		1.8
	Pork	7	[0.6-16.5]										92.9					7.1
	Beef	0	[0.0-7.7]										100					
Streptomycin	Broiler meat	18	[8.9-30.4]										80.4			1.8	1.8	16.1
	Pork	7	[0.6-16.5]										90.5	2.4				7.1
	Beef	2	[0.1-11.5]										97.8				2.2	
Teicoplanin	Broiler meat	0	[0.0-6.4]		100													
	Pork	0	[0.0-8.6]		100													
	Beef	0	[0.0-7.7]		100													
Vancomycin	Broiler meat	0	[0.0-6.4]				100											
	Pork	0	[0.0-8.6]				100											
	Beef	0	[0.0-7.7]				100											
Avilamycin	Broiler meat	0	[0.0-6.4]			85.7	14.3											
	Pork	0	[0.0-8.6]			78.6	21.4											
	Beef	0	[0.0-7.7]			71.7	28.3											
Flavomycin	Broiler meat	0	[0.0-6.4]		26.8	42.9	28.6	1.8										
	Pork	2	[0.1-12.9]		54.8	26.2	16.7					2.4						
	Beef	0	[0.0-7.7]		54.3	26.1	15.2	4.3										
Salinomycin	Broiler meat	0	[0.0-6.4]			89.3	5.4	5.4										
	Pork	0	[0.0-8.6]			100												
	Beef	0	[0.0-7.7]			100												
Linezolid	Broiler meat	0	[0.0-6.5]	7.3	36.4	36.4	20.0											
	Pork	0	[0.0-8.6]	11.8	8.8	52.9	26.5											
	Beef	0	[0.0-7.7]		26.1	41.3	32.6											

Vertical lines indicate breakpoints for resistance

a) 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

were observed between pigs and pork, where resistance to tetracycline, penicillin, erythromycin, kanamycin and streptomycin were significantly different. The occurrence of resistance to tetracycline in pigs has remained around 60% since 1996, whereas the resistance in pork has remained below 25% (Figure 12). In broilers/broiler meat of domestic origin, differences were observed for penicillin and quinupristin/dalfopristin, whereas in cattle and domestically produced beef no differences were found.

With the exception of enterococci from broiler meat, where the level of tetracycline resistance was significantly higher than in human isolates, resistance levels in enterococci from healthy humans were similar to levels seen in isolates from food products. These observations seem to be consistent with the assumption that antimicrobial resistance in isolates from food are reflected in the occurrence of resistance in healthy humans.

Table 34. Distribution of MICs and occurrence of resistance among Enterococcus faecium *from healthy humans in the community (n=40), Denmark* DANMAP 2002

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	13	[4.2-26.8]			85.0	2.5					12.5 a)						
Chloramphenicol	3	[0.0-13.2]					15.0	77.5	5.0	2.5							
Florfenicol	0	[0.0-8.8]				15.0	85.0										
Penicillin	13	[4.2-26.8]				35.0	37.5	15	7.5	5							
Erythromycin	8	[1.6-20.4]			65.0	17.5	10.0	2.5	2.5		2.5						
Gentamicin	0	[0.0-8.8]										100					
Kanamycin	10	[2.8-23.7]										40.0	25	17.5	7.5	7.5	2.5
Streptomycin	3	[0.0-13.2]										97.5				2.5	
Teicoplanin	0	[0.0-8.8]		87.5	10.0	2.5											
Vancomycin	0	[0.0-8.8]				92.5	7.5										
Quinupristin/dalfopristin	3	[0.0-13.2]		30.0	10.0	57.5	2.5										
Avilamycin	8	[1.6-20.4]			5.0	27.5	35.0	25.0	7.5								
Salinomycin	0	[0.0-8.8]			45.0	47.5	5.0	2.5									
Linezolid	0	[0.0-8.8]				25.0	75.0										

Vertical lines indicate breakpoints for resistance

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

Table 35. Distribution of MICs and occurrence of resistance among Enterococcus faecalis *from healthy humans in the community(n=50), Denmark* DANMAP 2002

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	36	[22.9-50.8]			64.0						36.0a)						
Chloramphenicol	14	[5.8-26.7]				4.0	16.0	62.0	4.0	10.0	4.0						
Florfenicol	0	[0.0-7.1]				52.0	48.0										
Penicillin	0	[0.0-7.1]				38.0	58.0	4.0									
Erythromycin	20	[10.3-33.7]			60.0	18.0	2.0			2.0	18.0						
Gentamicin	0	[0.0-7.1]										100					
Kanamycin	18	[8.6-31.4]										80.0	2.0			4.0	14.0
Streptomycin	14	[5.8-26.7]										72.0	6.0	4.0	4.0	2.0	12.0
Teicoplanin	0	[0.0-7.1]		100.0													
Vancomycin	0	[0.0-7.1]			82.0	18.0											
Avilamycin	0	[0.0-7.1]			40.0	50.0	8.0	2.0									
Flavomycin	0	[0.0-7.1]		2.0	34.0	46.0	16.0	2.0									
Salinomycin	0	[0.0-7.1]			92.0	4.0	4.0										
Linezolid	0	[0.0-7.1]					86.0	14.0									

Vertical lines indicate breakpoints for resistance

a) 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

Differences in resistance in isolates from animals and humans were observed particularly between pigs and humans for which resistance to tetracycline, penicillin, erythromycin and streptomycin differed.

Between isolates from broilers and humans, differences were observed for penicillin and quinupristin/dalfopristin. Between cattle and humans no differences were observed. This is in accordance with the assumption that meat may be contaminated with enterococci from the environment and food handlers during processing, but other sources are possible. In most cases levels of resistance found in *E. faecalis* from humans did not differ from the resistance levels found in isolates from food. However, between broilers and humans the observed resistance to chloramphenicol was different and between pigs and humans a difference was observed for tetracycline resistance.

Table 36. Occurrence of resistance (%) among Enterococcus faecium *from food animals, foods of Danish and imported origin and healthy humans in the community, Denmark*

DANMAP 2002

Compound	Pigs	Pork	Cattle	Beef a)		Broilers	Broiler meat b)		Healthy humans
	Danish %	Danish %	Danish %	Danish %	Imported %	Danish %	Danish %	Imported %	%
Tetracycline	53	7	0	0	3	7	10	59	13
Chloramphenicol	1	0	0	0	0	1	0	3	3
Florfenicol	0	0	0	0	0	1	0	0	0
Penicillin	38	0	0	0	0	61	15	17	13
Erythromycin	30	0	13	3	3	22	6	24	8
Gentamicin	1	0	0	0	0	1	4	0	0
Kanamycin	21	0	0	0	3	4	2	7	10
Streptomycin	24	0	0	0	3	5	0	7	3
Teicoplanin	2	0	0	0	0	5	0	3	0
Vancomycin	2	0	0	0	0	5	0	3	0
Quinupristin/dalfopristin	13	0	0	0	0	28	0	0	3
Avilamycin	0	0	0	0	0	10	2	17	8
Salinomycin	0	0	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0	0
Number of isolates	194	28	15	32	31	102	48	29	40

a) country of origin is unknown for 1 isolate
b) country of origin is unknown for 2 isolates

Tabel 37. Occurrence of resistance (%) among Enterococcus faecalis *from food animals, foods of Danish and imported origin and healthy humans in the community, Denmark*

DANMAP 2002

Compound	Pigs	Pork	Beef		Broilers	Broiler meat	Healthy humans
	Danish %	Danish %	Danish %	Imported %	Danish %	Danish %	%
Tetracycline	83	33	13	35	45	46	36
Chloramphenicol	2	0	0	0	0	0	14
Florfenicol	0	0	0	0	0	0	0
Penicillin	0	0	0	0	0	0	0
Erythromycin	35	10	0	0	25	21	20
Gentamicin	6	5	0	0	0	3	0
Kanamycin	16	8	0	0	0	0	18
Streptomycin	30	8	0	4	4	18	14
Teicoplanin	0	0	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0
Avilamycin	0	0	0	0	0	0	0
Flavomycin	0	3	0	0	6	0	0
Salinomycin	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0
Number of isolates	238	40	23	23	69	39	50

Text box 2

Copper resistance among *Enterococcus faecium* in Denmark

Large quantities of copper are used as supplements in the feed for food animals in Denmark. It is added as a mineral supplement, but as a side effect it will influence bacteria present in the gut. The effect of copper is probably to inhibit part of the bacterial population in the intestinal tract, similar to what is believed to be part of the mode of action of antimicrobial growth promoters. In general, piglets (< 35 kg live weight) receive 175 ppm and slaughter pigs 35 ppm $CuSO_4$ in their feed. Copper is also included in chicken feed, but in lower concentration than for pigs (25 ppm). These concentrations are higher than the minimum inhibitory concentrations for most bacteria, and bacteria in the gut would be expected to develop resistance to copper in the same way as they have developed resistance to therapeutic or growth promoting antimicrobials.

We have recently identified a cluster of genes that confers resistance to copper in the bacterium *Enterococcus faecium* [Hasmann, H, Aarestrup, FM. Antimicrob. Agents Chemother. 2002; 46: 1410-1416]. The genes are commonly found among isolates from pigs, but are also observed in *E. faecium* from other food animals as well as in isolates from humans. The genes are, in the case of the pig isolates, located on transferable plasmids and are linked to genes encoding resistance to macrolides (erythromycin and tylosin) and glycopeptides (vancomycin and avoparcin). When comparing copper resistance levels among *E. faecium* from Denmark to isolates from Spain and Sweden it can be seen, that the level of resistance in *E. faecium* from Spain and Denmark are of the same magnitude (56% and 75%, respectively). In contrast to this, only 6 % of the Swedish isolates contained the copper resistance genes. This coincides well with the fact that Spain allows the same high amounts of copper as Denmark in the feed for piglets, whereas Sweden only accepts low amounts of copper (35 ppm), regardless of the age of the animals [Aarestrup, F.M. *et al*. 2002. Appl. Environ. Microbiol. 68:4127-4129].

The question is now, if the use of copper in the feed is actually selecting for copper resistance in *E. faecium* in the gut and more importantly, if resistance towards copper co-selects for resistance towards macrolides and glycopeptides due to the location of the three resistance mechanisms on the same plasmid among our porcine isolates. The antimicrobial effect of copper depends on – among other things – the pH in the section of the intestine where the bacteria in question are present. Should it turn out to be the case that the levels of copper used as mineral supplement do select for resistance, it means that the continued usage of copper could lead to maintenance of resistance towards the two therapeutic antibiotics, regardless of the discontinued use of tylosin and avoparcin as growth promoters. This question is currently under investigation at the Danish Veterinary Institute.

Further information: Research scientist Henrik Hasman (hha@vetinst.dk)

Text box 3

Surveillance of antibiotic resistance in faecal bacteria isolated from Danish healthy volunteers from the community

Background: Monitoring of resistance in faecal bacteria from healthy volunteers has been done sporadically in Denmark. A new surveillance was started in the middle of March 2002 as a collaboration between the Danish Zoonosis Centre, the Danish Veterinary Institute, and the Department of Gastrointestinal and Parasitic Infections and the National Center for Antimicrobials & Infection Control at Statens Serum Institut. The aim of this project is to conduct a continuous surveillance of resistance among the indicator bacteria *Escherichia coli, Enterococcus faecium* and *Enterococcus faecalis* isolated from faeces samples obtained from 200 Danish healthy volunteers per year. *E. coli*, *E. faecium* and *E. faecalis* have been chosen as indicator bacteria because they can be found in animal faeces, foodstuff and human faeces, thus representing possible routes of transfer of resistant bacteria, or resistant genes, from animals via foodstuff to humans ("farm to table" principle).

Methods: Subjects were randomly selected for participation in the surveillance from the Danish Civil Register (CPR) system , which is a continuously updated register of all residents in Denmark. With a calculated response rate of 20% and starting in the middle of March, a total of 760 individuals were invited to participate in 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 selection algorithm was used to generate birthdates and genders of the individuals to be invited in the study. A letter including information about the study and a consent form was mailed to the selected individuals and they were asked to confirm their willingness to participate by returning the signed form. A package including a questionnaire and a faecal test tube was subsequently sent to the respondents. Faecal test tubes were mailed to the Department of Gastrointestinal and Parasitic Infections, Statens Serum Institut. The questionnaire covered information on medications, foreign travel, and consumption of eggs, meat, dairy products, and contact with animals including pets. Subjects were asked to complete the questionnaire as soon as possible after returning the faecal sample and were then contacted by telephone for an interview.
A random *E. coli* isolate, a random *E. faecium* isolate and a random *E. faecalis* isolate were selected from each person if possible. Furthermore, a selective method was used to detect vancomycin resistant enterococci (Appendix 1). All susceptibility testing was done with Sensititre plates (Appendix 1), including growth promoters and clinical antibiotics.
The scientific ethics committee for Copenhagen and Frederiksberg municipalities approved the protocol.

