



# **DANMAP 2000**

**DANMAP 2000 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark**

**Statens Serum Institut  
Danish Veterinary & Food Administration  
Danish Medicines Agency  
Danish Veterinary Laboratory**



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*Due to unforeseen technical difficulties data for food isolates could not be included. However, the isolates have been collected and the results will be presented with the results for 2001.*

## Sammendrag

DANMAP 2000 er den 5. rapport fra overvågningsprogrammet "Danish Integrated Antimicrobial Resistance Monitoring and Research Programme". Rapporten beskriver udviklingen i resistens blandt zoonotiske bakterier og ikke-zoonotiske sygdomsfremkaldende bakterier fra dyr og mennesker og blandt indikator bakterier fra dyr. Den beskriver endvidere udviklingen i forbrug af antibiotika. Overvågningsprogrammet omfatter tillige bakterier fra fødevarer. Resistensundersøgelserne af bakterier fra fødevarer indsamlet i 2000 forelå på grund af tekniske problemer ikke ved rapportens deadline. De vil i stedet blive præsenteret i rapporten for 2001.

### Forbrug af antibiotika

Der er ofte diskussion om hvorledes det samlede forbrug af antimikrobielle midler (antibiotika) fordeler sig på dyr og mennesker. Grunden er, at der i de færreste lande findes nøjagtige opgørelser over forbruget af antibiotika. DANMAP har hidtil rapporteret forbruget som Definerede Døgn Doser (DDD) pr. 1000 indbyggere. Dette er den eneste opgørelsesmåde, der giver mulighed for at sammenligne udvikling i forbrug af antibiotika med forskellig styrke over tid, men den har den ulempe at den umuliggør sammenligning med forbruget til dyr. Derfor rapporterer DANMAP 2000 forbruget til mennesker – foruden DDD – som kg aktivt stof (Figur 1, side 9). Som det fremgår af figuren går langt den største del af det samlede forbrug af antibiotika i Danmark til dyr. I 1997, det sidste år med uhindret brug af vækstfremmere, blev der anvendt ca. 41.000 kg til mennesker, svarende til omtrent 25% af det samlede forbrug til dyr.

Forbruget af vækstfremmere fortsatte det fald, der har fundet sted siden 1994 og nåede nul i 2000. Derimod er forbruget til behandling af dyr steget i de senere år. Stigningen var ganske betydelig fra 1999 til 2000, idet forbruget øgedes fra 61.900 kg til 80.600 kg. Stigningen er større end stigningen i husdyrproduktionen og omfattede specielt antibiotika (tetracykliner, makrolider eller aminoglycosider) der især bruges ved vand- eller fodermedicinering af svin. Oplysninger fra praktiserende dyrlæger tyder på, at selv om der i forbindelse med ophør med brug af vækstfremmere har været problemer med fravænningsdiarré blandt smågrise i en del besætninger, skyldes det høje forbrug nu i højere grad problemer med infektion med *Lawsonia intracellularis*. Der er dog også en del der tyder på, at der har været et stigende overforbrug i de senere måneder. Der er initiativer under forberedelse til at imødegå denne udvikling.

Forbruget af fluorokinoloner i husdyrbesætninger steg en smule fra 1999 til 2000, men er stadig langt lavere end i 1998 (se tekstboks på side 14). Forbruget af fluorokinoloner til mennesker overstiger det til dyr, men også her er der sket et fald i de senere år. Derimod er forbruget af glycopeptider (især vancomycin), som kun anvendes på hospitaler steget fra 25 kg i 1997 til 37 kg i 2000. Årsagen til stigningen kendes ikke. Den kan imidlertid have en sammenhæng men et stigende antal infektioner med methicillin resistente *Staphylococcus aureus* hos mennesker.

### Resistens blandt zoonotiske bakterier

For *Salmonella* er sammenligning af resistens ofte kompliceret på grund af tilstedeværelse og spredning af resistente kloner i nogle smittereservoirer og ikke i andre. For eksempel, selv om forekomsten af *Salmonella* Typhimurium er lav hos kvæg er en stor del af de isolater der indgår i DANMAP penta-resistente DT104, hvilket indebærer at den samlede resistensforekomst bliver høj. På samme måde skyldes forekomsten af kinolonresistens (nalidixansyre-resistens) hos *Salmonella* Enteritidis spredningen af en bestemt resistent bakterieklon. Generelt gælder det dog at resistensforekomsten blandt *Salmonella* er forholdsvis lav, især resistens overfor kinoloner. Blandt *S. Typhimurium* fra svin er der sket en stigning i tetracyclinresistens fra 14% til 25% fra 1999 til 2000, som ikke kan forklares ved en stigning i andelen af DT104. Der var også en stigning i tetracyclinresistens fra 11 til 17% blandt ikke-DT104 fra indenlandsk erhvervede tilfælde af *S. Typhimurium* infektion hos mennesker. Begge disse stigninger kan have en sammenhæng med stigningen i tetracyclinforbruget til svin. Der er generelt god sammenhæng mellem de resistensniveauer vi har fundet blandt *Salmonella* bakterier fra produktionsdyr og *Salmonella* fra indenlandsk erhvervede tilfælde af salmonellose hos mennesker, når der tages højde for at en del af disse kan skyldes importerede levnedsmidler.

Der er sket set et fald i resistens overfor makrolider blandt *Campylobacter coli* fra svin i forbindelse med ophøret med at bruge makrolider som vækstfremmere. Fra 1999 til 2000 er der dog sket en stigning i makrolidresistens, formentlig som direkte følge af det øgede forbrug af tylosin til behandling. Derimod er resistens overfor kinoloner faldet hos *C. coli* fra svin, som et resultat af det faldende forbrug af fluorokinoloner til svin siden 1998. *C. coli* er kun ansvarlig for ca. 5% af tilfældene af

campylobacterinfektion hos mennesker, hvorimod *Campylobacter jejuni* tegner sig for over 90%. For *C. jejuni* fra kvæg og slagtekyllinger var der i modsætning til *C. coli* en stigning i kinolonresistens fra 1999 til 2000. For isolater fra kvæg var stigningen ganske betydelig, fra 3% til 15% af isolaterne. Vi kan ikke umiddelbart forklare denne udvikling på baggrund af udviklingen i kinolonforbruget. Ser man på *C. jejuni* fra mennesker er procentdel bakterier med resistens overfor kinoloner steget støt siden 1997 og har nu nået 24% for indenlandsk erhvervede tilfælde. Den høje forekomst hos de indenlandske tilfælde af kinolonresistens sammen med et niveau af tetracyclinresistens som vi ikke ser blandt isolater fra dyr tyder på, at der kan være kilder til campylobactersmitte som ikke indgår i DANMAP overvågningen, sandsynligvis importeret fjerkræ. Det forhold at næsten 25% af de humane campylobacterisolater er resistente overfor kinoloner er bekymrende på grund af konsekvenserne for effektiv behandling af mennesker.

#### Resistens hos indikatorbakterier

Vi indsamler prøver af tarmindehold fra slagtedyr til undersøgelse for indikator bakterier (*Escherichia coli*, *Enterococcus faecium* og *Enterococcus faecalis*). Prøverne indsamles, så resultaterne afspejler resistensforholdene i den del af husdyrpopulationen der udøver et smittepres med resistente bakterier på fødevarer. Der indgår endnu ikke indikator bakterier fra raske mennesker i programmet. Dette ventes dog at ske fra 2. halvdel af 2001.

For enterokokker afspejler resistensudviklingen ret nøje udviklingen i antibiotikaforbrug. Ophøret med brug af vækstfremmere har resulteret i faldende resistens overfor de pågældende antibiotika. Den meget komplicerede sammenhæng mellem antibiotika-forbrug og udvikling i resistens illustreres af, at forekomsten af virginiamycinresistens hos *E. faecium* fra svin steg i 2000, selv om virginiamycin overhovedet ikke blev brugt. Mere detaljerede undersøgelser viste at den sandsynlige forklaring på udviklingen var at en bestemt, multiresistent *E. faecium* klon, som også var resistent overfor virginiamycin, blev udbredt i svinebesætninger. Hvorledes denne spredning har fundet sted er ikke afklaret. Faldet i makrolidresistens er også stagneret i 2000. En del af forklaringen er spredningen af den omtalte multiresistente bakterieklon, men det stigende forbrug af makrolider til behandling af svin kan også have spillet ind.

For *E. coli* fra raske dyr gælder at der har været faldende resistens overfor en række antibiotika i de seneste år. Hvad angår *E. coli* fra svin er det interes-

sant, at der ikke har været nogen stigning i resistens overfor tetracyclin. Stigningen i tetracyclinforbrug til svin har særligt fundet sted blandt de yngre aldersgrupper, men den stigning i resistens det har afstedkommet (se nedenfor) har ikke bredt sig til den aldersgruppe, som bliver undersøgt ved slagting. Den sandsynlige forklaring herpå er det forhold, at for *E. coli* afløser den ene type den anden efterhånden som dyret bliver ældre, således at de resistente typer afløses af ikke-resistente. Dette kan således også tyde på, at de gener, der koder for tetracyclinresistens endnu ikke har haft tid til at sprede sig i nævneværdig grad indenfor bakteriepopulationen.

#### Resistens hos ikke-zoonotiske sygdomsfremkaldende bakterier

Alle disse bakterier stammer fra diagnostiske indsendelser. Hvad angår *E. coli* fra kalve (serotype F5) har resistensforekomsten være ret konstant de seneste 5 år. For isolater fra fjerkræ (serotyperne O2 og O78) har udviklingen været noget mere variabel, men dette kan skyldes det forholdsvis beskedne antal isolater, der indgår. Ser man på *E. coli* fra svin (serotype O149) har der været en stigning i tetracyclinresistens i 2000. Dette afspejler sandsynligvis det stigende tetracyclinforbrug i den aldersgruppe, hvor *E. coli* O149 er årsag til sygdom. Det skal dog bemærkes at stigningen er sket på baggrund af et forholdsvis lavt niveau i 1999 i forhold til de foregående år. Udviklingen hos *E. coli* fra svin viser et fald i kinolonresistens, som falder sammen med det betydelige fald i forbruget af fluorokinoloner til svin. Der er et meget beskedent forbrug af gentamicin til behandling i danske husdyrbesætninger. På trods heraf var 12% af *E. coli* fra kalve resistente overfor gentamicin.

For *Streptococcus pneumoniae* hos mennesker har vi tidligere udtrykt bekymring for den stigende forekomst af resistens overfor erythromycin og penicillin. Stigningen i erythromycinresistens fortsatte i 2000, medens der var et lille fald i resistens overfor penicillin. For andre bakterier isoleret fra syge mennesker har resistensforekomsten været ret stabil de seneste 5 år. Vi er dog bekymrede over udviklingen i antallet af infektioner med methicillinresistente *Staphylococcus aureus* (MRSA). I 1994 var der 34 tilfælde, men i 2000 97 tilfælde. Tyve til 30 procent af tilfældene er påvist i almen praksis, resten på hospitaler. Det er endnu ikke klart, hvorvidt de afspejler en stigning i antallet af importerede tilfælde eller om infektionerne er erhvervet i Danmark. Foreløbige undersøgelser tyder på at en stor andel af tilfældene fra hospitaler stammer fra udbrud på hospitalsafdelinger.

## Konklusioner

I de 5 år, hvor DANMAP overvågningen har forløbet, har den givet adskillige væsentlige bidrag til vores forståelse af antibiotikaresistens. Vi har for eksempel gjort den erfaring, at selv om udviklingen i resistensforekomst kan skifte meget pludseligt kan det modsatte også være tilfældet. Det betyder at der kan gå adskillige år før intervention, for eksempel at forbruget af et bestemt antibiotikum ophører, et nyt bliver taget i anvendelse eller forbruget af et eksisterende stof øges, kommer til udtryk i form af ændringer i resistensforekomst. Det er derfor nødvendigt med løbende overvågning for at kunne følge udviklingen.

Det er også blevet klart, at co-selektion er meget vigtig med hensyn til udvikling i forekomst af bestemte resistensfænotyper. Et eksempel herpå er, at resistens overfor kloramfenikol stadig er udbredt blandt visse bakterier fra husdyrbesætninger, på trods af at kloramfenikol ikke har været tilladt til produktionsdyr siden 1978. Et andet eksempel er den store forskel i

faldet i vancomycinresistens blandt *E. faecium* fra henholdsvis slagtekyllinger og svin efter avoparcinforbuddet i 1995.

Som det fremgår af denne og tidligere DANMAP rapporter bidrager såvel importerede fødevarer som infektioner erhvervet i udlandet til resistensforholdene hos mennesker. Selv om betydningen af importerede fødevarer er forholdsvis beskeden i Danmark, hvor de fleste fødevarer af animalsk oprindelse er hjemligt produceret, vil dette ikke være tilfældet for lande, der importerer de fleste fødevarer.

DANMAP har et betydeligt forskningselement, som det fremgår fra den oversigt over videnskabelige publikationer, der er anført i Appendiks 2. Et af de områder hvor der er behov for en yderligere forskningssindsats er sammenhængen mellem klonal spredning af resistente bakterier i forhold til anvendelsen af antibiotika. Der er et påtrængende behov for at få den relative betydning af disse to faktorer belyst.

## Summary

DANMAP 2000 is the fifth report from the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It reports trends in resistance among zoonotic bacteria and non-zoonotic pathogenic bacteria from food animals and humans and indicator bacteria from food animals. The report also describes trends in use of antimicrobial agents. The DANMAP monitoring also includes resistance in bacteria from foods, however, due to technical difficulties results for isolates collected in 2000 were not available for this year's report.

### Consumption of antimicrobials

It is often a subject of discussion what proportions of the total amounts of antimicrobials are used in humans, in comparison with animals. We have previously reported the consumption in humans in Defined Daily Doses (DDD) per 1,000 population per day. While using DDD as unit of measurement permits comparison of the use of antimicrobials of unequal potency it makes it very difficult to compare consumption in humans with usage in animals. Therefore, DANMAP 2000 also provides data on human consumption as amount of active compound, i.e. kg. (Figure 1, page 9). Looking at the figure it is clear that of the total usage of antimicrobials in Denmark, far the largest quantity is used in food animals. In 1997, the last year with unrestricted use

of antimicrobial growth promoters, the quantity used in humans amounted to about 25% of the total usage in animals.

In animals, the use of antimicrobial growth promoters the use of oral compounds (tetracyclines, macrolides and aminoglycosides), mainly in pigs. Reports from practising veterinarians suggest that the initial problems in some herds with diarrhoea in weaned pigs following the discontinuation of antimicrobial growth promoters have been superseded by problems with *Lawsonia intracellularis* infections. There are, however, also indications that there has been increasing overuse of antimicrobials in recent months. Initiatives are presently underway to attempt to solve this problem.

The use of fluoroquinolones in food animals increased marginally from 1999 to 2000 but remains at much lower levels than in 1998 (see text box on page 14). The use of fluoroquinolones in humans exceeds the use in animals, but has also shown a decline in recent years. The consumption of glycopeptides (hospital use only), while still low, has increased from 25 kg in 1997 to 37 kg in 2000. The reason for the increase is not known; however, it may be associated with an increased incidence of infection with methicillin resistant *Staphylococcus aureus* in humans.

### Resistance in zoonotic bacteria

For *Salmonella*, comparison of resistance is complicated because of the presence and spread of resistant clones in some reservoirs and not in others. For example, while the overall prevalence of *Salmonella* Typhimurium is low in cattle, a high proportion of the isolates available for testing is penta-resistant *S. Typhimurium* DT104. Similarly, a high prevalence of resistance to quinolones (nalidixic acid) in *Salmonella* Enteritidis is caused by the spread of a particular resistant clone. In general, however, resistance levels in *Salmonella* are relatively low, in particular quinolone resistance. Among *S. Typhimurium* from pigs there was an increase in tetracycline resistance not explained by an increase in the proportion of DT104. Tetracycline resistance also increased in domestically acquired human infections. These increases may both be associated with the increase in usage of tetracycline in pigs. There is good agreement between resistance levels in *Salmonella* from food animal reservoirs and in *Salmonella* isolates from domestically acquired human cases of salmonellosis, even though resistant bacteria in imported foods also are known to contribute to resistance in humans.

Among *Campylobacter*, we found for *C. coli* in pigs, that resistance to macrolides declined following the stop for use of macrolide growth promoters. There was, however, an increase in resistance to macrolides from 1999 to 2000, coinciding with the increased use of macrolides for treatment of pigs. *C. coli* accounts for only about 5% of human cases of campylobacteriosis while *Campylobacter jejuni* is responsible for over 90%. In 2000 we have found decreasing quinolone resistance among *C. coli* from pigs. In contrast, it increased in *C. jejuni* from broilers and from cattle. Among isolates from humans this increase has occurred continuously since 1997. The high level of quinolone resistance in *C. jejuni* from domestically acquired cases of campylobacteriosis and a level of tetracycline resistance not seen in isolates from food animal reservoirs is an indication that there are reservoirs of *Campylobacter* infection not included in the DANMAP programme, probably imported poultry. The presence of quinolone resistance in about 25% of *Campylobacter* isolates is worrying because of the possible adverse implications for human health.

### Resistance in indicator bacteria

We collect faecal samples from animals at slaughter to isolate *Escherichia coli* and enterococci (*E. faecium* and *E. faecalis*) for susceptibility testing. The samples are collected so that the results provide a

measure of antimicrobial resistance in the general population of food animals and of the exposure of the food chain to resistant bacteria. We have not yet collected community samples of enterococci from humans; however, a programme for collection of such isolates is planned for implementation in the second half of 2001.

For enterococci the trends in resistance reflect rather closely changes in antimicrobial usage. The discontinued use of antimicrobial growth promoters is reflected in decreasing resistance to the antimicrobials in question. However, the complexity of the association between usage and occurrence of resistance is illustrated by our finding in 2000 that streptogramin resistance in *E. faecium* from pigs increased, even though streptogramins were not used. Detailed analyses indicated that the likely explanation was due to dissemination in the pig population of a particular multi-resistant *E. faecium* clone, which was resistant to streptogramins. The routes of spread have not been determined. The decline in macrolide resistance has also levelled out from 1999 to 2000. Part of the explanation is the spread of the resistant clone responsible for the increase in streptogramin resistance, but the increased use of macrolides for treatment may also have played a role.

Among indicator *E. coli* resistance to a number of antimicrobials has declined in recent years. For isolates from pigs this is interesting, because it means the increased use of tetracycline, in young pigs is not yet reflected in increased levels of resistance among *E. coli* in the older age groups that are sampled at slaughter. The likely explanation is the succession of types that takes place in the gut of an animal, as it grows older, so that resistant serotypes are replaced by non-resistant. This may also indicate that tetracycline resistance genes have not yet spread within the bacterial populations to a noticeable extent.

### Resistance in non-zoonotic pathogens

These bacteria all originate from diagnostic submissions. For *E. coli* from cattle (serotype F5) resistance levels have been rather stable the last 5 years. For isolates from poultry (serotypes O2 and O78) the trend has been more variable due to the relatively small number of isolates. Among isolates from pigs (serotype O149) it is interesting to see that in contrast to indicator *E. coli* from pigs at slaughter resistance to tetracycline has increased in 2000. The level of tetracycline resistance in 1999, however, was



quite low compared with previous years. Nevertheless the trend coincides well with the increased usage of tetracyclines that has taken place in this age group. We have also observed a decline in quinolone resistance in *E. coli* from pigs, which coincides with the decline in fluoroquinolone use in pigs. There is very limited use of gentamicin in Danish food animals. In spite of this, 12% of *E. coli* from young calves are resistant to gentamicin.

In *Streptococcus pneumoniae* from humans we have previously expressed concern about increasing levels of resistance to erythromycin and to penicillin. The increase in erythromycin resistance continued in 2000, although there was a small decline in penicillin resistance. Resistance among other isolates from diagnostic submissions from humans has for most part been stable over the last 5 years. We are concerned, however, about the development in incidence of infection with methicillin resistant *Staphylococcus aureus* (MRSA). In 1996 there were 34 cases, compared with 97 in 2000. Twenty to 30% of the cases were from primary care. It is not yet clear whether the increase is related to an increase in the number of imported cases or whether they are acquired domestically. Preliminary results indicate that a substantial number of the hospital cases corresponded to outbreaks.

### Conclusions

In the 5 years DANMAP has been running it has made several important contributions to our under-

standing of antimicrobial resistance. For example, we have found that even though changes can happen quickly, they can also take a long time to manifest themselves. Therefore, effects of intervention, for example discontinuation of use, introduction of a new antimicrobial or increasing use of an existing one may take several years to affect the resistance prevalence and should be evaluated over a long period of time.

We have also experienced just how important co-selection can be in affecting trends in occurrence of particular resistance phenotypes. This is seen from the continuing occurrence of, for example, chloramphenicol resistance in isolates from cattle, even though chloramphenicol has not been used in food animals for more than 20 years and from the different rates of decline in glycopeptide resistance in *E. faecium* from broilers and pigs, respectively.

As seen from this and previous DANMAP reports imported food and infections acquired abroad certainly play a role in the overall picture. While the role of imported foods is relatively modest in Denmark, where most food is produced domestically, this would not be the case for countries that import most of their food.

An area that deserves a dedicated research effort is the interaction between clonal spread of resistant bacterial strains and use of antimicrobials. The relative importance of these two factors should be quantified.