Results: From the middle of March 2002 to December 2002, faeces from 109 persons were collected. Forty *E. faecium* isolates, 50 *E. faecalis* isolates and 92 *E. coli* isolates were obtained from these samples. None of the randomly selected enterococcal isolates were resistant to vancomycin, teicoplanin or gentamicin. Twenty-four of the 92 *E. coli* isolates were resistant to one antibiotic or more. Nine isolates were multi-resistant (resistant to four antibiotics or more). Resistance to sulfamethoxazole, ampicillin, tetracycline, streptomycin and trimethroprim was the most common, with 14%, 14%, 11%, 10% and 10%, respectively. Two isolates were resistant to ciprofloxacin. None of the isolates were resistant to gentamicin.

Conclusion:
Antibiotic resistance in *E. coli* and enterococci from faeces of healthy human volunteers was less frequent than in clinical isolates of the same species. In particular, resistance towards ampicillin and sulfonamides was less than half of that in clinical *E. coli* isolates. Some isolates, however, showed the same multiple resistance patterns often found in isolates from hospitals. These results showed that there is a pool of antibiotic resistance genes in the general population outside hospitals, which may represent a risk for ineffective treatment if these strains should cause infections in the persons carrying these bacteria.

Further information: Anette M. Hammerum, PhD (ama@ssi.dk)

Escherichia coli from food animals

Table 38 presents the MIC distribution and occurrence of resistance in *E. coli* from animals at slaughter (indicator *E. coli*). The levels of resistance among indicator *E. coli* isolates from pigs, broilers and cattle were generally low and no statistically significant changes in the proportions of resistant isolates occurred from 2001 to 2002. Figure 13 presents the trends in resistance to selected antimicrobials from1996 to 2002.

Escherichia coli from food

A total of 372 isolates of *E. coli* were collected from broiler meat, turkey meat, beef and pork at retail outlets. One hundred and six (28%) of these isolates originated from imported foods. Resistance to ampicillin, tetracycline, sulfonamide, trimethoprim and streptomycin was most frequently observed (Table 39). In 2002, the occurrence of resistance among *E. coli* isolates from foods has remained almost unchanged as compared to previous years.

Among food isolates, 2 isolates from turkey meat, 1 from Danish and 1 from imported turkey meat were ciprofloxacin resistant. Furthermore, 1 isolate from pork, 7 isolates from broiler meat and 3 isolates from turkey meat had ciprofloxacin MIC values between 0.12 and 2.0 and were resistant to nalidixic acid.

Table 38. Distribution of MICs and occurrence of resistance among Escherichia coli *from broilers (n=120), cattle (n=96) and pigs (n=293), Denmark*

DANMAP 2002

Compound	Animal species	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Broilers	5	[1.9-10.6]							93.3	0.8	0.8		0.8	4.2 a)					
	Cattle	6	[2.3-13.1]							83.3	10.4			2.1	4.2					
	Pigs	26	[21.0-31.4]							64.5	9.6			2.0	23.9					
Chloramphenicol	Broilers	0	[0.0-3.0]							4.2	59.2	35.0	1.7							
	Cattle	0	[0.0-3.8]							11.5	47.9	39.6	1.0							
	Pigs	4	[1.9-6.6]							7.2	51.5	35.8	1.7	2.0	1.0	0.7				
Florfenicol	Broilers	0	[0.0-3.0]							8.3	75.8	15.0	0.8							
	Cattle	0	[0.0-3.8]							7.3	45.8	44.8	2.1							
	Pigs	0	[0.0-1.3]							9.6	46.4	38.6	5.5							
Ampicillin	Broilers	19	[12.6-27.4]						9.2	40.8	30.0	0.8		3.3	15.8					
	Cattle	4	[1.2-10.3]						8.3	49.0	35.4	3.1			4.2					
	Pigs	11	[7.6-15.1]						12.3	47.1	27.0	2.4	0.3		10.9					
Ceftiofur	Broilers	0	[0.0-3.0]					96.7	3.3											
	Cattle	0	[0.0-3.8]					99.0	1.0											
	Pigs	0	[0.0-1.3]					99.3	0.3	0.3										
Cephalothin	Broilers	8	[4.1-14.8]							5.8	21.7	42.5	21.7	3.3	0.8	4.2				
	Cattle	4	[1.2-10.3]							2.1	15.6	62.5	15.6	4.2						
	Pigs	1	[0.4-3.5]							5.1	22.2	51.9	19.5	1.0		0.3				
Sulfamethoxazole	Broilers	26	[18.3-34.6]												72.5	0.8	0.8			25.8
	Cattle	6	[2.3-13.1]												93.8					6.3
	Pigs	23	[18.5-28.5]												74.7	2.0				23.2
Trimethoprim	Broilers	7	[2.9-12.7]								93.3				6.7					
	Cattle	3	[0.7-8.9]								96.9				3.1					
	Pigs	6	[3.7-9.5]								93.9				6.1					
Apramycin	Broilers	<1	[0.02-4.6]								85.8	13.3	0.8							
	Cattle	0	[0.0-3.8]								92.7	7.3								
	Pigs	<1	[0.01-1.9]								94.5	5.1	0.3							
Gentamicin	Broilers	0	[0.0-3.0]						98.3	1.7										
	Cattle	0	[0.0-3.8]						99.0	1.0										
	Pigs	<1	[0.01-1.9]						98.0	1.0	0.7		0.3							
Neomycin	Broilers	2	[0.2-5.9]							98.3				0.8	0.8					
	Cattle	3	[0.7-8.9]							96.9				3.1						
	Pigs	3	[1.4-5.8]							96.6	0.3		0.3	0.7	2.0					
Spectinomycin	Broilers	3	[0.5-7.1]									20.0	69.2	5.0	3.3	1.7	0.8			
	Cattle	2	[0.3-7.3]									22.9	74.0	1.0			2.1			
	Pigs	25	[20.1-30.3]									16.0	51.2	4.1	3.8	12.3	12.6			
Streptomycin	Broilers	7	[2.9-12.7]								54.2	38.3	0.8	3.3	3.3					
	Cattle	7	[3.0-14.5]								71.9	20.8		2.1	1.0	4.2				
	Pigs	34	[28.4-39.5]								42.3	20.1	3.8	13.3	8.9	11.6				
Ciprofloxacin	Broilers	0	[0.0-3.0]	87.5	1.7	5.0	5.0	0.8												
	Cattle	0	[0.0-3.8]	100																
	Pigs	0	[0.0-1.3]	98.6	0.3	0.3	0.7													
Nalidixic acid	Broilers	11	[5.9-17.8]									89.2			0.8	7.5	2.5			
	Cattle	0	[0.0-3.8]									100								
	Pigs	1	[0.2-3.0]									98.3	0.7	0.3		0.7				
Colistin	Broilers	0	[0.0-3.0]								99.2	0.8								
	Cattle	0	[0.0-3.8]								100									
	Pigs	0	[0.0-1.3]								100									

Vertical lines indicate breakpoints for resistance. The dotted line indicates ciprofloxacin breakpoint for resistance applied for human isolates

a) 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

Escherichia coli from healthy human volunteers from the community

From March 2002 to December 2002 faecal samples from 109 persons were collected and 92 *E. coli* isolates were subsequently isolated. Table 40 presents the MIC distribution and occurrence of resistance of the 92 isolates. Ten percent of the *E. coli* isolates were resistant to four antimicrobials or more, whereas 74% were susceptible to all antimicrobials tested. Resistance to sulfamethoxazole, ampicillin, tetracycline, streptomycin and trimethroprim were most common.

Ciprofloxacin/nalidixic acid resistance was observed in 2% of the isolates, but none exceeded the NCCLS breakpoint for ciprofloxacin. In contrast to isolates from food animals and food, where the NCCLS breakpoint of 4 μg/ml for ciprofloxacin was used, a breakpoint of 0.12 μg/ml was used for isolates from humans (Please see Appendix 1, page 61). None of the isolates were resistant to gentamicin.

Escherichia coli from farm to table

The occurrences of resistance in food animals, food and healthy humans in the community are compared in Table 41. Resistance levels in *E. coli* from healthy human volunteers were similar to resistance levels in *E. coli* from Danish foods and Danish food animals although differences were observed in a few cases. In *E. coli* from Danish pigs spectinomycin resistance and tetracycline resistance were more frequently found than in *E. coli* from humans. Isolates of *E. coli* from Danish pigs were less often resistant to trimethoprim than *E. coli* from Danish pork. Resistance levels in *E. coli* from Danish and imported foods were similar. Only tetracycline resistance in imported broiler and turkey meat was significantly higher than in the corresponding Danish products.

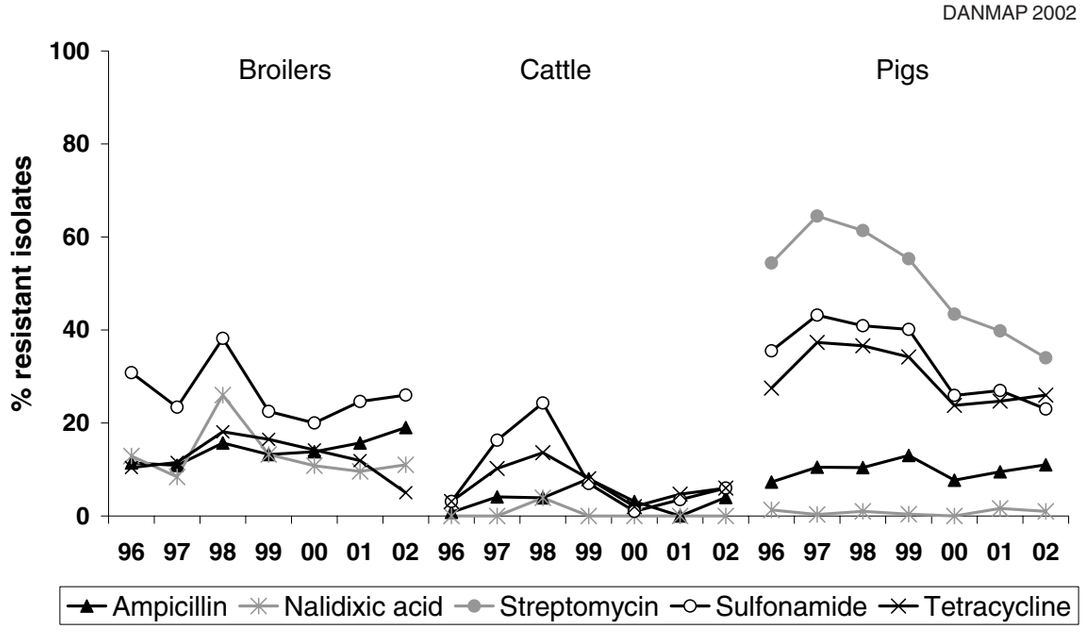

Figure 13. Trends in resistance to some selected antimicrobials among Escherichia coli *from food animals, Denmark*

Table 39. Distribution of MICs and occurrence of resistance among Escherichia coli *from beef (n=147), pork (n=69), broiler meat (n=92) and turkey meat (n=64), Denmark*

DANMAP 2002

Compound	Food Type	%Resistant	[95% Confidence interval]	Distribution (%) of MICs															
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Beef	11	[6.4-17.1]							86.4	2.0	0.7	0.7	2.7	7.5 a)					
	Pork	26	[16.3-38.1]							71.0	1.4	1.4		1.4	24.6					
	Broiler meat	20	[12.0-29.1]							79.3		1.1		8.7	10.9					
	Turkey meat	52	[38.7-64.2]							45.3	1.6	1.6	3.1	6.2	42.2					
Chloramphenicol	Beef	1	[0.2-4.8]							8.2	77.6	12.2	0.7			1.4				
	Pork	4	[0.9-12.2]							26.1	62.3	7.2		1.4		2.9				
	Broiler meat	3	[0.7-9.2]							23.9	64.1	8.7				3.3				
	Turkey meat	3	[0.4-10.8]							31.3	57.8	7.8				3.1				
Florfenicol	Beef	1	[0.2-4.8]							7.5	81.6	8.8	0.7			1.4				
	Pork	0	[0.0-5.2]							36.2	58.0	5.8								
	Broiler meat	0	[0.0-3.9]							35.9	60.9	3.3								
	Turkey meat	0	[0.0-5.6]							40.6	56.3	3.1								
Ampicillin	Beef	10	[5.3-15.5]						17.0	61.9	10.2		1.4		9.5					
	Pork	22	[12.7-33.3]						26.1	43.5	8.7				21.7					
	Broiler meat	20	[12.0-29.1]						37.0	38.0	3.3	2.2			19.6					
	Turkey meat	22	[12.5-34.0]						32.8	39.1	4.7		1.6		21.9					
Ceftiofur	Beef	0	[0.0-2.5]					100												
	Pork	0	[0.0-5.2]					100												
	Broiler meat	0	[0.0-3.9]					97.8	2.2											
	Turkey meat	0	[0.0-5.6]					100												
Cephalothin	Beef	0	[0.0-2.5]							1.4	13.6	59.2	25.9							
	Pork	3	[0.4-10.1]							1.4	24.6	49.3	21.7	2.9						
	Broiler meat	4	[1.2-10.8]							6.5	30.4	42.4	16.3	2.2		2.2				
	Turkey meat	8	[2.6-17.3]								26.6	45.3	20.3	7.8						
Sulfonamide	Beef	7	[3.8-13.0]												89.8	2.0	0.7			7.5
	Pork	23	[13.9-34.9]												73.9	2.9				23.2
	Broiler meat	18	[11.1-27.9]												80.4	1.1				18.5
	Turkey meat	20	[11.3-32.2]												73.4	4.7	1.6			20.3
Trimethoprim	Beef	5	[1.9-9.6]								94.6	0.7			4.8					
	Pork	20	[11.6-31.7]								79.7				20.3					
	Broiler meat	13	[6.9-21.7]								83.7	3.3			13.0					
	Turkey meat	19	[10.1-30.5]								81.3				18.8					
Apramycin	Beef	0	[0.0-2.5]								95.2	4.8								
	Pork	0	[0.0-5.2]								94.2	5.8								
	Broiler meat	0	[0.0-3.9]								92.4	7.6								
	Turkey meat	0	[0.0-5.6]								93.8	6.3								
Gentamicin	Beef	0	[0.0-2.5]						100											
	Pork	0	[0.0-5.2]						100											
	Broiler meat	0	[0.0-3.9]						97.8	2.2										
	Turkey meat	0	[0.0-5.6]						100											
Neomycin	Beef	3	[1.1-7.8]							95.9	0.7			1.4	2.0					
	Pork	0	[0.0-5.2]							100										
	Broiler meat	2	[0.3-7.6]							96.7	1.1				2.2					
	Turkey meat	5	[1.0-13.1]							93.8	1.6			1.6	3.1					
Spectinomycin	Beef	2	[0.4-5.8]									2.0	89.8	5.4	0.7	1.3	0.7			
	Pork	10	[4.2-19.8]									13.0	59.4	7.2	10.1	7.2	2.9			
	Broiler meat	4	[1.2-10.8]									10.9	72.8	9.8	2.2	2.2	2.2			
	Turkey meat	8	[2.6-17.3]								1.6	21.9	57.8	4.7	6.3	3.1	4.7			
Streptomycin	Beef	10	[5.3-15.5]								11.6	75.5	3.4	0.7	1.4	7.5				
	Pork	26	[16.3-38.1]								17.4	49.3	7.2	5.8	2.9	17.4				
	Broiler meat	16	[9.4-25.5]								20.7	58.7	4.3	2.2	7.6	6.5				
	Turkey meat	27	[16.3-39.1]								25.0	42.2	6.3	3.1	1.6	21.9				
Ciprofloxacin	Beef	0	[0.0-2.5]	100																
	Pork	0	[0.0-5.2]	98.6			1.4													
	Broiler meat	0	[0.0-3.9]	91.3	1.1	1.1	3.3	2.2		1.1										
	Turkey meat	3	[0.38-10.8]	90.6	1.6		3.1	1.6				3.1								
Nalidixic acid	Beef	0	[0.0-2.5]									100								
	Pork	1	[0.0-7.8]									97.1	1.4			1.4				
	Broiler meat	9	[3.8-16.4]									91.3		1.1	3.3	2.2	2.2			
	Turkey meat	8	[2.6-17.3]									90.6	1.6		1.6	1.6	4.7			
Colistin	Beef	0	[0.0-2.5]								100									
	Pork	0	[0.0-5.2]								100									
	Broiler meat	0	[0.0-3.9]								98.9	1.1								
	Turkey meat	0	[0.0-5.6]								100									

Vertical lines indicate breakpoints for resistance. The dotted line indicates ciprofloxacin breakpoint for resistance applied for human isolates

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

Table 40. Distribution of MICs and occurrence of resistance among Escherichia coli *from healthy humans in the community (n=92), Denmark* DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	12	[6.1-20.4]							83.8	2.2			2.2	9.8 a)					
Chloramphenicol	1	[0.0-5.9]							1.1	59.8	36.9	1.1		1.1					
Florfenicol	0	[0.0-3.9]							1.1	50.0	47.8	1.1							
Ampicillin	14	[7.7-23.0]						3.3	46.7	53.9			14.1						
Ceftiofur	0	[0.0-3.9]					97.8	2.2											
Cephalothin	0	[0.0-3.9]							1.1	28.3	47.8	22.8							
Sulfonamide	14	[7.7-23.0]												83.7	1.1	1.1			14.1
Trimethoprim	10	[4.6-17.8]								90.2				9.8					
Apramycin	3	[0.7-9.2]								75.0	21.7	3.3							
Gentamicin	0	[0.0-3.9]						98.9		1.1									
Neomycin	1	[0.0-5.9]							92.4	6.5				1.1					
Spectinomycin	3	[0.7-9.2]									9.8	78.3	4.3	4.3	2.2	1.1			
Streptomycin	10	[4.6-17.8]								32.6	48.9	8.7	2.2	3.3	4.3				
Ciprofloxacin	2	[0.3-7.6]	97.8			1.1	1.1												
Nalidixic acid	2	[0.3-7.6]									97.8				2.2				
Colistin	0	[0.0-3.9]								98.9	1.1								

Vertical lines indicate breakpoints for resistance

a) 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. Occurrence of resistance (%) among Escherichia coli *from Danish animals, food of Danish and imported origin and healthy humans in the community, Denmark* DANMAP 2002

Compound	Broilers	Broiler meat		Turkey meat		Cattle	Beef		Pigs	Pork	Humans
	Danish	Danish	Imported	Danish	Imported	Danish	Danish	Imported	Danish	Danish	
	%	%	%	%	%	%	%	%	%	%	%
Tetracycline	5	7	52	30	81	6	9	12	26	24	12
Chloramphenicol	0	0	11	3	4	0	0	4	4	5	1
Florfenicol	0	0	0	0	0	0	0	4	0	0	0
Ampicillin	19	14	33	19	27	4	7	14	11	20	14
Ceftiofur	0	0	0	0	0	0	0	0	0	0	0
Cephalothin	8	4	4	5	12	4	0	0	1	3	0
Sulfonamide	26	11	33	14	27	6	5	12	23	21	14
Trimethoprim	7	5	30	5	35	3	4	6	6	20	10
Apramycin	1	0	0	0	0	0	0	0	0	0	3
Gentamicin	0	0	0	0	0	0	0	0	0	0	0
Neomycin	2	0	7	5	4	3	1	8	3	0	1
Spectinomycin	3	4	7	5	8	2	1	4	25	8	3
Streptomycin	7	9	33	19	35	7	5	16	34	26	10
Ciprofloxacin	0	0	0	3	4	0	0	0	0	0	0 a)
Nalidixic acid	11	9	11	8	8	0	0	0	1	2	2
Colistin	0	0	0	0	0	0	0	0	0	0	0
Number of isolates	120	57	27	37	26	96	96	50	293	66	92

a) 2% of isolates exhibited a MIC > 0.12μg/ml, all isolates exhibited a MIC < 4μg/ml

Resistance in bacteria from diagnostic submissions

Bacteria from food animals

DANMAP 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.

Escherichia *coli*

The following *E. coli* serotypes were included: O2 and O78 from poultry, F5 from cattle (young calves) and serotype O149 from weaned pigs.

The MIC distribution and the occurrence of resistance are presented in Table 42.

The limited number of *E. coli* isolates from poultry each year makes it difficult to demonstrate changes in resistance levels from year to year. With some caution, it is possible to study resistance trends over time. Generally, *E. coli* isolates from poultry have become less resistant and especially resistance to sulfonamide, tetracycline and nalidixic acid have decreased (Figure 14).

Among *E. coli* F5 from cattle, resistance to ampicillin, gentamicin, sulfonamide and tetracycline has remained

Table 42. Distribution of MICs and occurrence of resistance among Escherichia coli *from diagnostic submissions from broilers (n=16), cattle (n=101) and pigs (n=111), Denmark* DANMAP 2002

Compound	Animal species	% Resistant [95% Confidence interval]	Distribution (%) of MICs																
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	Broilers	6 [0.2-30.2]							93.8					6.3 a)					
	Cattle	84 [75.6-90.7]							15.8			1.0	4.0	79.2					
	Pigs	75 [65.7-82.5]							25.2				19.8	55.0					
Chloramphenicol	Broilers	0 [0.0-20.6]							18.8	81.3									
	Cattle	13 [7.0-21.0								35.6	51.5		1.0	1.0	10.9				
	Pigs	31 [22.2-40.1]							17.1	40.5	6.3	5.4	6.3		24.3				
Florfenicol	Broilers	0 [0.0-20.6]							18.8	81.3									
	Cattle	<1 [0.03-5.4]							1.0	54.5	41.6	2.0		1.0					
	Pigs	0 [0.0-3.3]							27.9	59.5	10.8	1.8							
Ampicillin	Broilers	13 [1.6-38.4]						6.3	75.0	6.3				12.5					
	Cattle	79 [70.0-86.6]						3.0	10.9	6.9				79.2					
	Pigs	45 [35.6-54.8]						18.0	30.6	4.5	0.9	0.9	1.8	43.2					
Ceftiofur	Broilers	0 [0.0-20.6]					100												
	Cattle	<1 [0.03-5.4]					99.0					1.0							
	Pigs	0 [0.0-3.3]					99.1	0.9											
Cephalothin	Broilers	25 [3.2-65.1]								12.5	50.0	12.5	12.5	12.5					
	Cattle	7 [2.8-13.8]								16.8	65.3	10.9	5.0	1.0	1.0				
	Pigs	6 [2.6-12.6]								28.8	48.6	16.2	1.8		4.5				
Sulfamethoxazole	Broilers	31 [11.0-58.7]												68.8				18.8	12.5
	Cattle	74 [64.6-82.4]												25.7					74.3
	Pigs	77 [67.6-84.1]												22.5	0.9				76.6
Trimethoprim	Broilers	6 [0.2-30.2]								93.8				6.3					
	Cattle	54 [44.2-64.4]								45.5				54.5					
	Pigs	38 [28.8-47.5]								61.3	0.9			37.8					
Apramycin	Broilers	0 [0.0-20.6]								93.8	6.3								
	Cattle	0 [0.0-3.6]								84.2	15.8								
	Pigs	17 [10.6-25.4]								82.9					17.1				
Gentamicin	Broilers	0 [0.0-20.6]						93.8	6.3										
	Cattle	<1 [0.03-5.4]						96.0	2.0		1.0		1.0						
	Pigs	14 [8.5-22.4]						80.2		2.7	2.7	7.2	4.5	2.7					
Neomycin	Broilers	0 [0.0-20.6]							100										
	Cattle	9 [4.2-16.2]							87.1	4.0			2.0	6.9					
	Pigs	36 [27.1-45.7]							63.1	0.9			9.0	27.0					
Spectinomycin	Broilers	0 [0.0-20.6]									6.3	93.8							
	Cattle	14 [7.8-22.2]									20.8	57.4	5.0	3.0	6.9	6.9			
	Pigs	57 [47.0-66.1]									11.7	19.8	8.1	3.6	18.0	38.7			
Streptomycin	Broilers	6 [0.2-30.2]								18.8	68.8	6.3			6.3				
	Cattle	70 [60.4-79.0]								15.8	12.9	1.0	10.9	22.8	36.6				
	Pigs	77 [68.6-84.9]								13.5	7.2	1.8	21.6	18.0	37.8				
Ciprofloxacin	Broilers	0 [0.0-20.6]	93.8			6.3													
	Cattle	<1 [0.03-5.4]	81.2		6.9	10.9					1.0								
	Pigs	0 [0.0-3.3]	78.4	6.3	7.2	6.3	0.9	0.9											
Nalidixic acid	Broilers	6 [0.2-30.2]									93.8				6.3				
	Cattle	19 [11.7-27.8]									81.2				6.9	11.9			
	Pigs	19 [12.1-27.5]									81.1		3.6	4.5	8.1	2.7			
Colistin	Broilers	0 [0.0-20.6]								100									
	Cattle	0 [0.0-3.6]								100									
	Pigs	0 [0.0-3.3]								100									

Vertical lines indicate breakpoints for resistance

a) 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

almost unchanged since 1996. From 2001 to 2002 a significant decrease in gentamicin resistance from 19% to 1% was observed (Figure 14).

Since 1999, ampicillin resistance has increased significantly among *E. coli* O149 from weaned pigs (Figure 14). From 2001 to 2002, the consumption of penicillins with extended spectrum has increased among weaned pigs (Table 6).