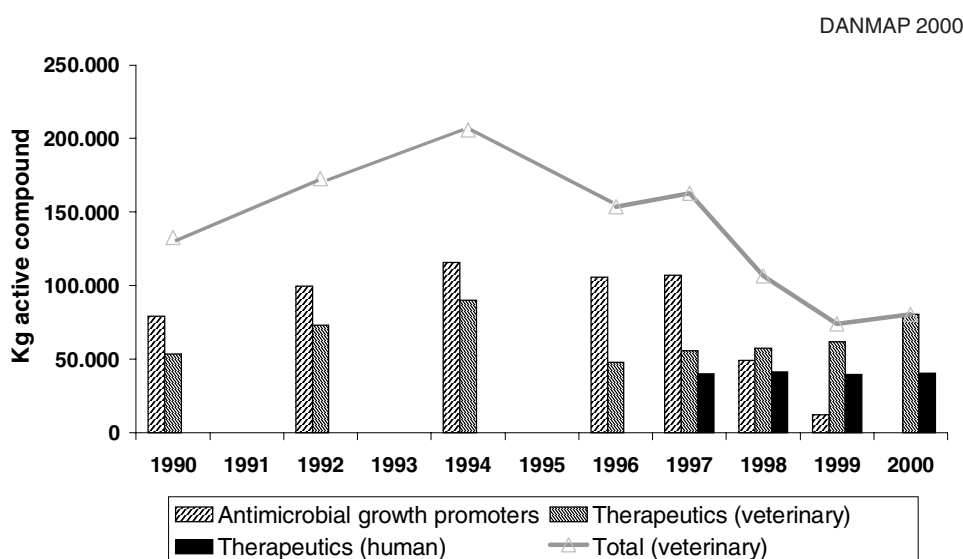


Figure 1. Trend in usage of antimicrobials for growth promotion (AGP) in food animals and therapy in food animals and humans, Denmark

## Demographic data

Table 1 shows the production of food animals. From 1999 to 2000, the number of poultry, cattle and pigs slaughtered decreased by 2.3%, 1.2% and 0.5%, respectively. The proportion of imported meat consumed has been estimated at 40% for beef, 20-

25% for poultry and 10% for pork. Table 2 shows detailed information on the distribution of the human population in counties and selected data on the health care system.

Table 1. Production of food animals and the production of meat, Denmark

DANMAP 2000

Year	Poultry		Cattle		Pigs	
	(x 1,000 heads)	Kg (millions)	(x 1,000 heads)	Kg (millions)	(x 1,000 heads)	Kg (millions)
1990	98,686	126	789	219	16,427	1,260
1992	111,536	150	862	236	18,559	1,442
1994	120,349	167	813	210	20,760	1,604
1996	111,495	165	789	198	20,530	1,592
1998	129,334	185	733	179	22,873	1,770
1999	140,116	197	700	173	22,534	1,781
2000	136,934	a)	691	a)	22,411	a)

a) Data on the production of meat in 2000 was not available

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

DANMAP 2000

County no.	County name	No. inhabitants	No. inh./sq. km	No. inh. /GP c)	No. bed-days d)	No. hospitals
		(1/1/2000)	(2000)	(1999)	(1999)	(1999)
1	Copenhagen Municipality a)	495,699	5,617	1,376	1,080,000 e)	5
2	Frederiksberg Municipality a)	90,327	10,300	1,611	-	1
3	Copenhagen County b)	613,444	1,166	1,598	606,000	3
4	Frederiksborg	365,306	271	1,650	327,000	3
5	Roskilde	231,559	260	1,677	228,000	2
6	West Zealand	295,086	99	1,579	272,000	4
7	Storstroem	259,106	76	1,540	271,000	5
8	Bornholm	44,337	75	1,310	43,000	1
9	Funen	471,974	135	1,547	537,000	10
10	South Jutland	253,482	64	1,566	226,000	4
11	Ribe	224,345	72	1,580	214,000	5
12	Vejle	347,542	116	1,595	378,000	6
13	Ringkoebing	272,857	56	1,623	259,000	5
14	Aarhus	637,122	140	1,582	694,000	9
15	Viborg	233,681	57	1,577	258,000	3
16	North Jutland	494,153	80	1,543	511,000	7
All	Denmark	5,330,020	124	1,561	5,909,000	73

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) are under the same administration.

## Consumption of antimicrobials

### Usage in food animals

We describe the usage of 3 groups of antimicrobials. Antimicrobials for **treatment** are all prescription-only medicines. Farmers may obtain them either directly from a veterinarian or by redeeming a veterinary prescription at a pharmacy. The Danish Medicines Agency collects information on sales of prescription-only medicines. These data are used in DANMAP. Antimicrobials for **growth promotion** include only those agents approved by the EU as feed additives, currently this is avilamycin and flavomycin and the ionophores (salinomycin and monensin). The distribution system for antimicrobial growth promoters is well structured and separated from that used for prescription-only medicines. Antimicrobials used as **coccidiostats** in poultry feed are also approved as feed additives in the EU. They are distributed through the same channels used for growth promoters. The Danish Plant Directorate collects statistics on sales

of growth promoters as well as coccidiostats. Please refer to Appendix 1 for details of data collection.

### Therapeutics

Table 3 shows the usage of therapeutic antimicrobials by main group and the route of administration. Table 4 shows the trend in usage between 1986 and 2000. It is apparent from Table 3 that the total usage has increased considerably from 1999 to 2000, when it reached 80,600 kg active compound with an additional 2,100 kg of sulfonamide-trimethoprim and 800 kg oxolinic acid used in aquaculture. Overall, the increase amounts to 30%, chiefly because of an increased use of oral compounds, mainly tetracyclines and macrolides/lincosamides. In 1999 the Danish Veterinary Laboratory initiated research projects to monitor the effects of withdrawing all use of antimicrobial growth promoters in pigs and poultry. The results of the projects will be available in early

Table 3. Usage of antimicrobials (kg active compound) for treatment of food animals by ATC-group and route of administration, Denmark

ATC-group Compound		DANMAP 2000									
		Oral		Injection		Intramammary		Intrauterine		Total	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
J01A	Tetracyclines	13,600	21,200	2,600	2,800	<25	<25	<25	0	16,200	24,000
J01C	Penicillins with extended spectrum	3,700	4,500	2,700	3,000	150	150	0	0	6,600	7,600
	Penicillins with narrow spectrum	0	0	14,500	14,700	150	100	50	50	14,700	14,800
J01D	Cephalosporins	0	0	50	50	<25	<25	0	0	50	50
J01E	Sulfonamides	0	0	0	0	0	0	1,000	1,000	1,000	1,000
	Sulfonamides + trimethoprim	3,400	3,500	3,400	3,500	<25	50	0	0	6,800 a)	7,000
J01F	Macrolides + lincosamides	3,400	9,200	1,900	1,800	<25	<25	0	0	5,300	11,100
J01G	Aminoglycosides	4,500	7,300	2,800	2,800	150	100	100	150	7,500	10,400
J01M	Quinolones	50	50	100	100	0	0	0	0	150 a)	150 a)
J01X	Others	3,300	4,200	300	300	<25	<25	0	0	3,600	4,500
J01	Total	32,000	50,000	28,400	29,100	500	400	1,200	1,200	61,900	80,600

a) Does not include consumption in aquaculture

Table 4. Trends in the estimated total usage of antimicrobials for treatment of food animals. Data 1986-1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses.

N. E. Rønn (Ed.). Data 1996-2000: Danish Medicines Agency

DANMAP 2000

ATC-group	Compound	1986	1988	1990	1992	1994	1996	1998	1999	2000
J01A	Tetracyclines	3,800	3,600	9,300	22,000	36,500	12,900	12,100	16,200	24,000
J01C	Penicillins with narrow spectrum	3,700	3,800	5,000	6,700	9,400	7,200	14,300	14,700	14,800
J01C/J01D	Penicillins with extended spectrum	850	1,000	1,200	2,500	4,400	5,800	6,700	6,600	7,600
J01E	Sulfonamides + trimethoprim a)	2,500	2,200	3,800	7,900	9,500	4,800	7,700	6,800	7,000
J01E	Sulfonamides	22,300	24,200	8,700	5,900	5,600	2,100	1,000	1,000	1,000
J01F	Macrolides + lincosamides b)	10,100	9,300	10,900	12,900	11,400	7,600	7,100	8,700	15,400
J01G	Aminoglycosides	7,800	7,400	7,700	8,500	8,600	7,100	7,800	7,500	10,400
J01M/J01X	Others a)	13,800	6,900	6,700	6,800	4,400	600	650	350	300
J01	Total	64,800	58,400	53,400	73,200	89,900	48,000	57,300	61,900	80,600

a) Does not include consumption in aquaculture

b) The macrolides include: spiramycin, tylosin, lincomycin and tiamulin

2002. However, analyses of diagnostic submissions and reports from veterinarians in practise indicate that some pig herds have suffered from problems caused by infections with *Lawsonia intracellularis* and it has been suggested that this may explain part of the increased usage of tetracyclines. Additionally, there appears to be some overuse.

The usage of antimicrobials should be standardised for the size of the population in which they are used. As a proxy for population we use production data. The production of meat has increased by 9.5% from 1996 to 1999, while milk production has remained stable at around 4.4 million tonnes. Since 1996, the quantities of antimicrobials given as injection and, in particular, orally have increased by a rate greater than animal production. In contrast, the quantities used for

intramammary and intrauterine administration have remained stable.

In spite of the recent increase in the use of therapeutics, the total usage of antimicrobials in Danish farm animals has declined very markedly. In total it was 206,000 kg for growth promotion and therapy in 1994 but fell to 80,900 kg in 2000 (Figure 2). The decrease started with the ban on avoparcin in May 1995 and simultaneously, but not directly related, the issue of regulations restricting veterinarians' sale of antibiotics to farmers and the use of antibiotics for prophylaxis.