Apramycin was first marketed in Denmark in 1998 and is used for treatment of infections in pigs and cattle. Isolates with certain gentamicin resistance genotypes are also cross-resistant to apramycin. In 2001, resistance to gentamicin increased for the first time among *E. coli* from pigs and in 2002 the tendency was the same (Figure 14). In 2001, the consumptions of gentamicin and apramycin in pigs were 96 and 671 kg active compound, respectively. In

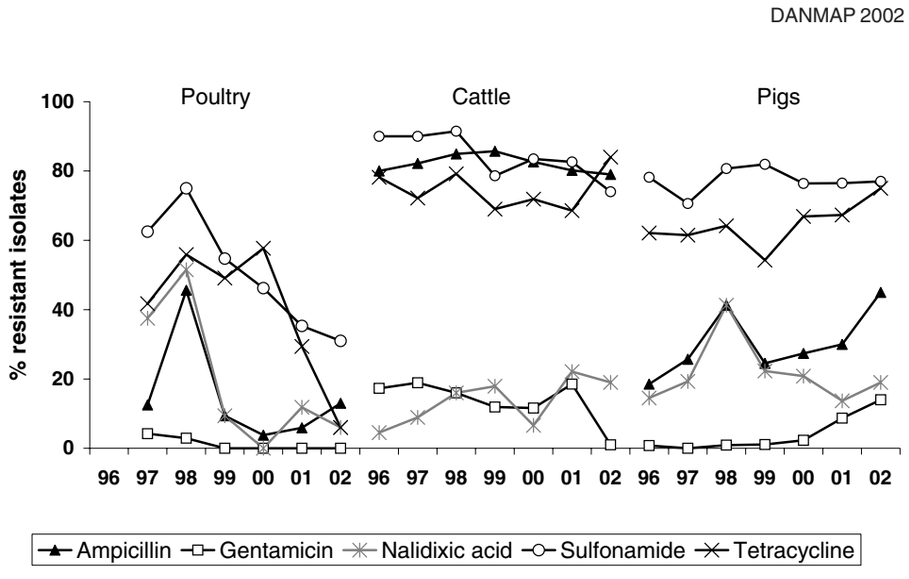

Figure 14. Trends in resistance to selected antimicrobials among Escherichia coli *from diagnostic submissions from animals, Denmark*

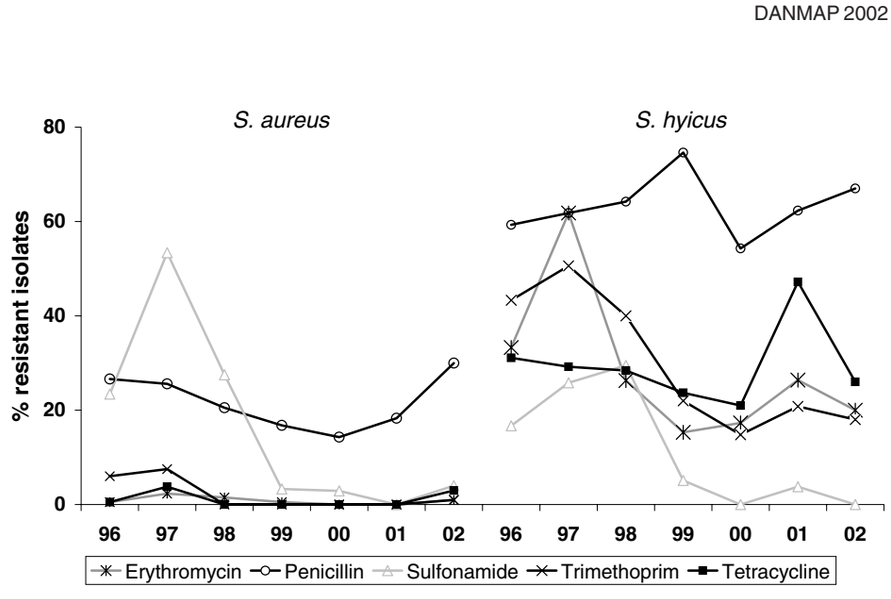

Figure 15. Trends in resistance to selected antimicrobials among staphylococci from diagnostic submissions from cattle (S. aureus) and pigs (S. hyicus), Denmark

Table 43. Distribution of MICs and occurrence of resistance among Staphylococcus aureus *from cattle (n=101), Denmark* DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512 >512
Tetracycline	3	[0.6-8.4]	80.2 13.9 2.0 1.0 | 2.0 1.0 a)
Chloramphenicol	0	[0.0-3.6]	2.0 20.8 76.2 1.0 |
Florfenicol	0	[0.0-3.6]	3.0 34.7 62.4
Oxacillin	0	[0.0-3.6]	93.1 4.0 3.0 |
Penicillin	30	[21.0-39.6]	69.3 1.0 | 1.0 3.0 5.9 5.0 9.9 2.0 3.0
Ceftiofur	0	[0.0-3.6]	1.0 15.8 60.4 22.8 |
Sulfamethoxazole	4	[1.1-9.8]	17.8 28.7 30.7 14.9 2.0 2.0 | 4.0
Trimethoprim	<1	[0.03-5.4]	66.3 25.7 5.0 2.0 | 1.0
Erythromycin	<1	[0.03-5.4]	3.0 55.4 39.6 1.0 | 1.0
Gentamicin	0	[0.0-3.6]	98.0 2.0 |
Kanamycin	0	[0.0-3.6]	95.0 4.0 1.0 |
Spectinomycin	6	[2.2-12.5]	1.0 1.0 92.1 | 5.9
Streptomycin	4	[1.1-9.8]	14.9 48.5 29.7 3.0 | 3.0 1.0
Ciprofloxacin	0	[0.0-3.6]	54.5 36.6 8.9 |
Vancomycin	0	[0.0-3.6]	100 |
Virginiamycin	0	[0.0-3.6]	99.0 1.0 |
Quinupristin/dalfopristin	<1	[0.03-5.4]	76.2 22.8 | 1.0
Avilamycin	0	[0.0-3.6]	40.6 48.5 10.9 |
Bacitracin	<1	[0.03-5.4]	87.1 7.9 4.0 | 1.0
Tiamulin	0	[0.0-3.6]	2.0 30.7 65.3 1.0 | 1.0

Vertical lines indicate breakpoints for resistance
a) 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 44. Distribution of MICs and occurrence of resistance among Staphylococcus hyicus *from pigs (n=76), Denmark* DANMAP 2002

Compound	% Resistant [95% Confidence interval]		Distribution (%) of MICs 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512 >512
Tetracycline	26	[16.9-37.7]	72.4 1.3 | 1.3 21.1 3.9 a)
Chloramphenicol	0	[0.0-4.7]	68.4 31.6 |
Florfenicol	0	[0.0-4.7]	1.3 80.3 18.4 |
Oxacillin	0	[0.0-4.7]	98.7 1.3 |
Penicillin	67	[55.4-77.5]	31.6 1.3 | 1.3 1.3 6.6 2.6 9.2 13.2 22.4 10.5
Ceftiofur	0	[0.0-4.7]	2.6 44.7 51.3 1.3 |
Sulfamethoxazole	0	[0.0-4.7]	28.9 44.7 14.5 6.6 2.6 2.6 |
Trimethoprim	18	[10.5-29.0]	60.5 10.5 6.6 3.9 | 1.3 17.1
Erythromycin	20	[11.5-30.5]	1.3 32.9 46.1 | 19.7
Gentamicin	0	[0.0-4.7]	100 |
Kanamycin	5	[1.5-12.9]	89.5 2.6 | 2.6 5.3
Spectinomycin	13	[6.5-22.9]	42.1 44.7 | 1.3 11.8
Streptomycin	43	[32.1-55.3]	13.2 38.2 3.9 1.3 | 2.6 6.6 13.2 21.1
Ciprofloxacin	5	[1.5-12.9]	89.5 2.6 1.3 | 1.3 2.6 2.6
Vancomycin	0	[0.0-4.7]	52.6 47.4 |
Virginiamycin	1	[0.03-7.1]	89.5 9.2 | 1.3
Quinupristin/dalfopristin	1	[0.03-7.1]	88.2 10.5 | 1.3
Avilamycin	0	[0.0-4.7]	53.9 44.7 1.3 |
Bacitracin	1	[0.03-7.1]	7.9 50.0 40.8 | 1.3
Tiamulin	13	[6.5-22.9]	11.8 34.2 34.2 1.3 1.3 3.9 | 5.3 7.9

Vertical lines indicate breakpoints for resistance
a) 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

2002 these figures were 18 and 548 kg active compound.

Staphylococci

Isolates of *Staphylococcus aureus* originated from cases of bovine mastitis, while *Staphylococcus 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 Table 43 and 44, respectively. Trends in resistance to some selected antimicrobials among staphylococci from diagnostic submissions from cattle and pigs are presented in Figure 15.

Isolates of *S. aureus* were susceptible to most of the antimicrobials tested. The only significant change from 2001 to 2002 was a continued increase in resistance to penicillin. Among isolates of *S. hyicus* a significant decrease in resistance to tetracycline, from 47% in 2001 to 26% in 2002, was observed. The consumption

of tetracycline in pigs decreased slightly in the same period (Table 6). However, this decrease may not be the only explanation for the decrease in resistance. Transmission of resistant clones of *S. hyicus* may confound the association between usage of antimicrobials and the levels of resistance observed.

Bacteria from humans

For *Streptococcus pneumoniae* and *Staphylococcus aureus*, this report includes data covering the whole country. For *Escherichia coli* and coagulase-negative staphylococci, this report includes data from the clinical microbiology laboratories serving the Copenhagen and Frederiksberg municipalities, which have status of counties, and the counties of Copenhagen, Roskilde, West Zealand, Storstroem, Funen, South Jutland, Ribe, Ringkoebing, Aarhus, Viborg and North Jutland, which represent 87% of the Danish population. Some of these laboratories did not routinely test for resistance in *Streptococcus pyogenes* and the data for this microorganism cover only 56% of the population. More information on demographics is presented in Table 2, page 10.

Streptococcus pneumoniae

As the national reference centre, the Streptococcus Unit at the Statens Serum Institut performs typing and susceptibility testing on *S. pneumoniae* isolates referred by the Danish local clinical microbiology laboratories. In 2002, susceptibility testing was performed on 1,089 non-duplicate isolates from blood or spinal fluid samples. Resistance to penicillin in *S. pneumoniae* isolates is a major problem worldwide. In Denmark, this type of resistance was rare until 1995 when it started to increase to reach a peak at approximately 4% in 1999. Since 2000 there has been a slight decrease in the percentage of penicillin non-susceptible *S. pneumoniae*, which was 2.4% in 2002 (Figure 16).

Resistance to macrolides in *S. pneumoniae* has been increasing since 1992 and reached 5% among isolates from blood and spinal fluid in 2000. This increase seems to have stopped and the percentage of macrolide-resistant *S. pneumoniae* was 4.7% in 2002, a level similar to the one observed in 2001 (Figure 16). There has been no major change in the level of total macrolide use, which has remained at about 2 DDD/1,000 inhabitant-days since 1994; however, there were changes in the distribution of the macrolides used, with an increasing use of azithromycin and to a lesser extent of clarithromycin (Figure 2, page 18). In Denmark, the prevalence of macrolide-resistant *S. pneumoniae* started to increase following the introduction of azithromycin and then showed a parallel increase to consumption of this macrolide. Since 1999, there has been no further increase in azithromycin consumption, which could well be the cause for the interruption observed in the increase in macrolide-resistant *S. pneumoniae*.

Streptococcus pyogenes

In 2001, we introduced surveillance of macrolide resistance in *S. pyogenes* (group A streptococci or GAS). Until now, resistance to macrolides has been a minor problem in Denmark, but increasing resistance worldwide has called for closer monitoring. In 2002, 6,856 GAS from various clinical samples in ten counties were examined. Resistance (non-susceptibility) to macrolides was 2.5% [95% C.I.: 2.2-2.9], with county-to-

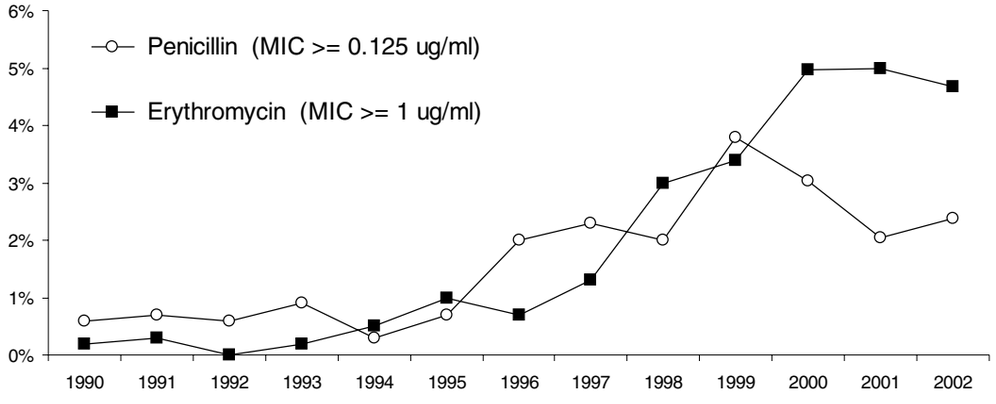

Figure 16. Resistance (%) to selected antimicrobials in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark*

county variations ranging from 1.0% to 4.2%. There was no significant change in macrolide resistance as compared to 2001. Preliminary reports on the distribution of macrolide resistance genes in Danish GAS isolates in 2000-2002 have shown that *erm*(A), *erm*(B) and *mef*(A) genes were all found in Danish GAS isolates, with *erm*(B) being the most frequent. Additionally, molecular typing of GAS isolated in 2000-2002 found 17 different patterns among macrolide-resistant GAS, thus suggesting that macrolide resistance in GAS in Denmark is not a clonal problem, but probably the consequence of the ecological pressure due to macrolide use.