In 2000, a total of 63 kg of cephalosporins were used in animals with 16 kg of active compound as intramammary injections and 47 kg for parenteral

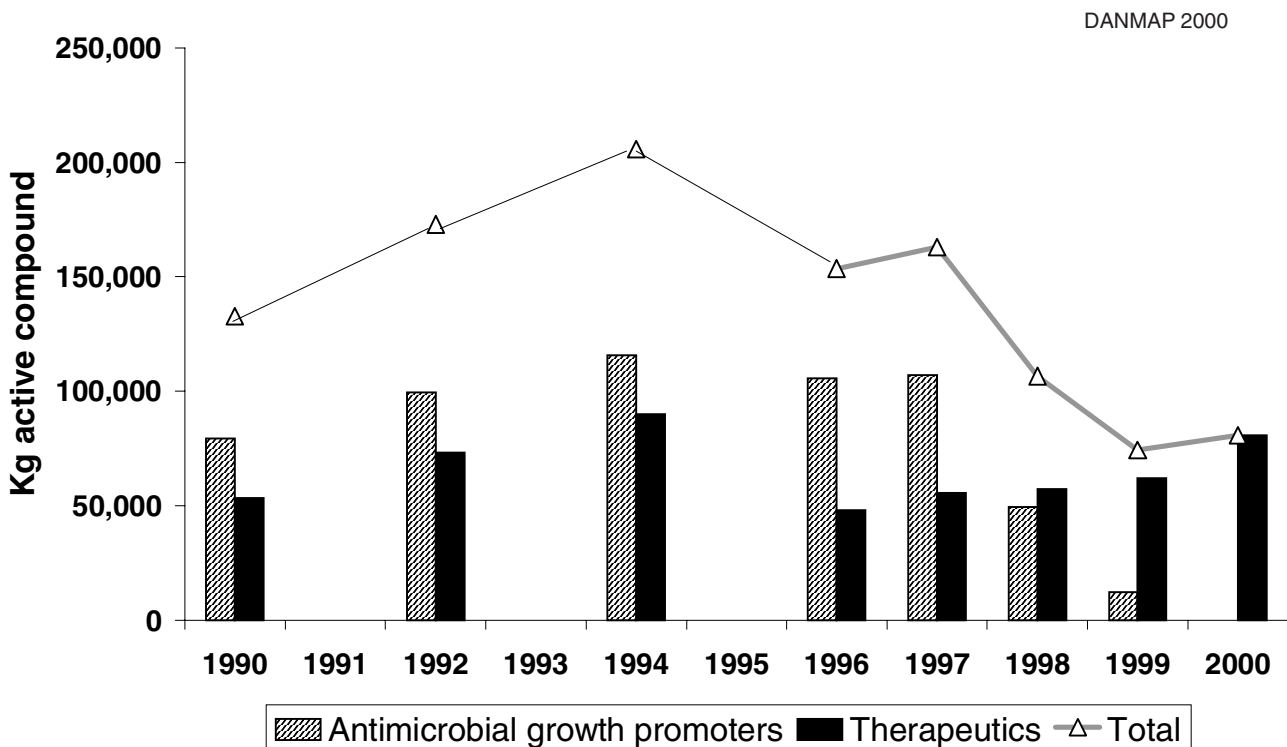


Figure 2. Trend in usage of antimicrobials for growth promotion (AGP) and therapy in food animals, Denmark. Use of coccidiostats is not included

injection. This represents an increase compared with 1999, where the total usage was 57 kg.

In 1999 we reported a dramatic decline in the usage of fluoroquinolones due to the withdrawal of an oral formulation used in pigs. While the usage has remained low in 2000 compared to 1998, there was nevertheless an increase of 14% compared with 1999. The textbox on page 14 provides a more detailed description of the trend in usage of fluoroquinolones.

### Growth promoters and coccidiostats

While four antimicrobial agents are still permitted for use as growth promoters within the EU, Danish farming organisations decided in 1998 on a voluntary

stop for all use of antimicrobial growth promoters (AGPs). This stop became complete in 1999, when AGPs were phased out in weaned pigs. Accordingly, the usage of antimicrobials as growth promoters in 2000 in Danish food animals was zero (Table 5).

Table 6 shows the usage of coccidiostats. Coccidia are intestinal parasites causing diarrhoea. They are a particular problem in poultry and almost all broiler feed contains coccidiostats to control the problem. The usage of coccidiostats declined by almost 9,500 kg in 2000 and while poultry production also declined the magnitude of decline cannot explain the decreased usage of coccidiostats. We have not been able to determine the cause of the decrease.

Table 5. Usage of antimicrobial growth promoters (kg active compound), Denmark

DANMAP 2000

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

Table 6. Usage of coccidiostats in poultry (kg active compound), Denmark

DANMAP 2000

Coccidiostats	1990	1992	1994	1996	1998	1999	2000
Amprolium/Ethopabate	3,562	2,716	2,342	1,339	275	839	-
Dimetridazole	-	-	-	38	-	106	-
DOT	-	-	300	-	-	13	-
Monensin	-	108	1,016	3,405	3,709	8,664	3,962
Robenidine	33	295	858	293	367	85	-
Metichlorpindol/							
Methylbenzoat	89	1,503	3,360	4,857	930	155	-
Lasalocid	75	-	5	773	1,677	895	606
Halofuginone	-	-	19	8	-	2	-
Narasin	1,588	5,157	6,370	3,905	3,177	5,806	5,073
Salinomycin	7,783	10,298	6,018	4,531	7,884	8,812	6,338
Nicarbazine	-	-	-	115	36	4	-
Narasin/Nicarbazine	-	-	-	-	-	32	20
Nifursol	-	395	-	146	234	79	-
Diclazuril	-	-	18	34	3	1	-
<b>Total</b>	<b>13,569</b>	<b>20,472</b>	<b>20,306</b>	<b>19,444</b>	<b>18,292</b>	<b>25,493</b>	<b>15,999</b>

## Trend in usage of fluoroquinolones

Fluoroquinolones were approved for use in animals in Denmark in 1993. However, prior to granting marketing permission, there had been some sales on the basis of temporary permits. The products have been marketed as formulations for injection, as premixes for use in pigs and as liquid formulations for use mainly in poultry and to a lesser extent in calves.

The guidelines for prudent use of antibiotics formulated by the Danish Veterinary Laboratory in consultation with veterinarians and other experts, recognise that on the basis of resistance patterns, fluoroquinolones should not be first-choice agents for treatment of infections in Danish farm animals. In late 1998 the Danish Veterinary and Food Administration issued a recommendation to all veterinarians to restrain their prescription of fluoroquinolones. Early 1999, a major pharmaceutical company withdrew a widely used premix for pigs. The company's move was motivated by a concern about development of resistance and by a wish to safeguard the future use of fluoroquinolones in food animals. The Veterinary and Food Administration is considering legislation to further restrict the use of fluoroquinolones to situations where susceptibility testing shows they are necessary.

The effect of these initiatives is shown on Figure 3. The usage declined from just over 400 kg active compound in 1998 to under 150 kg in 1999. In addition to the premix withdrawn by the company, the decline also affected the oral formulation for poultry and the injectables. This indicates that the recommendations about prudent use of fluoroquinolones may have had an effect on usage. The decreasing use in pigs has been accompanied in a decline in quinolones resistance among *E. coli* O149 isolated from diarrhoea in young pigs. There was an increase in fluoroquinolone usage in 2000 compared with 1999, but the total in the usage is still significantly lower than before the intervention. Some of the oral formulations for poultry may have been used in calves. The decline in fluoroquinolone usage has not been associated with an increase in the usage of cephalosporins, another group of potent broad-spectrum antimicrobials.

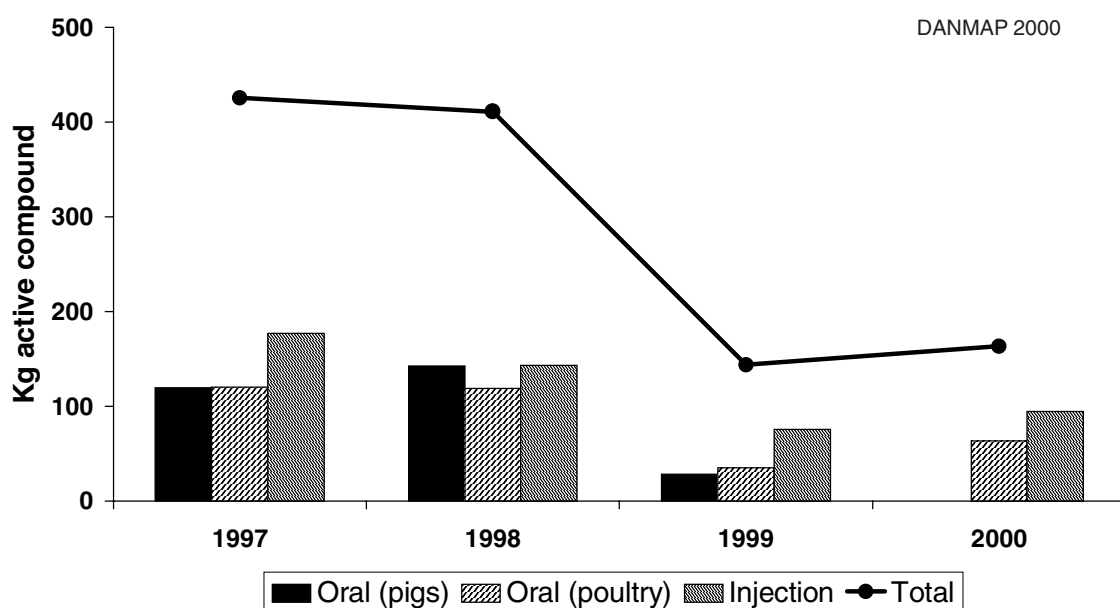


Figure 3. Trend in usage of fluoroquinolones in food animals, Denmark

## Consumption in humans

### Overall

In 2000, overall consumption of antibacterials for systemic use (ATC Group J01, 2001 definition) in humans in Denmark amounted to 26.5 million DDDs

or 13.6 DDD/1,000 inhabitant-days. To allow comparison with consumption in food animals, we also calculated consumption in kg of active compounds (Table 7). In 2000, approximately 40 tonnes of antibacterials were used in humans in Denmark.

*Table 7. 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 8 and 9). 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 a number of kilograms of active compound.*

		DANMAP 2000			
ATC group a)	Therapeutic group	Year			
		1997	1998	1999	2000 (low - high estimates) b)
J01AA	Tetracyclines	1,692	1,692	1,590	1,701
J01B	Amphenicols	1	1	0	1
J01CA	Penicillins with extended spectrum	5,513	5,467	5,181	5,135
J01CE	Beta-lactamase sensitive penicillins	18,813	19,947	18,790	19,782
J01CF	Beta-lactamase resistant penicillins	1,913	2,115	2,416	2,654
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	48	55	51	51
J01D	Cephalosporins and related substances	660	657	685	727 (343-1,111)
J01EA	Trimethoprim and derivatives	245	256	258	263
J01EB	Short-acting sulfonamides	3,498	3,493	3,289	3,148
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	337	322	279	285
J01FA	Macrolides	4,227	4,536	4,147	4,040 (2,909-5,172)
J01FF	Lincosamides	28	38	33	33 (27-40)
J01G	Aminoglycosides	32	31	32	32
J01MA	Fluoroquinolones	320	343	321	290 (206-375)
J01MB	Other quinolones	15	17	16	0
J01XA	Glycopeptides	25	27	32	37
J01XC	Steroid antibacterials (fusidic acid)	74	73	78	70
J01XD	Imidazoles	128	127	140	154
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	137	141	141	149
J01XX	Other antibacterials (methenamine)	2,233	2,132	1,956	1,792 (1,434-2,150)
J01	Antibacterials for systemic use (Total) c)	39,938	41,469	39,436	40,344 (38,379-42,309)

a) From the 2001 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 2000, extremes values, i.e. estimations using the lowest and the highest DDD, are given in parentheses

c) Does not include polymyxins

*Table 8. Consumption of antibacterials for systemic use in human primary health care (DDD/1,000 inhabitant-days), Denmark*