Staphylococcus aureus

A detailed analysis of data from the MRSA register maintained by the Staphylococcus Unit at the Statens Serum Institut for the period 1999-2002, completed by an analysis of the patients' discharge letters, are presented in Figure 17. For the past 20 years, methicillin-resistant *S. aureus* (MRSA) has represented less than 1% of *S. aureus* blood isolates and more than one half of these MRSA strains have been acquired outside Denmark. This pattern has recently changed. Imported cases of infection or colonisation only represented 21% of reported MRSA in 2002, as compared to 45% in 1999 (Figure 17). During the same period, MRSA infections acquired in Denmark have increased from 34 cases in 1999 to 68 cases in 2002 (preliminary results). Of these, a larger fraction was reported from hospitals in 2002 (46%) as compared to 2001 (27%) (Figure 17).

These results are presently being completed by an analysis of the pulsed-field gel electrophoresis (PFGE) profiles of all MRSA strains reported in 2002. Preliminary results show that the epidemic clone EDK97-1, which was responsible for 2/3 of MRSA infections from primary health care in 2001 (see DANMAP 2001), is still present in the Danish community. This analysis analysis should also help understand the increase in hospital MRSA cases in 2002.

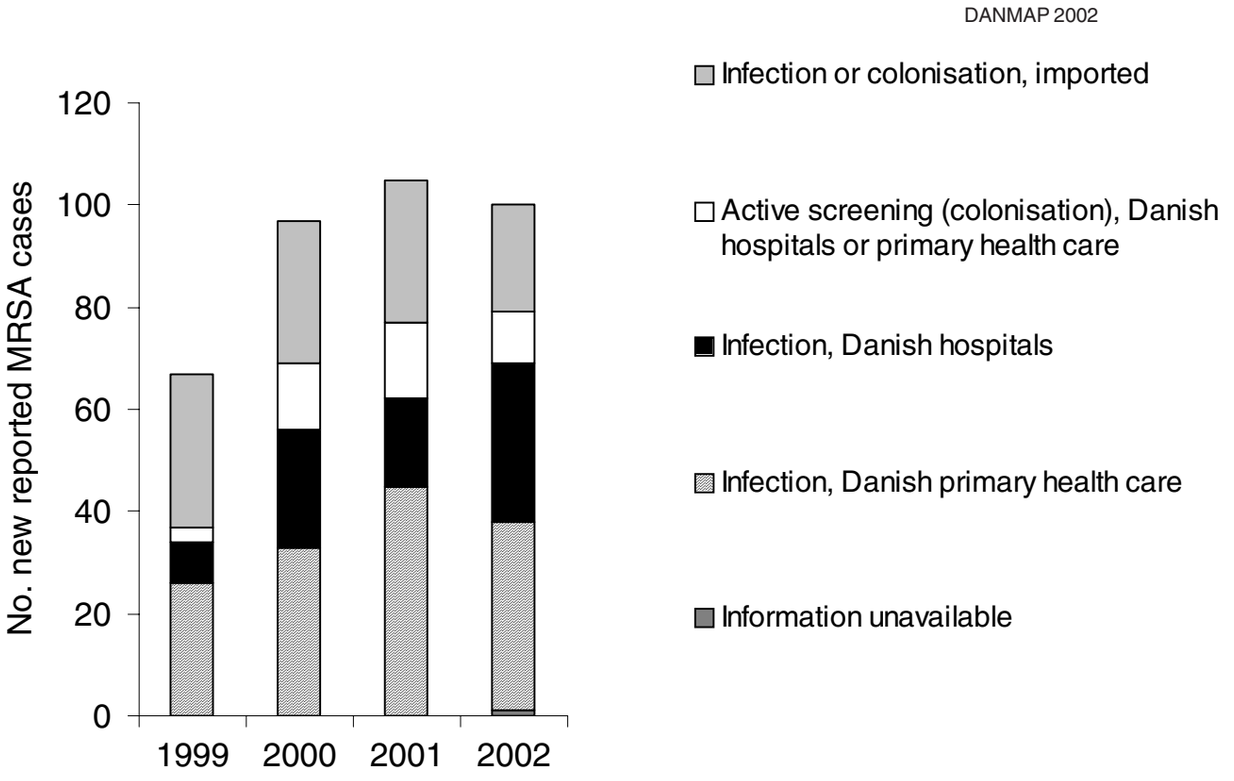

Figure 17. New reported cases of human infection or colonisation by methicillin-resistant Staphylococcus aureus *(MRSA), Denmark (2002, preliminary results)*

Escherichia coli

The results for thirteen counties for the period 1995-2002 are presented in Figures 18 to 23. As the number of laboratories that participate in DANMAP increases, it is becoming more difficult to represent and make sense of the data that have been collected over time since the beginning of the project. However, we still find it too early to pool these data from various origins since standardisation of surveillance methods, e.g. algorithm for removal of duplicate isolates, has not yet been achieved. This stresses the need for a national working group on antimicrobial resistance surveillance to clearly define these methods before a Danish electronic surveillance system is implemented.

Figures 18 to 20 show the levels of resistance to selected antimicrobials among *E. coli* blood isolates. As in 2001, with the exception of a few counties, data from 2002 confirm that the general increase in ampicillin resistance in *E. coli* blood isolates has stopped and remains between 30 and 50%. The large variations observed in some counties in 2000-2001 or in 2001-2002 are likely to be due to the smaller number of isolates tested in these counties. Similar to what has been reported during the past years, gentamicin and cefuroxime resistance in *E. coli* blood isolates remained low in 2002. Figures 21 to 23 show the levels of resistance to selected antimicrobials among *E. coli* urine isolates. The results are presented separately for isolates from hospitals (hosp) and from primary health care (phc). Despite a resistance level of 30 to 40%, sulfonamides still represent the drug of choice for treating urinary tract infections in Denmark. One should be aware that, in primary health care, a significant proportion of urine samples originates from complicated cases and is submitted to the laboratory because of treatment failure. This therefore represents a selected population. Data on resistance in *E. coli* from uncomplicated cases of urinary tract infection from a time-limited study are presented in Text Box 4 and confirm this hypothesis. Additionally, one cannot exclude differences in the frequency of sampling among counties, which precludes any comparison of resistance levels. However, if each county is considered separately, sulfonamide resistance was always higher in primary health care than in hospitals. This observation is consistent with the fact that sulfonamide use is very low in Danish hospitals (Table 13, page 19). Ampicillin resistance in *E. coli* urine isolates was between 30 and 50%. Finally, ciprofloxacin resistance in *E. coli* urine isolates remained very low in 2002.

Coagulase-negative staphylococci

Figures 24 to 26 show the levels of resistance to selected antimicrobials among coagulase-negative staphylococci blood isolates from thirteen counties. As in most other countries, penicillin resistance was almost 80% in Denmark. Methicillin resistance varied amoung counties from less than 10% to more than 50%. However, it is possible that differences in the level of resistance were merely the consequences of the procedure for selection of isolates that are submitted for susceptibility testing. For example, the laboratories reporting the highest percentage of methicillin-resistant coagulase-negative staphylococci mentioned that they only perform susceptibility testing in isolates of clinical significance. Caution is therefore warranted when trying to make comparisons of resistance levels among counties. Finally, erythromycin resistance in coagulase-negative staphylococci blood isolates ranged from 20 to more than 50% depending on the county.

Legends for Figures 18 - 26

County (Laboratory)	Code
Copenhagen & Frederiksberg Municipality (Hvidovre Hospital/Rigshospitalet)	CPF (H/R)
Copenhagen County	CPC
Roskilde	ROS
West Zealand	WZE
Storstroem	STO
Funen	FU
South Jutland	SJU
Ribe	RIB
Ringkoebing	RIN
Aarhus	AAR
Viborg	VIB
North Jutland	NJU

hosp = hospital, phc= primary health care

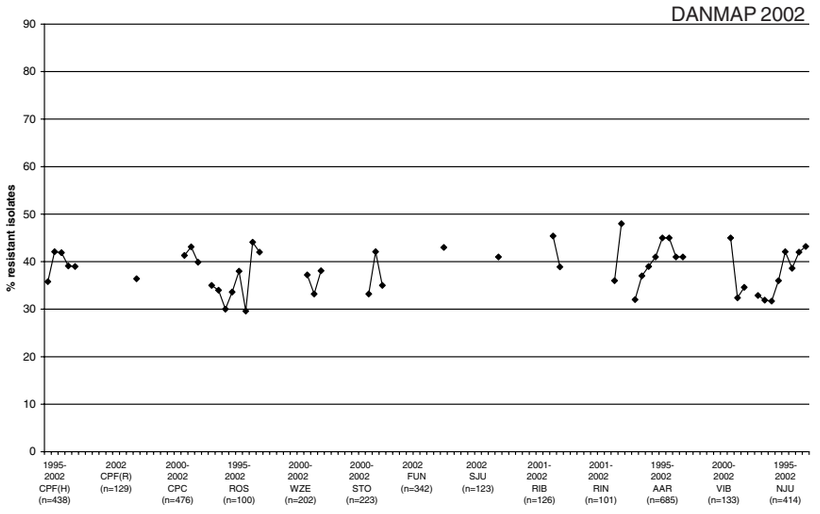

Figure 18. Resistance (%) to ampicillin in Escherichia coli *blood isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.

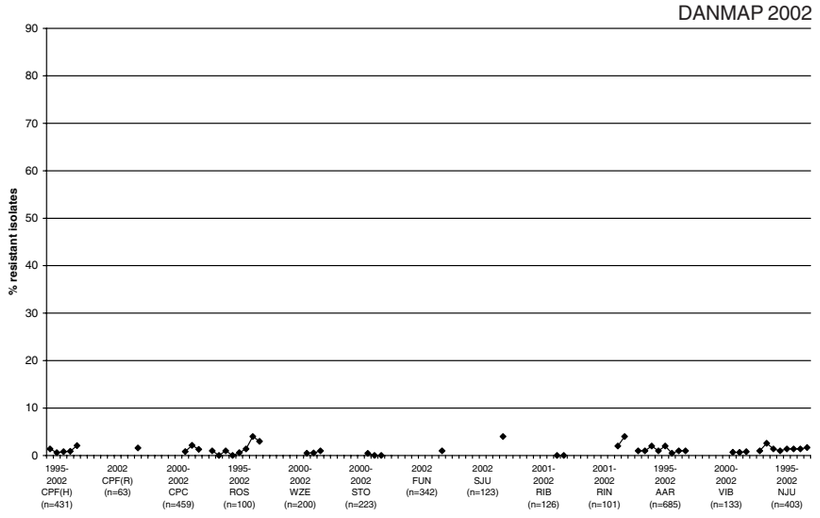

Figure 19. Resistance (%) to gentamicin in Escherichia coli *blood isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.

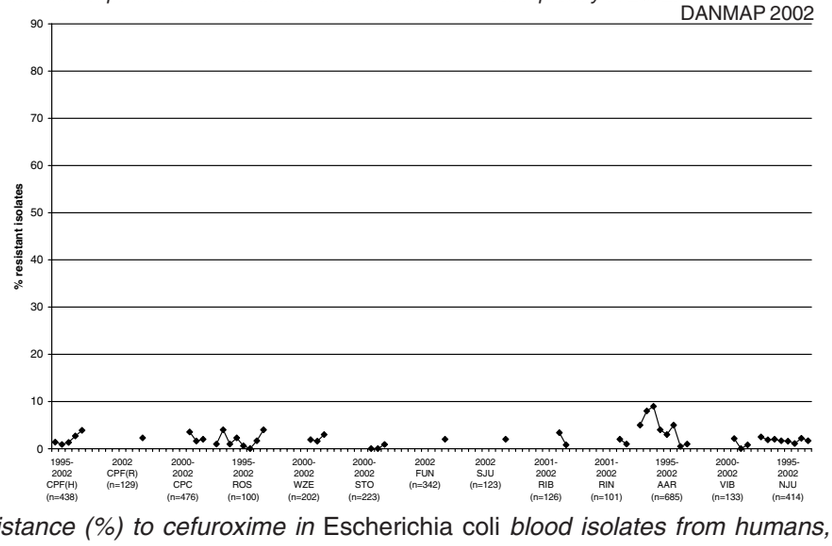

Figure 20. Resistance (%) to cefuroxime in Escherichia coli *blood isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.

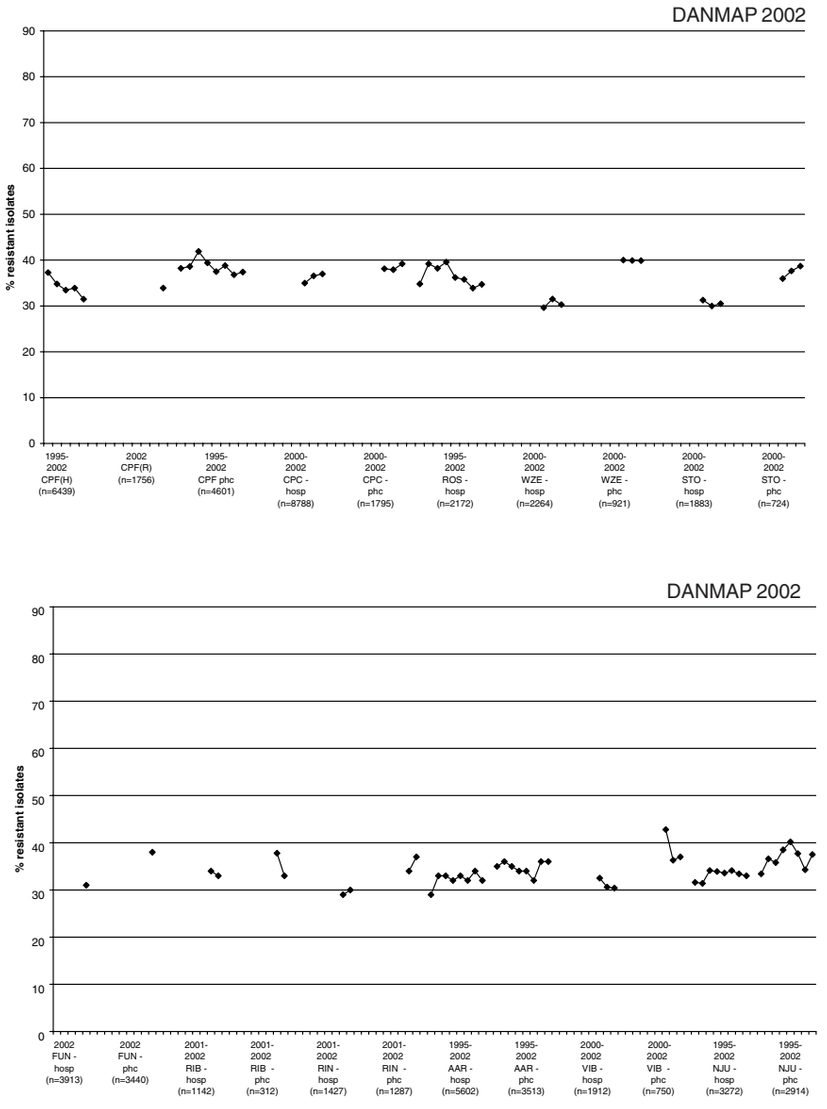

Figure 21. Resistance (%) to sulfonamide in Escherichia coli *urine isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.

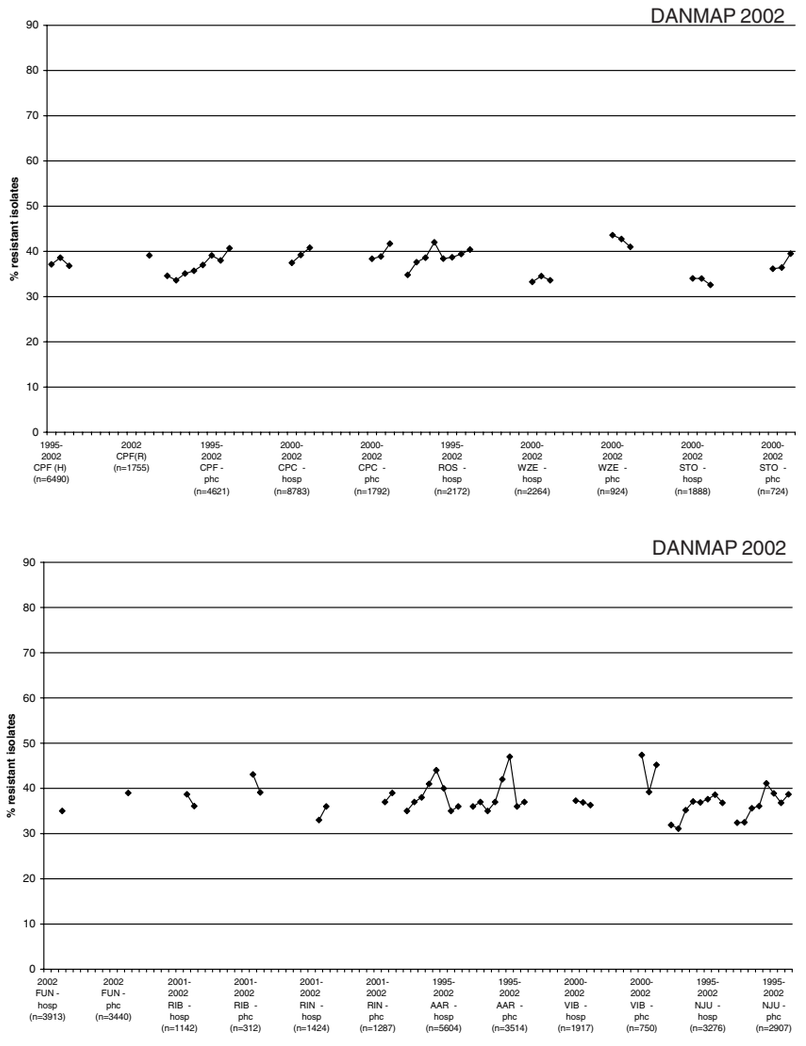

Figure 22. Resistance (%) to ampicillin in Escherichia coli *urine isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.

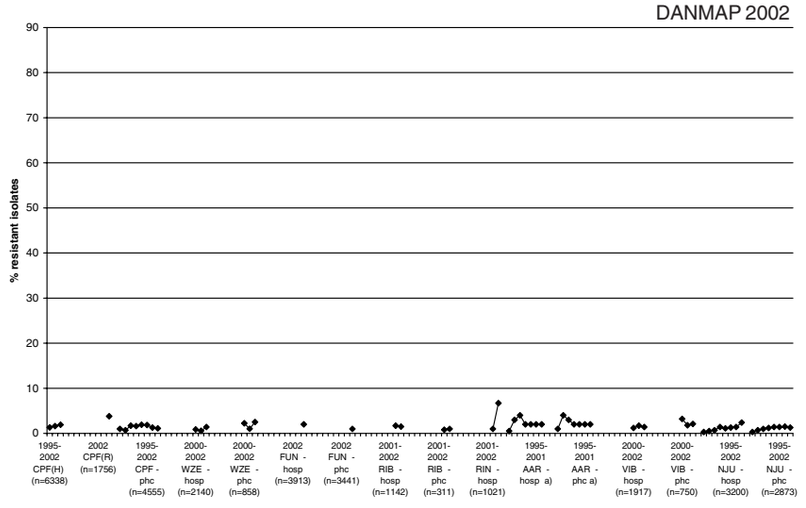

Figure 23. Resistance (%) to ciprofloxacin in Escherichia coli *urine isolates from humans, Denmark.*
The number n in parentheses represents the number of isolates tested for susceptibility in 2002.
a) not tested in 2002

Text box 4

Prevalence of antibiotic resistance rates depends upon the clinical setting: Experience from a study of *Escherichia coli* from cases of urinary tract infection

The frequency of antibiotic resistance in human pathogens, as also reported in the present DANMAP report, depends on the clinical setting from where the strains are sampled and this should be borne in mind when comparing figures or using the data as basis for choice of empiric treatment.

In a recent study by Kerrn *et al.* (J Antimicrob Chemother 2002; 50: 513-6), we reported on the susceptibility of *E. coli* - the most common urinary pathogen- towards antimicrobials commonly used for the treatment of urinary tract infection (UTI) in Denmark. *E. coli* isolates were obtained from the following sources:

- community acquired UTIs - general practioners in the Roskilde county/region were asked to submit cultures from patients with symptoms of UTI and to note for each patient, whether it was an uncomplicated UTI (female patient of age > 16 years, no UTI within previous 6 months, no signs of upper UTI) or a complicated UTI (all other cases of community acquired UTI),
- hospital acquired UTIs from the two hospitals in the Roskilde region,
- community acquired bacteraemia cases from the Roskilde region, where UTI was the focal infection for the bacteraemia.

Table 45 presents the frequencies of resistance to 5 antimicrobials commonly used to treat UTIs. Overall, the frequency of resistance was the lowest in *E. coli* from uncomplicated cases of community acquired UTIs, while there were no significant differences among isolates from complicated cases of community acquired UTIs and from hospital acquired UTIs.

Further study of the strains revealed that co-resistance between ampicillin and sulfamethizole was present in approximately 80% of the resistant strains. Concomitant conjugative transfer of both resistance traits was proven in 20 of the *E. coli* isolates studied.

This study confirms that stratification on the source of isolates might provide very different resistance frequencies and must be taken into account when reporting susceptibility results.

Table 45. Occurrence of resistance (%) among Escherichia coli *from various clinical samples*

Compound	Community acquired UTI		Hospital acquired UTI %	Bacteraemia %
	Uncomplicated %	Complicated %		
Ampicillin	20	34	47 [a)]	42
Mecillinam	0	2	7	0
Trimethoprim	10	24	28 [a)]	20
Sulfamethizole	22	39	47 [a)]	44
Nitrofurantoin	0	3	0	1
Number of isolates	59	88	75	74

a) Significantly different from the value in community acquired uncomplicated UTI (Fisher exact test, $P < 0.05$).

Further details about this study can be obtained from Niels Frimodt-Møller, MD DSci, National Center for Antimicrobials and Infection Control, Statens Serum Institut, Copenhagen, Denmark. E-mail: nfm@ssi.dk

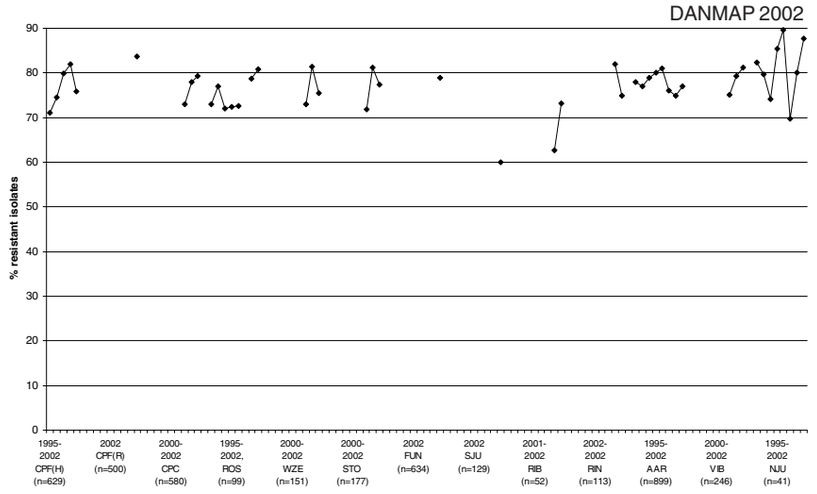

Figure 24. Resistance (%) to penicillin in coagulase-negative staphylococci blood isolates from humans, Denmark. *The number n in parentheses represents the number of isolates tested for susceptibility in 2002.*

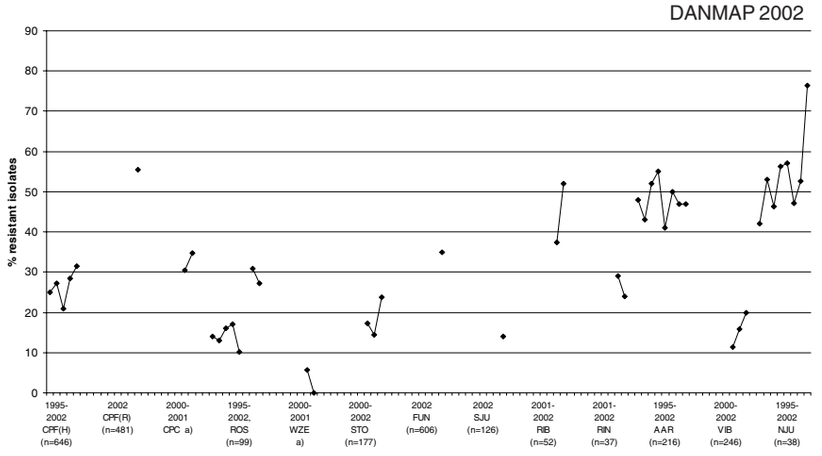

Figure 25. Resistance (%) to methicillin/oxacillin in coagulase-negative staphylococci blood isolates from humans, Denmark. *The number n in parentheses represents the number of isolates tested for susceptibility in 2002.*
a) not tested in 2002