		DANMAP 2000				
ATC group a)	Therapeutic group	Year				% Change (1999-2000)
		1997	1998	1999	2000	
J01AA	Tetracyclines	0.98	0.98	0.93	0.98	+ 6
J01CA	Penicillins with extended spectrum	2.39	2.39	2.29	2.30	0
J01CE	Beta-lactamase sensitive penicillins	4.57	4.81	4.48	4.70	+ 5
J01CF	Beta-lactamase resistant penicillins	0.34	0.40	0.48	0.52	+ 10
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.02	0.03	0.02	0.02	0
J01DA	Cephalosporins and related substances	0.02	0.03	0.02	0.02	+ 5
J01EA	Trimethoprim and derivatives	0.30	0.32	0.32	0.33	+ 2
J01EB	Short-acting sulfonamides	0.41	0.41	0.38	0.37	- 5
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.08	0.04	0.03	0.03	+ 5
J01FA	Macrolides	2.03	2.28	2.17	2.02	- 7
J01FF	Lincosamides	0.01	0.01	0.01	0.01	- 5
J01MA	Fluoroquinolones	0.22	0.23	0.20	0.15	- 25
J01XB	Polymyxins	0.03	0.02	0.03	0.03	- 3
J01XC	Steroid antibacterials (fusidic acid)	0.02	0.02	0.02	0.02	- 10
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.35	0.36	0.36	0.38	+ 5
J01XX	Other antibacterials (methenamine)	0.46	0.43	0.40	0.36	- 9
J01	Antibacterials for systemic use (Total)	12.24	12.76	12.14	12.25	+ 1

a) From the 2001 edition of the Anatomical Therapeutic Chemical (ATC) classification system

**Primary health care sector**

Table 8 presents the consumption of antibacterials for systemic use in primary health care from 1997 to 2000 calculated using the 2001 update of the ATC classification. This update of the classification now includes antibacterials such as nitrofurantoin and methenamine among group J01 and the data presented in this report are not comparable with the data presented in the DANMAP 99 report. In 2000, beta-lactamase sensitive penicillins (ATC Group J01CE, e.g. penicillin G and V) represented almost 40% of the total antimicrobial use in the Danish primary health care sector. Other frequently used antimicrobials were penicillins with extended spectrum (mostly amoxicillin, pivampicillin and pivmecillinam) and macrolides, representing approximately 19% and 16%, respectively. In

comparison, fluoroquinolones, combinations of penicillins including beta-lactamase inhibitors (essentially amoxicillin+clavulanate) and cephalosporins only represented 1.2%, 0.2% and 0.2% of the total consumption in primary health care, respectively.

Total consumption in primary health care did not change markedly between 1999 and 2000, although small increases or decreases were observed within specific classes. The largest relative decrease was observed for fluoroquinolones. Figure 4 shows that consumption of fluoroquinolones in primary health care steadily decreased after removal of their subsidisation in May 1999. This decrease stopped and consumption started to increase again when this economical disincentive, which specifically addressed

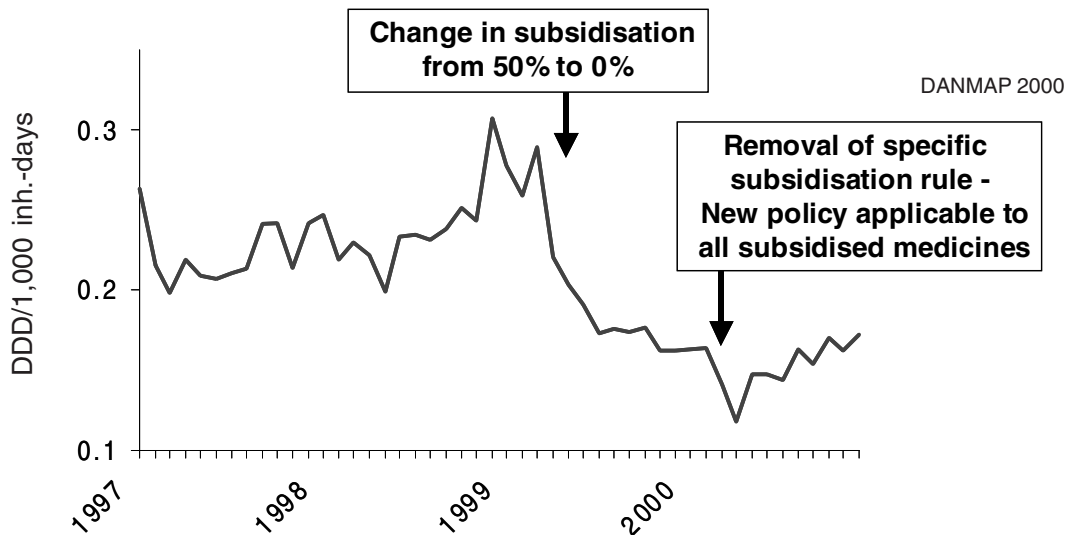


Figure 4. Monthly consumption of fluoroquinolones (J01MA) in primary health care, Denmark, January 1997-December 2000

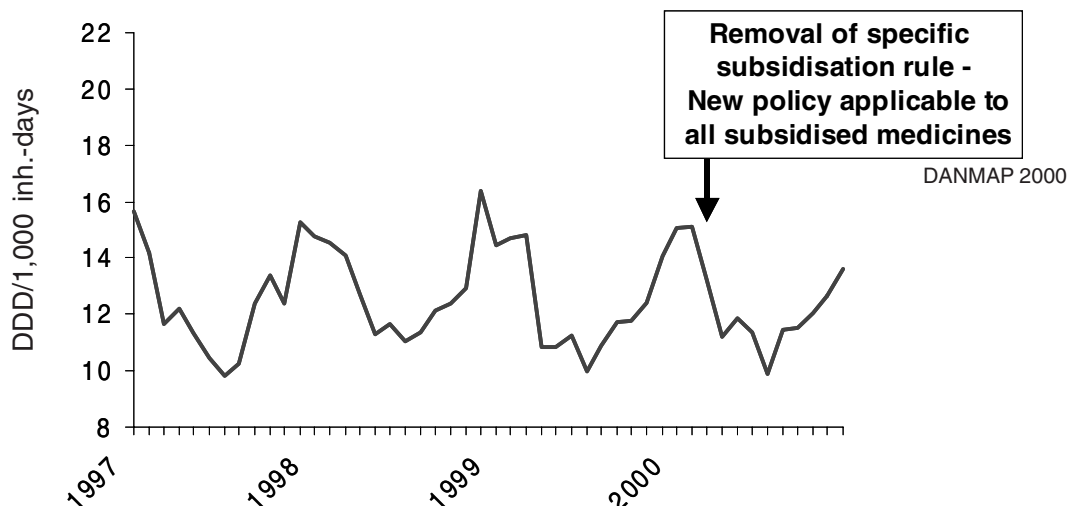


Figure 5. Monthly consumption of antibacterials for systemic use (J01, 2001 definition) in primary health care, Denmark, January 1997-December 2000



fluoroquinolones and certain other antimicrobials, was removed and replaced by a new policy applicable to all subsidised medicines. Starting 1<sup>st</sup> March 2000, adults must have purchased 500 DKK worth of prescription medicines during the past year before the insurance system starts subsidising 50% of the price. This rule does not apply to children below 18 years of age for whom medicines systematically receive 50% subsidisation. For both adults and children, 75% subsidisation applies to annual purchases from 1,200 to 2,800 DKK and 85% subsidisation applies for persons purchasing over 2,800 DKK worth of medicines within a 12-month period. At the present time, it appears that this new policy has had no impact on total consumption of antibacterials in primary health care (Figure 5).

Although the amounts of antimicrobial use in primary health care in Denmark are still one of the lowest in the world, differences remain among counties. With less than 11 DDD/1,000 inhabitant-days in 2000, Aarhus and Bornholm counties had the lowest consumption while 6 other counties used more than 13 DDD/1,000 inhabitant-days (Figure 6). Although there have been variations in the level of antimicrobial use in primary health care since 1994, the ranking of Danish counties according to their antimicrobial use has remained much the same and there is presently no explanation for these differences.

**Hospitals**

Table 9 presents the consumption of antibacterials for systemic use in hospitals from 1997 to 2000. Total consumption in hospitals was 428 DDD/1,000 bed-

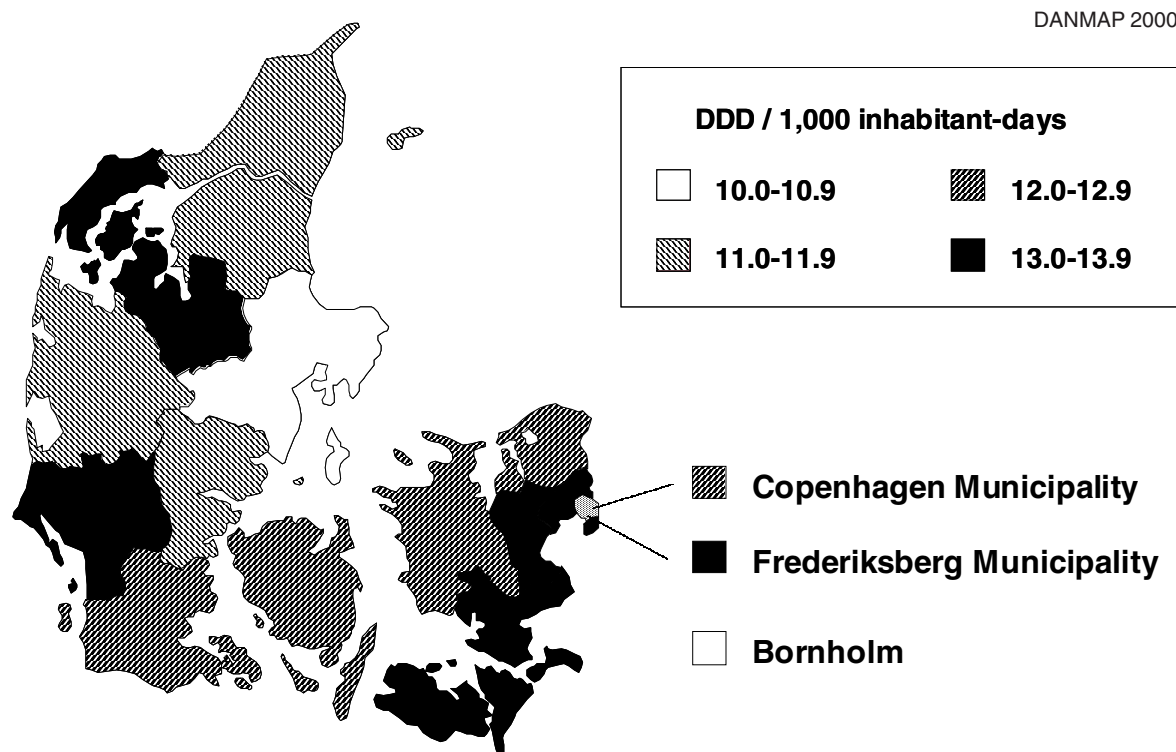


Figure 6. Consumption of antibacterials for systemic use (J01, 2001 definition) in human primary health care by county, Denmark

days in 1999 and estimated at approximately 450 DDD/1,000 bed-days in 2000. These data represent a first attempt to express consumption in Danish hospitals using a measurement unit recommended by WHO to control for variations in hospital activity, namely DDD per 1,000 bed-days. The number of DDD in each hospital may comprise small amounts of antibacterials given to outpatients, whereas the 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 show that antimicrobial consumption in Danish hospitals is among the lowest.

Although the prescription of antimicrobials in Danish hospitals can be described as conservative, there has been a regular increase in hospital antimicrobial consumption overall and for specific classes including cephalosporins, fluoroquinolones and glycopeptides

(Table 9). This increase was mainly due to a 7% increase in the number of DDDs of antimicrobials dispensed by hospital pharmacies (numerator) from approximately 2.3 million DDDs in 1997 to 2.5 million DDDs in 1999, while there was only a 2% decrease in the total number of hospital bed-days registered in Denmark (denominator) between 1997 to 1999. However, the average length of stay in Danish hospitals decreased from 5.7 days in 1997 to 5.4 days in 1999. An explanation for the increase in hospital antimicrobial consumption could therefore be that, as a result of this policy of earlier discharge, bed-days registered by hospitals now originate from sicker patients, possibly with more serious infections. It could also be that, at the time of discharge, patients receive antimicrobials for use at home, which we registered under hospital use. Finally, it could be that to expedite discharges patients now receive more intensive treatment. Nevertheless, this increase is of concern and deserves close monitoring in the future.

Table 9. Consumption of antibacterials for systemic use (DDD/1,000 bed-days) in 63 public hospitals or groups of hospitals which represent more than 95% of the total DDDs used in hospitals in Denmark. Psychiatric hospitals, private hospitals and one rehabilitation center were excluded

ATC group a)	Therapeutic group	Year				DANMAP 2000
		1997	1998	1999	2000 b)	Estimated % change (1999-2000)
J01AA	Tetracyclines	3.3	3.2	2.7	2.8	+ 4
J01CA	Penicillins with extended spectrum	108.6	109.6	108.1	112.5	+ 4
J01CE	Beta-lactamase sensitive penicillins	77.2	85.9	91.7	98.4	+ 7
J01CF	Beta-lactamase resistant penicillins	43.2	44.4	46.8	52.4	+ 12
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.3	0.4	0.4	0.9	+ 125
J01DA	Cephalosporins	44.7	46.5	49.9	53.7	+ 8
J01DF	Monobactams	0.6	0.1	0.1	0.2	+ 100
J01DH	Carbapenems	3.6	2.4	3.2	3.9	+ 22
J01EA	Trimethoprim and derivatives	4.0	4.2	3.6	3.6	0
J01EB	Short-acting sulfonamides	12.4	12.7	12.3	12.0	- 2
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	4.4	12.9	13.6	13.9	+ 3
J01FA	Macrolides	33.7	34.0	32.1	32.0	0
J01FF	Lincosamides	1.3	1.8	1.4	1.6	+ 14
J01GB	Aminoglycosides	19.5	19.5	20.2	21.0	+ 4
J01MA	Fluoroquinolones	14.3	15.1	18.4	22.7	+ 23
J01XA	Glycopeptides	2.1	2.3	2.8	3.2	+ 14
J01XB	Polymyxins	0.4	0.2	0.3	0.4	+ 33
J01XC	Steroid antibacterials (fusidic acid)	2.5	2.4	2.6	2.3	- 12
J01XD	Imidazoles	13.9	14.0	15.8	17.6	+ 11
J01XE	Nitrofurantoin derivatives (nitrofurantoin)	0.6	0.5	0.3	0.4	+ 33
J01XX	Other antibacterials (methenamine)	1.7	1.8	1.5	1.4	- 7
J01	Antibacterials for systemic use (Total)	392.4	413.9	428.0	456.9	+ 7

a) From the 2001 edition of the Anatomical Therapeutic Chemical (ATC) classification system

b) Estimated consumption using the exact number of DDDs and an estimate of the number of bed-days in 2000 based on 1999 data and preliminary data available for the first 6 months of 2000

In 2000, all penicillins combined represented approximately 60% of hospital antimicrobial use in Denmark. Cephalosporins (mainly cefuroxime) and fluoroquinolones (mainly ciprofloxacin) only represented 11.7% and 5% of total hospital use, respectively. Tetracyclines, combinations of penicillins incl. beta-lactamase inhibitors, carbapenems and glycopeptides each represented less than 1% of total hospital use. Figure 7 presents the distribution of consumption in Danish hospitals. Consumption of macrolides varied the most among hospitals.

Additionally, some hospitals were obviously outliers regarding their consumption of fluoroquinolones, combinations of penicillins incl. beta-lactamase inhibitors, carbapenems, glycopeptides, but also beta-lactamase sensitive penicillins. These outliers were generally, but not always, major university hospitals to which very sick patients are referred. This analysis stresses the importance of controlling for patient case-mix before attempting to benchmark hospitals or hospitalisation units.

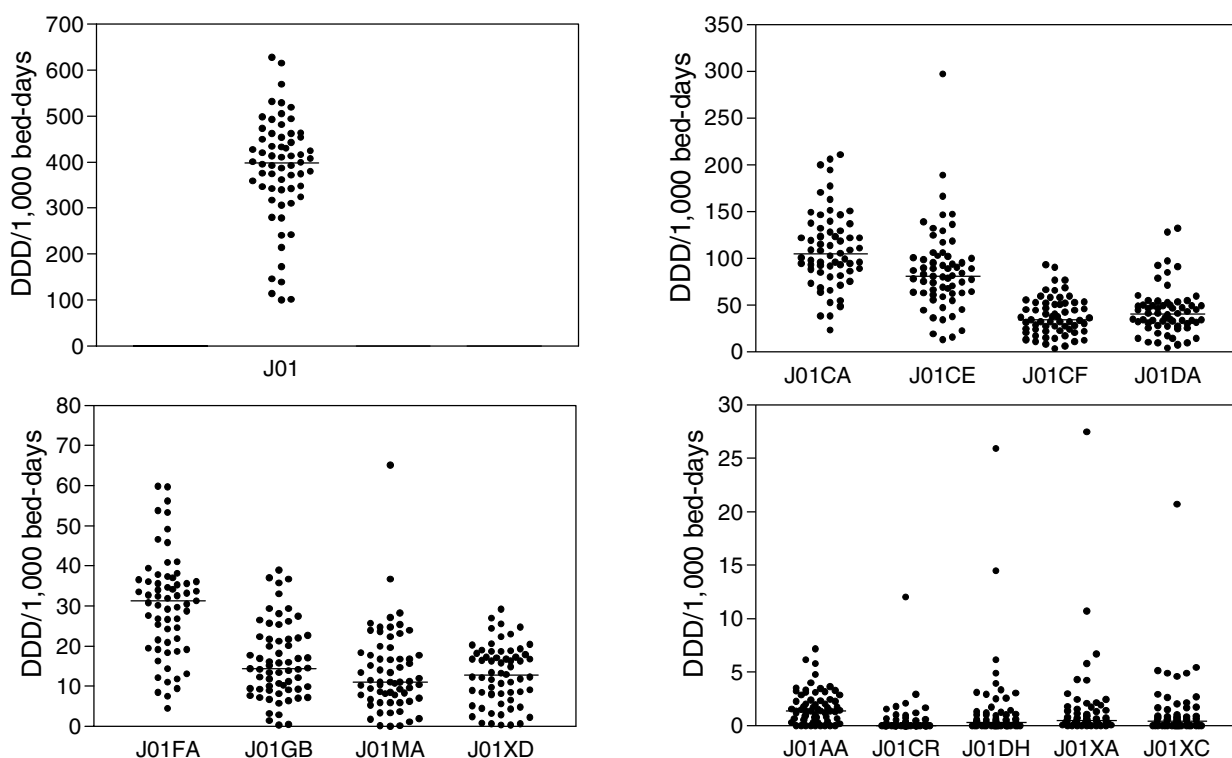


Figure 7. Distribution of consumption of antibacterials for systemic use among 63 hospitals or groups of hospitals, Denmark, 1999. Consumption was not available separately for some hospitals belonging to a group. Psychiatric hospitals, private hospitals and one rehabilitation center were excluded. Each dot represents one hospital. The horizontal bar represents the median

Legend for ATC codes:

- |   |  |
|---|--|
| J01, Antibacterials for systemic use                                | J01DH, Carbapenems                           |
| J01AA, Tetracyclines  | J01FA, Macrolides                            |
| J01CA, Penicillins with extended spectrum                           | J01GB, Aminoglycosides                       |
| J01CE, Beta-lactamase sensitive penicillins                         | J01MA, Fluoroquinolones                      |
| J01CF, Beta-lactamase resistant penicillins                         | J01XA, Glycopeptides                         |
| J01CR, Combinations of penicillins, incl. beta-lactamase inhibitors | J01XC, Steroid antibacterials (fusidic acid) |
| J01DA, Cephalosporins   | J01XD, Imidazoles                            |

## New method to investigate the effect of interventions and examine the relationship between antimicrobial use and antimicrobial resistance

Since the beginning of DANMAP, the effect of interventions to control antimicrobial use and the subsequent effect on resistance have been studied by comparing yearly levels of both indicators. In 2000, we started using time series analysis for this purpose. Time series are series of data collected over time and at much shorter intervals than the study period, i.e. months or weeks rather than years or quarters. For example, examination of the monthly consumption of fluoroquinolones in primary health care allowed us to show a reduction of consumption following removal of subsidisation of these antimicrobials in May 1999 and a slow increase in consumption following interruption of this economical incentive specifically directed at fluoroquinolones and its replacement by a new subsidisation policy affecting all subsidised medicines in March 2000 (Figure 4, page 16). Specific intervention models are presently being developed using this method which is presented by the Cochrane Effective Practice and Organisation of Care Group (EPOC) as one of the very few reliable tools to investigate the effect of interventions in health care organisations ([http://www.abdn.ac.uk/public\\_health/hsru/epoc/](http://www.abdn.ac.uk/public_health/hsru/epoc/)).

Additionally, such time series can also be used to compare consumption and resistance data. For example, the percentage of tetracycline-resistant *Staphylococcus aureus* from blood samples followed variations of the number prescriptions of tetracyclines in human primary health care, including the sharp decrease consecutive to the cessation of the subsidisation of tetracyclines in January 1996 (Figure 8). Similarly, we found a relationship between the monthly consumption of azithromycin in human primary health care and erythromycin resistance among *Streptococcus pneumoniae* isolates from sterile body sites (Figure 9). Examination of the series is essential but not sufficient to demonstrate a relationship and specific transfer function models are being developed for this purpose following an innovative method which has recently been described by López-Lozano and colleagues for the Spanish project, ViResiST (López-Lozano JM, et al. Int J

Antimicrob Agents 2000;14:21-3; Monnet DL & López-Lozano JM. Clin Microbiol Infect, in press).