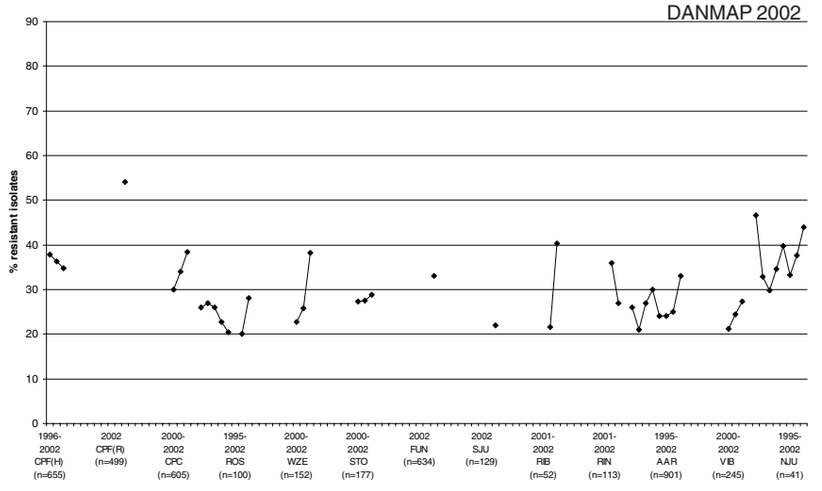

Figure 26. Resistance (%) to erythromycin in coagulase-negative staphylococci blood isolates from humans, Denmark. *The number n in parentheses represents the number of isolates tested for susceptibility in 2002.*

Appendix 1
Materials and methods

Data on consumption of antimicrobials

Antimicrobials in animals
In Denmark, all antimicrobials used in therapy are prescription-only medicines and must be distributed through pharmacies. The pharmacy either sells the medicines to veterinarians for use in practice or for re-sale to farmers, or will sell directly to the animal owner on presentation of a prescription. By law, the profit that veterinarians can make on the sale of medicines is severely limited and thus veterinarians have little financial enticement to sell medicines. Accordingly, an estimated 80% of all antimicrobials used for therapy in food animals are sold to farmers by pharmacies on the basis of a veterinary prescription.

In previous DANMAP reports, data on antimicrobial usage in animals was based on sales figures reported by the pharmaceutical industries. In contrast, VetStat collects data on all medicine prescribed by veterinarians for use in animals. Furthermore the VetStat database contains data on the consumption of coccidiostatics and antimicrobial growth promoters. VetStat started to collect data from 1st August 2000. From 2001 onwards data have been published on a monthly basis, and are available on the Vetstat homepage http://vetstat.vetinst.dk.

Data are reported by pharmacies, large animal practices and feed mills. Basically, the reported data includes date of sale, drug identity and quantity, identification of the prescribing veterinarians and identification of the farm where the medicine was used, codes for animal species, age and disease. Data on companion animals and horses are reported with fewer details. Data from all three sources are reported at least once a month.

All prescription medicines except premix for use in medicated feed can only be sold through a pharmacy. This includes medicines ordered by the veterinarians for use in their own practice. In this case the pharmacy will add an identification number for the veterinary practice. The pharmacy reports data to the Danish Medicines Agency, which forwards them to the VetStat database.

Veterinarians are required by law to report data on all medicines used or resold in practice for treatment of production animals. Accordingly, VetStat contains information about medicines supplied to veterinary practioners, and if used in production animals, details of the circumstances under which it was used.

Feed mills record the total sale of medicated feed and feed containing coccidiostatics. In case the farmer imports feed containing coccidiostatics, he will be held responsible for reporting these data to VetStat.

Data from pharmacies, veterinarians, feed mills and import by farmers (feed containing coccidiostatic only) are send to a central database where they are merged with data from the central farm register. The central farm register contains information at farm level about animal species and number of animals within different age groups. Antibacterials used in humans and/or animals are presented in Table A1.

Through the VetStat homepage veterinarians and farmers have access to data concerning their own clients and herds, respectively. For comparative purposes all data are standardised, which makes it possible for farmers to compare their own use with a mean usage on a regional or national level. In order to make meaningful comparisons Animal Daily Dosages (ADD) were defined. The ADD is calculated for each formulation and is the daily dosage required to treat an animal of a certain weight. It should be noted that ADDs primarily are technical units for statistical purposes.

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

In Denmark, all antimicrobials for use in humans are prescription-only medicines. All antimicrobials are sold by pharmacies in defined packages. Each package is uniquely identified by a code, which can be related to the size of the package (by content and in Defined Daily Doses or DDD), to the code of the antimicrobial in the Anatomical Therapeutic Chemical (ATC) classification

system, and to the name of the 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 subsidisation 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. On-line transfer of the transactions in real time is being established.

The present report includes data on the consumption of antimicrobials for systemic use, or group J01 of the 2002 update of ATC classification system, 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).

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 subclinical 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 Veterinary Institute (DVI) 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 98%, 80% and 95%, respectively, of the total production of these animal species in Denmark. Accordingly, the bacterial isolates

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

Antibacterials, which are only used in animals are mentioned in italics. Antibacterials, which are used both in humans and in animals are underlined.

DANMAP 2002

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*	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, *procaine penicillin*, *penethamate hydroiodide*
J01CF/*QJ51CF*	Beta-lactamase resistant penicillins	Dicloxacillin, *cloxacillin*, flucloxacillin, *nafcillin*
J01CR/*QJ01CR*	Comb. of penicillins, incl. beta-lactamase inhibitors	Amoxicillin/clavulanate, piperacillin/tazobactam
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*	Short-acting sulfonamides	Sulfamethizole, *sulfadimidine*
J01EE/*QJ01EW*	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfamethoxazole/trimethoprim, *sulfadiazine/trimethoprim*, *sulfadoxine/trimethoprim*, *sulfatroxazole/trimethoprim*
J01FA/*QJ01FA*	Macrolides	Erythromycin, *spiramycin*, roxithromycin, clarithromycin, azithromycin, *tylosin*, *tilmicosin*
J01FF/*QJ01FF*	Lincosamides	Clindamycin, *lincomycin*
J01G/A07AA/ *QJ01G/QA07AA* b)	Aminoglycosides	*Streptomycin*, *dihydrostreptomycin*, tobramycin, gentamicin, *neomycin*, netilmicin, *apramycin*
J01MA/*QJ01MA*	Fluoroquinolones	Ofloxacin, ciprofloxacin, norfloxacin, moxifloxacin, *enrofloxacin*, *danofloxacin*, *marbofloxacin, difloxacin*
QJ01MB	Other quinolones	*Oxolinic acid*
J01XA	Glycopeptides	Vancomycin, teicoplanin
J01XB/A07AA/*QA07AA* b)	Polymyxins	Colistin
J01XC	Steroid antibacterials	Fusidic acid
J01XD/P01AB/*QJ01XD* b)	Imidazole derivatives	Metronidazole
J01XE/*QJ01XE*	Nitrofurane derivatives	Nitrofurantoin
J01XX/*QJ01XX*	Other antibacterials	*Spectinomycin*, methenamine, linezolid, *tiamulin, valnemulin*

a) Antibiotics for intramammary use in animals are included. Antibiotics only used topically in humans or in animals are not included in this table
b) 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

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 DVI only one isolate of each serotype per farm is selected for the DANMAP report. The DVI 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 DVI, the Steins Laboratory in Holstebro 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 both Danish and imported foods. The food samples were collected according to the guidelines for microbiological examination of foods from the Danish Veterinary and Food Administration (Vejledning om mikrobiologisk kontrol af fødevarer, ISBN: 87-90978-46-3).

Isolates from humans
Salmonella, Campylobacter. A random sample of isolates grown from faeces samples submitted to the Department of Gastrointestinal and Parasitic Infections at Statens Serum Institut for microbiological diagnostic in 2002 were tested for antimicrobial susceptibility. Exact figures of the proportion tested 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 healthy volunteers in the community (NorMat)
To monitor the level of resistance among healthy individuals a running surveillance comprising approximately 200 stool samples per year were initiated in 2002. Subjects selected for participation in the surveillance were found through the Danish Civil Register system (CPR), which is a continuously updated register of all residents in Denmark. With a calculated response rate of 20% and starting in the middle of March in total 760 individuals were invited to participate in the study. A selection algorithm was used to generate birthdays and genders of the individuals to be invited in 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 about the study and a consent form was mailed to the selected individuals, and they were asked to confirm their willingness to participate by returning the signed form. Faecal test tubes were mailed to Department of Gastrointestinal and Parasitic Infections, Statens Serum Institut.
The scientific ethics committee for Copenhagen and Frederiksberg municipalities approved the protocol.

Staphylococcus aureus. All blood isolates collected nationwide are sent to the Staphylococcus Unit (reference laboratory) at the Statens Serum Institut for confirmation of susceptibility results, phage typing and pulsed-field gel electrophoresis (PFGE) typing.

Streptococcus pneumoniae. All blood and spinal fluid isolates collected nationwide are sent to the Streptococcus Unit at the Statens Serum Institut 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 Copenhagen and Frederiksberg municipalities (Hvidovre Hospital and Rigshospitalet), Copenhagen county, Roskilde county, West Zealand county, Storstroem county, Funen county, South Jutland county, Ribe county, Ringkoebing county, Aarhus county, Viborg county, and North Jutland county.

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 BPW and incubated overnight at 37°C. A plate with Modified Semi-solid Rappaport-Vassiliadis medium was inoculated with 100 ml of pre-

enrichment broth 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 microaerophilic atmosphere with 3% 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 ml of this enrichment culture was inoculated onto CCD agar and incubated as described above. *Campylobacter*-like colonies were identified by their catalase activity, by their ability to hydrolyse hippurate and indoxyl acetate, and by their susceptibility to cephalothine.

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

Enterococci. Enterococci from pigs and cattle were isolated and identified by the following procedure. One drop of faecal material suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz agar and incubated for 2 days at 42°C. Up to three colonies showing a morphology typical of *E. faecalis* and *E. faecium* were sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified according to the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, ribose, 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 (Becton Dickinson). Cultures were streaked on Slanetz agar and incubated for 48 h at 37°C. Colonies that morphologically resembled *E. faecium* and *E. faecalis* were identified to species level by using standard biochemical and physiological tests as described above. A subset of all isolates verified as *E. faecium* and *E. faecalis* were subjected to antimicrobial susceptibility testing.

Examination of food samples

The isolation of indicator organisms from food samples was performed by the RFCA. Subsequently, the isolates were sub-cultured 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 strain of *E. coli* and/or enterococci from each food sample were tested for antimicrobial susceptibility.

The isolation method for *E. coli* employed 5 grams of food, which was incubated at 44°C for 18-24 hours in 45 ml of MacConkey- or laurylsulfate-broth. The broth culture was streak-inoculated onto violet red bile agar and incubated for 48 hours 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).

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 using the procedure described under 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 DVI.

Thermotolerant *Campylobacter* spp. was isolated by a semi-quantitative method. Twenty-five grams of food

sample were mixed 1:4 with Mueller-Hinton bouillon supplemented with sodium pyrovate 0.25 mg/l, sodium metabisulphite 0.25 mg/l, ferro 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. 1 ml from each dilution was enriched under microaerophilic conditions for 24 hours at 42ºC in 9 ml of Mueller-Hinton bouillon with supplement (as described above). After pre-enrichment 10 µl was streaked on to mCCDA and further incubated under microaerophilic conditions for 24-48 hours at 42ºC. mCCDA plates were examined for the presence of *Campylobacter*-like colonies. Suspected 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 faeces samples using the SSI Enteric Medium (SSI rød plade, SSI Diagnostika, Copenhagen, Denmark) and enrichment using a 0.6% selenite medium (SSI Diagnostika).

***Campylobacter* spp.** were isolated from faeces samples using a modified CCDA medium (SSI Diagnostika). Species identification was performed with hybrid test and indoxyl acetate test.

Enterococci from healthy humans in the community 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 35ºC. 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 35ºC. 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 20 strep tests (BioMérieux, France) and PCR (Poulsen, RL *et al.,* APMIS 1999; 107: 404-412 and Dutka-Malen S *et al*. J. Clin. Microbiol., 1995; 33:24-27).