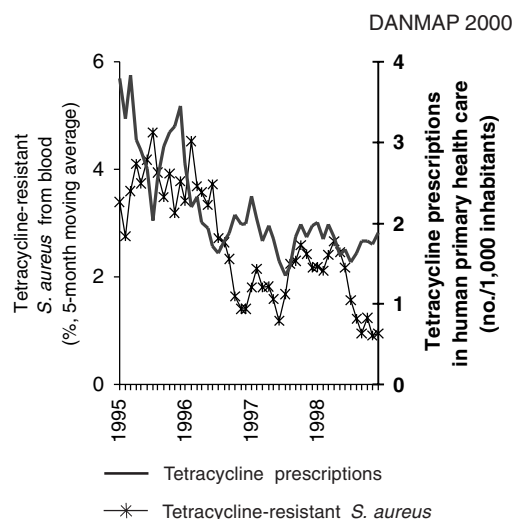


Figure 8. Monthly percent tetracycline-resistant *Staphylococcus aureus* and monthly prescriptions of tetracyclines (J01AA) in primary health care, Denmark, January 1995-December 1998

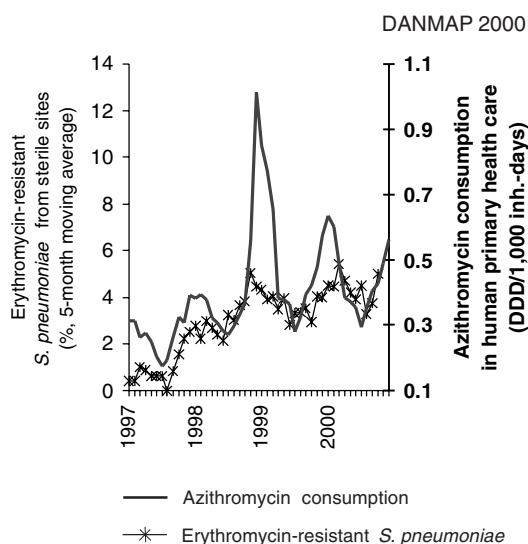


Figure 9. Monthly percent erythromycin-resistant *Streptococcus pneumoniae* and monthly azithromycin consumption (J01FA10) in primary health care, Denmark, January 1997-December 2000

## Resistance in zoonotic bacteria

### Salmonella

#### Salmonella from food animals

Tables 10, 11 and 12 show the *Salmonella* serotype and phage type distributions of isolates from food animals. *Salmonella* isolates from pigs and poultry were mainly from subclinical infections, while the majority of isolates from cattle were from clinical cases of salmonellosis. Only 1 isolate per herd or flock was included in this report. Because *Salmonella* is more frequently isolated from pigs than cattle or poultry, the sample from pigs was randomly chosen from among all *Salmonella* pig isolates that were serotyped at the Danish Veterinary Laboratory. In

Table 10. Distribution (%) of *Salmonella* serotypes isolated from animals and humans among the isolates selected for susceptibility testing, Denmark

DANMAP 2000				
Serotypes	Poultry	Cattle	Pigs	Humans
Agona	1		1	3
Enteritidis	27	<1	<1	52
Hadar	5	<1		3
Infantis	11		5	1
Newport				1
Senftenberg	2		<1	1
Thompson	1			2
Typhimurium	14	45	64	19
Virchow				2
Others incl. not typable	39	53	29	17
Number of isolates	100	117	340	2,343 a)

a) Isolates from a cruise ship outbreak are included

Table 11. Distribution (%) of *Salmonella* Typhimurium phages types from animals and humans among the isolates selected for susceptibility testing, Denmark

DANMAP 2000				
Phage type	Poultry	Cattle	Pigs	Humans
1				7
12	43	33	44	20
66	14	2	10	5
104/104b		35	6	11
120		2	2	8
135			1	3
170	7	8	6	4
193		4	7	4
U292		2		4
U302			<1	4
Others incl. not typable	36	15	23	31
Number of isolates	14	52	215	421

contrast, the *Salmonella* samples from cattle and poultry consisted of 1 isolate from each herd or flock where *Salmonella* was detected. *Salmonella* Typhimurium DT104 accounted for a large proportion of isolates from cattle herds (Table 11). This resulted from trace-back efforts, which occur whenever DT104 is detected in a herd. Therefore, the data in Table 11 overestimate the *S. Typhimurium* DT104 prevalence relative to other serotypes among Danish cattle herds.

Table 13 shows the occurrence of resistance among all *Salmonella* serotypes from animals in 2000. Table 14 shows the resistance in *Salmonella* Enteritidis from broilers and layers and *S. Typhimurium* from broilers, layers, cattle and pigs. Similar to 1999, none of the *Salmonella* isolates studied in 2000 were resistant to ciprofloxacin. Four (15%) of 27 *S. Enteritidis* isolates were resistant to nalidixic acid in 2000 compared with 8 (20%) of 40 isolates in 1999. All isolates resistant to nalidixic acid originated from the table egg sector with all farms of origin receiving day old chickens from the same hatchery. The high prevalence of nalidixic acid resistance among *S. Enteritidis* was therefore most likely due to a clonal spread caused by trade with day old chickens harbouring nalidixic acid resistant stains. In total, 15 (3%) of 557 *Salmonella* isolates from food animals (all serotypes) were resistant to nalidixic acid. All 15 isolates had reduced susceptibility to ciprofloxacin (4, 6 and 5 isolates had ciprofloxacin MIC values of 0.12, 0.25 and 0.5, respectively).

Table 12. Distribution (%) of *Salmonella* Enteritidis phages types from animals and humans among the isolates selected for susceptibility testing, Denmark

DANMAP 2000		
Phage type	Poultry	Humans
1b	23	<1
1		10
2	5	<1
4	9	23
6	5	9
6a		2
6b	5	<1
8	41	22
13a	5	2
21	4	4
34		11
Others incl. not typable	5	16
Number of isolates	22	810

Table 13. Susceptibility and occurrence of resistance (%) among *Salmonella enterica* from food animals, Denmark

ATC-group	Compound	<i>Salmonella enterica</i>										DANMAP 2000	
		Poultry					Cattle						Pigs
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	2 - >32	2	2	4	2 - >32	2	>32	25	2 - >32	2	>32	22
J01B	Chloramphenicol	2 - 8	8	8	0	2 - >64	4	>64	15	2 - >64	4	8	6
	Florfenicol	2 - 8	4	4	0	2 - 64	4	32	15	2 - 32	4	8	3
J01C	Ampicillin	1 - >32	1	2	3	1 - >32	1	>32	16	1 - >32	1	2	9
	Ceftiofur	0.5 - 1	1	1	0	0.5 - 2	0.5	1	0	0.5 - 2	0.5	1	0
J01E	Sulfonamid	32 - >512	64	128	5	32 - >512	32	>512	24	32 - >512	32	>512	23
	Trimethoprim	4 - 4	4	4	0	4 - >32	4	4	3	4 - >32	4	4	6
J01G	Apramycin	4 - 8	4	4	0	4 - 8	4	4	0	4 - >64	4	4	0
	Gentamicin	1 - 1	1	1	0	1 - 2	1	1	0	1 - 16	1	1	1
	Neomycin	2 - >32	2	2	2	2 - >32	2	2	1	2 - >32	2	2	7
	Spectinomycin	16 - 64	32	32	0	16 - >128	32	>128	15	16 - >128	32	>128	14
	Streptomycin	4 - >64	8	16	7	4 - >64	8	>64	25	4 - >64	8	>64	20
J01M	Ciprofloxacin	0.03 - 0.5	0.03	0.06	0	0.03 - 0.25	0.03	0.03	0	0.03 - 0.25	0.03	0.03	0
	Nalidixic acid	4 - >128	4	16	10	4 - >128	4	4	2	4 - >128	4	4	1
J01X	Colistin	4 - 8	4	4	0	4 - 8	4	4	0	4 - 4	4	4	0
Number of isolates		100					117					340	

Table 14. Susceptibility and occurrence of resistance among *Salmonella Enteritidis* and *Salmonella Typhimurium* from food animals, Denmark

ATC-group	Compound	<i>S. Enteritidis</i>										<i>S. Typhimurium</i>										DANMAP 2000
		Poultry					Cattle					Poultry					Pigs					
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	
J01A	Tetracycline	2 - >32	2	2	7	2 - >32	2	>32	43	2 - >32	2	>32	25	2 - >32	2	>32	25	2 - >32	2	>32	25	
J01B	Chloramphenicol	2 - 8	8	8	0	2 - >64	8	>64	32	2 - >64	8	>64	9	2 - >64	4	8	9	2 - >64	4	8	9	
	Florfenicol	2 - 4	4	4	0	2 - 64	4	32	32	2 - 64	4	32	5	2 - 32	4	8	5	2 - 32	4	8	5	
J01C	Ampicillin	1 - 2	2	0	2	1 - >32	1	>32	36	1 - >32	1	>32	12	1 - >32	1	>32	12	1 - >32	1	>32	12	
J01D	Ceftiofur	0.5 - 1	1	1	0	0.5 - 1	0.5	1	0	0.5 - 1	0.5	1	0	0.5 - 2	0.5	1	0	0.5 - 2	0.5	1	0	
J01E	Sulfonamid	32 - >512	64	128	4	32 - >512	64	>512	47	32 - >512	64	>512	29	32 - >512	32	>512	29	32 - >512	32	>512	29	
	Trimethoprim	4 - 4	4	4	0	4 - 4	4	4	0	4 - 4	4	4	7	4 - >32	4	4	7	4 - >32	4	4	7	
J01G	Apramycin	4 - 4	4	4	0	4 - 4	4	4	0	4 - 8	4	4	0	4 - 8	4	4	0	4 - 8	4	4	0	
	Gentamicin	1 - 1	1	1	0	1 - 2	1	1	0	1 - 2	1	1	0	1 - 2	1	1	0	1 - 2	1	1	0	
	Neomycin	2 - >32	2	2	2	2 - 2	2	2	2	2 - 2	2	2	9	2 - >32	2	2	9	2 - >32	2	2	9	
	Spectinomycin	16 - 32	32	32	0	16 - >128	32	>128	32	16 - >128	32	>128	14	16 - >128	32	>128	14	16 - >128	32	>128	14	
	Streptomycin	4 - 8	4	4	0	8 - >64	16	>64	43	8 - >64	8	>64	23	4 - >64	8	>64	23	4 - >64	8	>64	23	
J01M	Ciprofloxacin	0.03 - 0.5	0.03	0.5	0	0.03 - 0.03	0.03	0.03	0	0.03 - 0.03	0.03	0.03	0	0.03 - 0.25	0.03	0.03	0	0.03 - 0.25	0.03	0.03	0	
	Nalidixic acid	4 - >128	4	>128	15	4 - 4	4	4	0	4 - 8	4	4	1	4 - >128	4	4	1	4 - >128	4	4	1	
J01X	Colistin	4 - 4	4	4	0	4 - 4	4	4	0	4 - 4	4	4	0	4 - 4	4	4	0	4 - 4	4	4	0	
Number of isolates		27					14					53					216					

Figure 10 presents the level of resistance to selected antimicrobials from 1996 to 2000. From 1996 to 1999, the level of tetracycline resistance in *S. Typhimurium* isolates from pigs was almost constant. A significant increase in tetracycline resistance from 14% to 25% occurred between 1999 and 2000, which was concomitant to a pronounced increase in tetracycline consumption (Table 4, page 11). From 1996 to 2000, there was a trend towards increased resistance to tetracycline among *S. Typhimurium* and *S. Enteritidis* isolates from poultry. During the same period resistance to sulfonamide decreased significantly among *S. Typhimurium* isolates from poultry.