Selective method for isolation of vancomycin resistant enterococci

Ten µl of the faeces suspension were added to 5 ml Enterococcosel broth incubated overnight. Cultures were streaked on to Bile Aesculin agar with 16 µg/ml vancomycin and incubated for 2 days at 35ºC. Colonies showing morphology typical of *Enterococcus ssp.* were sub-cultivated on 5% blood agar plates. The isolates were identified as using API 20 strep tests (BioMérieux, France) and PCR (Poulsen, RL *et al.,* APMIS 1999; 107: 404-412 and Dutka-Malen S *et al*. J. Clin. Microbiol., 1995; 33:24-27).

Escherichia coli from healthy humans in the community 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 (SSI rød plade, SSI Diagnostika). 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).

Escherichia coli*, coagulase-negative staphylococci and *Streptococcus pyogenes were isolated on various commercial media used in clinical microbiology laboratories.

Susceptibility testing

Isolates from animals and foods

Plate dilution was used to test the susceptibility of *Campylobacter* isolates to all animicrobials included in the panel.

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 according to NCCLS guidelines and incubated aerobically at 37ºC for 18-22 hours. The MIC was defined as the lowest concentration of antimicrobial with no visible growth. The breakpoints used are shown in Table A2.

The following strains were 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 stains. The MIC values for the strains were evaluated in accordance to NCCLS guidelines and tests re-done if the values were out of range. With plate dilution, all 4 control strains were included on each plate.

Isolates from humans

Salmonella, Campylobacter. Until 31st August 2002, susceptibility testing for *Salmonella* spp. isolates was performed using the tablet diffusion method (Neo-Sensitabs®, Rosco A/S, Roskilde, Denmark) on Danish

Table A2 Breakpoints and range of dilutions used for testing bacteria from animals and food. Isolates with MIC higher than or equal to the figures shown were considered resistant, Denmark DANMAP 2002

Antimicrobial agent	*E. coli, Salmonella*		Staphylococci		Enterococci		*Campylobacter*	
	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range
Ampicillin	32	1-32					32	1-32
Apramycin	16	4-64						
Avilamycin			16	2-32	16	1-32		
Bacitracin			128	16-256	128	8-256		
Ceftiofur	8	0.5-8	8	0.12-16				
Cephalothin	32	2-64						
Chloramphenicol	32	2-64	32	2-64	32	2-64	32	1-64
Ciprofloxacin	4 a)	0.03-4	4	0.12-8			4	0.03-16
Colistin	16	4-64					64	0.5-64
Erythromycin			8	0.12-16	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	1-32	1,024	128-2,048	16	0.5-32
Kanamycin			64	4-128	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	0.5-8				
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
Sulfamethoxazole	512	64-1024	512	8-512			512	8-512
Quinupristin/dalfopristin b)			4	1-32	4	0.5-32		
Teicoplanin					16	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	1-32	32	2-32		
Virginiamycin			8	1-32				

a) >= 0.125 ug/ml was the ciprofloxacin breakpoint applied for all *Salmonella* isolates and for *E. coli* isolates from humans
b) The trade name is Synercid

Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined in Table A3. From 1st September 2002, susceptibility testing of *Salmonella* spp. was performed with Sensititre (Trek Diagnostic Systems Ltd.). The wells were inoculated according to NCCLS guidelines and incubated aerobically at 36ºC for 18 - 22 hours. The breakpoints used are shown in Table A2. *Escherichia* coli ATCC 25922 was used for quality control.

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

Staphylococcus aureus. The Staphylococcus Unit at the Statens Serum Institut is using the tablet diffusion method (Neo-Sensitabs®, Rosco A/S) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by Rosco A/S. Methicillin resistance was confirmed with a 3-h hybridisation assay for the detection of the *mecA* gene (Skov RL, et al. J. Antimicrob. Chemother. 1999; 43: 467-475).

Streptococcus pneumoniae. The Streptococcus Unit at the Statens Serum Institut screens for penicillin-resistant *S. pneumoniae* using a 1 microgram oxacillin tablet (Neo-Sensitabs®, Rosco A/S) on 10% horse blood agar (SSI Diagnostika). Penicillin MICs are determined using the E-test (AB

Table A3. Breakpoints used for gastrointestinal pathogens from humans. Isolates were considered resistant if they had an inhibition zone less than shown in the table, Denmark DANMAP 2002

Antimicrobial agent	Species	
	Salmonella enterica	*Campylobacter*
Ampicillin	28 mm	-
Apramycin	20 mm	24 mm
Ceftiofur	20 mm	-
Chloramphenicol	24 mm	33 mm
Colistin	17 mm	18 mm
Ciprofloxacin	- a)	27 mm
Erythromycin	-	27 mm
Gentamicin	22 mm	30 mm
Kanamycin	-	22 mm
Nalidixic acid	24 mm	27 mm
Spectinomycin	21 mm	30 mm
Streptomycin	21 mm	32 mm
Sulfonamide	20 mm	-
Tetracyclin	28 mm	32 mm
Trimethoprim	18 mm	-

a) Resistance to fluoroquinolones is based on susceptibility results for nalidixic acid

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 2002, the clinical microbiology laboratories serving Roskilde, Storstroem, Funen, Ribe, Ringkoebing and Viborg counties, as well as Rigshospitalet, which acts a the national referral hospital and serves part of the Municipality of Copenhagen, were using the tablet diffusion method (Neo-Sensitabs®, Rosco A/S) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by Rosco A/S. The clinical microbiology laboratory serving North Jutland county used the same tablets on Mueller-Hinton II agar (Becton-Dickinson, Franklin Lakes, NJ, USA, and SSI Diagnostika) and the breakpoints defined by the Swedish Reference Group for Antibiotics.

In 2002, the clinical microbiology laboratories serving the Copenhagen and Frederiksberg Municipalities (Hvidovre Hospital), West Zealand county, South Jutland county and Aarhus county were using the disk diffusion method (Oxoid, Basingstoke, UK) on Iso-Sensitest (ISA) medium with or without 5% horse blood (Oxoid). The clinical microbiology laboratory serving Copenhagen county was using the disk diffusion method (AB Biodisk, Solna, Sweden) on 5% horse blood Antibiotic Sensitivity Medium (PDM, AB Biodisk). 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 microtitre wells. The wells were inoculated according to NCCLS guidelines and incubated aerobically at 35ºC 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.

Performance test

Similarly to previous years a performance test was carried out to ascertain the comparability of antimicrobial susceptibility test results of the laboratories involved in the presentation of data.
The laboratory in Department of Gastrointestinal and Parasitic Infections, the Clinical Microbiology Laboratory and the National Center for Antimicrobials and Infection Control at the Statens Serum Institut, as well as the Danish Veterinary Institute and the Danish Veterinary and Food Administration received 5 *E. coli*, 5 *Salmonella* spp. and 5 *Enterococcus* spp. strains. A total of 908 antibiotic-bacterium susceptibility tests were performed and the overall results were 1.2% failures (Table A4). Failures corresponded to either misinterpretation of test results when similar MICs were found or to actual failures, i.e. > 2-fold differences in MICs. The highest failure rate was seen in susceptibility testing of enterococci versus bacitracin, which has been reported as a problem in previous years. The Sensititre microtitre system, a micro-broth dilution test, gave quite variable results for bacitracin, with MIC values for some enterococci varying from 8 to 256 mg/l depending on the laboratory. This was due to differences in reading the endpoint. MIC testing for bacitracin requires further development. However, an overall agreement of 98.8 % was considered excellent

Data handling

Data on animal isolates

The results of 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. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant breakpoint. Each isolate was identified by the bacterial species, including subtype as applicable and by the date and place of sampling and the species of animal. Information on the herd or flock of origin was also recorded. All handling and evaluation of results was carried out using PC SAS, v.8.

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 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, Campylobacter. Data on *Salmonella* spp. and *Campylobacter* spp. infections were exported from the Danish registry on gastro-intestinal infections (Microsoft® Access) maintained by the Department of Gastrointestinal and Parasitic Infections at the Statens Serum Institut. This register includes only one isolate per patient within a window of 6 months. Data on susceptibility testing of gastrointestinal pathogens are stored as zone diameters (mm) or MIC values (μg/ml) for *Salmonella* isolates tested since 1st September 2002 in a Microsoft® Excel database at the same department. Using the isolate identification number, the Danish register on gastro-intestinal infections 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 Epi Info v. 6.04c.

Staphylococcus aureus. Data on methicillin-resistant *Staphylococcus aureus* (MRSA) were exported from the Danish MRSA registry (Microsoft® Excel) maintained by the Staphylococcus Unit at the Statens Serum Institut. Patients are only registered in this database the first time they are diagnosed as being infected or colonised by MRSA. Additional information concerning the probable origin of MRSA isolates was obtained by contacting local clinical microbiology laboratories and from careful examination of the discharge letters. MRSA cases were then classified as imported infection or colonisation (patients who have been admitted in a foreign hospital, refugees and children adopted from foreign countries), active screening in a Danish hospital or in primary health care (surveillance samples to detect nasal or skin colonisation), infection acquired in a Danish hospital, or infection acquired in the Danish primary health care.

Streptococcus pneumoniae. Data on susceptibility testing of *Streptococcus pneumoniae* isolates are

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

DANMAP 2002

Antimicrobial agent	*Escherichia coli*		*Salmonella* spp.		*Enterococcus* spp.	
	S + I a)	R	S + I	R	S + I	R
Penicillin	-	-	-	-	12/12	3/3
Ampicillin	15/15	10/10	15/15	10/10	-	-
Amox/Clav	20/20	-	20/20	-	-	-
Oxacillin	-	-	-	-	-	-
Cefalothin	15/16	2/4	20/20	-	-	-
Ceftiofur	20/20	-	20/20	-	-	-
Erythromycin	-	-	-	-	8/8	12/12
Lincomycin	-	-	-	-	-	10/10
Tetracyclin	11/12	8/8	12/12	8/8	8/8	12/12
Chloramfenicol	16/16	4/4	12/12	8/8	8/8	12/12
Vancomycin	-	-	-	-	20/20	-
Teicoplanin	-	-	-	-	20/20	-
Linezolid	-	-	-	-	15/15	-
Synercid	-	-	-	-	6/6	9/9
Virginiamycin	-	-	-	-	-	-
Nalidixic acid	16/16	4/4	8/8	12/12	-	-
Ciprofloxacin	20/20	4/5	20/20	-	-	-
Neomycin	20/20	4/4	20/20	-	-	-
Streptomycin	12/12	8/8	12/12	8/8	9/9	6/6
Kanamycin	-	-	-	-	12/12	3/3
Apramycin	-	-	-	-	-	-
Gentamicin	24/25	-	20/20	5/5	16/16	4/4
Spectinomycin	20/20	-	8/8	12/12	-	-
Colistin	25/25	-	25/25	-	-	-
Sulfonamide	12/12	12/12	12/12	8/8	-	-
Trimethoprim	14/15	10/10	20/20	5/5	-	-
Sulfa/trim	-	-	-	-	-	-
Bacitracin	-	-	-	-	6/6	5/9
Tiamulin	-	-	-	-	-	-
Florfenicol	-	-	-	-	15/15	-
Avilamycin	-	-	-	-	15/15	-
Flavomycin	-	-	-	-	9/9	6/6
Salinomycin	-	-	-	-	15/15	-
Total	260/264 (98.5 %)	66/69 (95.7 %)	244/244 (100 %)	76/76 (100 %)	181/181 (100 %)	70/74 (94.6 %)

a) S+I: susceptible and intermediate, R: resistant.

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 using this software.

***Escherichia coli,* coagulase-negative staphylococci and *Streptococcus pyogenes*.** Thirteen clinical microbiology laboratories provided aggregated data on resistance levels in *Escherichia coli* blood and urine isolates and in coagulase-negative staphylococci blood isolates. Only nine of these laboratories were able to provide data on resistance levels in *Streptococcus pyogenes*.

In twelve of the thirteen laboratories, data were extracted from the laboratory information system, i.e. (1) 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), (2) MADS (Clinical Microbiology Laboratory, Skejby Hospital, Aarhus, Denmark) for Copenhagen Municipality (Rigshospitalet), Storstroem county (Næstved Hospital), South Jutland (Sønderborg Hospital), Ribe county (Esbjerg Hospital), Ringkoebing county (Herning Hospital), Aarhus county (Skejby Hospital) and Viborg county (Viborg Hospital), and (3) Funen's "Green System" for Funen county (Odense University Hospital). For Roskilde county, resistance data on *E. coli* and coagulase-negative staphylococci from blood samples were obtained from the laboratory information system at the Statens Serum Institut, 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 or on the first isolate taken from each patient during the study period.

Confidence limits

Estimation of exact 95% (two-sided) confidence intervals for proportions were based on binomial probability distributions as described in Armitage & Berrry (2001), Statistical Methods in Medical Research, 4th ed. 2001, Oxford: Blackwell Scientific Publications.

Appendix 2

2001

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