**Salmonella from humans**

In 2000, 2,308 human infections with zoonotic *Salmonella* serotypes were registered in Denmark. Between

1999 and 2000, the rate of *Salmonella* infections decreased from 62 to 43 cases per 100,000 inhabitants. The serotype and phage type distributions for *S. Enteritidis* and *S. Typhimurium* are shown in Tables 10, 11 and 12, respectively.

Antimicrobial resistance among human *S. Enteritidis* isolates remained low in 2000 with the exception of quinolones and to a lesser extent ampicillin (Table 15). Resistance was generally observed more frequently in isolates acquired abroad than in domestic isolates. For example in 2000, resistance to quinolones was 18.4% in isolates from cases known to have been acquired abroad compared with only 4.5% in isolates from domestic cases. The increasing quinolone resistance in domestically acquired *S. Enteritidis* is likely to be associated with

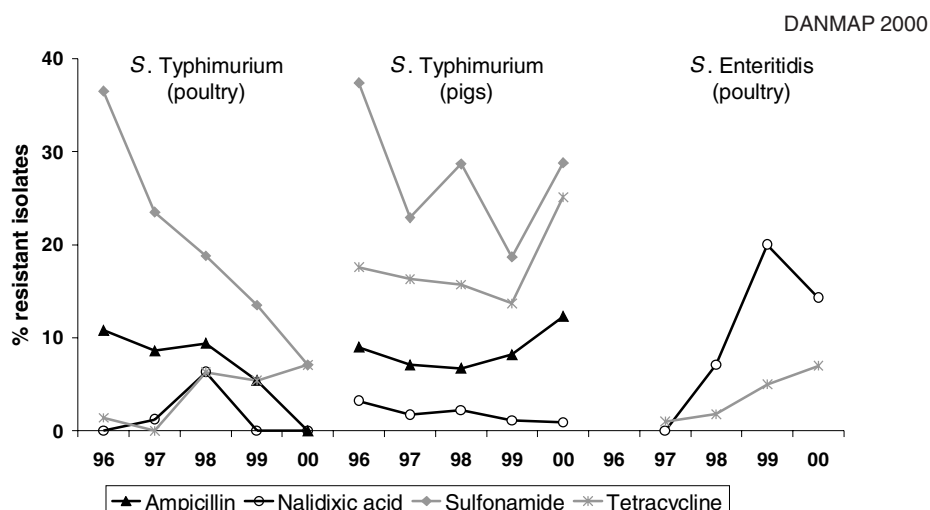


Figure 10. Trends in resistance to selected antimicrobials among Salmonella Typhimurium from poultry and pigs and Salmonella Enteritidis from poultry, Denmark

Table 15. Occurrence of resistance (%) among Salmonella Enteritidis isolated from humans by origin of infection, Denmark a)

ATC group	Compound	DANMAP 2000							
		Domestic				Acquired abroad			
		1997	1998	1999	2000	1997	1998	1999	2000
J01A	Tetracycline	<1	<1	2	<1	2	0	2	6
J01B	Chloramphenicol	<1	0	0	0	0	0	0	0
J01C	Ampicillin	2	1	1	2	4	10	3	2
J01D	Ceftiofur	0	0	0	0	0	0	0	0
J01E	Sulfamethizol	<1	<1	1	<1	2	0	0	0
J01G	Trimethoprim	<1	<1	1	0	2	0	2	0
	Apramycin	0	0	0	<1	0	0	0	0
	Gentamicin	<1	0	<1	0	0	0	2	0
	Kanamycin	0	0	<1	0	0	0	0	2
J01M	Spectinomycin	<1	<1	<1	0	2	0	0	0
	Streptomycin	<1	<1	0	<1	0	2	0	0
	Nalidixic acid	<1	<1	4	5	9	10	14	18
J01X	Colistin	<1	0	0	0	0	0	0	0
Number of isolates a)		598	368	430	243	46	51	58	49

a) The number of cases for which origin of infection was not specified and/or were not tested for susceptibility to antimicrobials was 51 in 1997, 111 in 1998, 184 in 1999 and 521 in 2000

consumption of imported poultry. Additionally, resistance to tetracycline was 6.1% in isolates from imported cases in 2000, compared with only 0.4% in isolates from domestic cases.

Among human *S. Typhimurium* isolates, antimicrobial resistance was in general much higher in isolates from cases acquired abroad than in isolates from domestic cases (Table 16). In 2000, domestic isolates showed a modest decrease in resistance, which contrasts with the increase observed until 1999. This result is due to the major national efforts carried out to control salmonellosis, and particularly *S. Typhimurium* DT104. As a result the percentage of penta-resistant DT104 and related phage types (DTU302, DT104b) among domestic *S. Typhimurium* isolates included in DANMAP decreased from 22.5%

in 1999 to 13.7% in 2000. However, one would have expected an even greater overall resistance decrease. Indeed, Table 17 shows that while the number of cases due to multi-drug resistant DT104 and similar phage types decreased, there was a general increase in resistance in other *S. Typhimurium* phage types. The increase in the percentage of resistance to trimethoprim, apramycin and gentamicin observed between 1999 and 2000 was related to an outbreak of *S. Typhimurium* DTU302, which was resistant to these antimicrobials.

#### Farm to table

Although *S. Enteritidis* was the predominant serotype in egg layers in Denmark, it was rare in broiler production, and insignificant in other animal species. Table 18 presents a comparison of resistance among

Table 16. Occurrence of resistance (%) among *Salmonella Typhimurium* isolated from humans by origin of infection, Denmark a)

DANMAP 2000

ATC group	Compound	Domestic				Acquired abroad			
		1997	1998	1999	2000	1997	1998	1999	2000
J01A	Tetracycline	18	25	30	27	36	50	52	47
J01B	Chloramphenicol	8	16	24	17	27	30	48	34
J01C	Ampicillin	10	23	28	23	36	40	55	41
J01D	Ceftiofur	0	0	<1	0	-	0	0	0
J01E	Sulfamethizol	17	27	35	29	50	40	52	50
	Trimethoprim	3	5	1	8	0	7	0	6
J01G	Apramycin	0	<1	<1	3	0	0	3	0
	Gentamicin	0	2	<1	7	0	0	3	6
	Kanamycin	-	<1	<1	4	-	3	0	3
	Spectinomycin	10	19	27	18	36	30	48	41
	Streptomycin	15	24	30	24	50	27	52	34
J01M	Nalidixic acid	2	7	2	2	5	3	7	6
J01X	Colistin	0	0	0	0	0	0	0	0
Number of isolates a)		595	440	502	300	22	30	31	32

a) The number of cases for which origin of infection was not specified and/or were not tested for susceptibility to antimicrobials was 98 in 1998, 1 in 1999 and 89 in 2000.

Table 17. Occurrence of resistance (%) among domestic *Salmonella Typhimurium* phage types from humans, Denmark

DANMAP 2000

ATC group	Compound	DT104, DT104b and DTU302		Other phage types	
		1999	2000	1999	2000
J01A	Tetracycline	94	90	11	17
J01B	Chloramphenicol	92	90	4	5
J01C	Ampicillin	94	90	9	12
J01D	Ceftiofur	0	0	<1	0
J01E	Sulfamethizol	96	95	17	19
	Trimethoprim	0	27	2	5
J01G	Apramycin	0	22	1	<1
	Gentamicin	<1	24	<1	4
	Kanamycin	0	5	<1	4
	Spectinomycin	94	95	7	6
	Streptomycin	93	83	12	14
J01M	Nalidixic acid	2	2	3	2
J01X	Colistin	0	0	0	0
Number of isolates		113	41	389	259



*S. Enteritidis* from Danish poultry and isolates from human cases acquired in Denmark and abroad. The resistance levels among *S. Enteritidis* isolates from Danish poultry and human isolates acquired in Denmark were quite similar, except for nalidixic acid and tetracycline resistance. All 4 nalidixic acid resistant *S. Enteritidis* were isolated from the layer production and all 4 were found in rearing flocks, which if infected with *Salmonella*, are slaughtered before they start to produce eggs. Therefore, the *S. Enteritidis* sample does not correctly reflect the exposure of consumers. One of 2 tetracycline resistant *S. Enteritidis* isolates originated from the broiler production, while the remaining isolate was from a layer flock. It is our experience that *Salmonella* in broilers generally has much less impact on human health than does *Salmonella* in table eggs. On the basis of comprehensive serotyping and phage typing studies, the Annual Report on Zoonosis in Denmark, estimates that Danish broilers are associated with a limited number of the human *Salmonella* cases. Furthermore, the *S. Enteritidis* sample size from poultry was small and the number of human *S. Enteritidis* isolates resistance tested was considerably smaller than in the previous years. Therefore it can not be ruled out that the isolates may represent a biased sample. Altogether, these factors are important to consider when comparing the resistance patterns of *S. Enteritidis* isolated from different sources. They all likely contribute to the differences in tetracycline resistance observed among *S. Enteritidis* isolates from poultry and human cases acquired in Denmark. The comparison of resistance levels among *S. Typhimurium* isolates (Table 19) was highly influenced by the frequency of *S. Typhimurium* DT104 in the

Table 18. Comparison of resistance (%) among *Salmonella Enteritidis* from food animals and human cases acquired domestically and abroad, Denmark

		DANMAP 2000		
ATC-group	Compound	Poultry	Humans	
		Danish	Domestic	Acquired abroad
J01A	Tetracycline	7	<1	6
J01B	Chloramphenicol	0	0	0
J01C	Ampicillin	0	2	2
J01D	Ceftiofur	0	0	0
J01E	Sulfonamide	4	<1	0
	Trimethoprim	0	0	0
J01G	Apramycin	0	<1	0
	Gentamicin	0	0	0
	Spectinomycin	0	0	0
	Streptomycin	0	<1	0
J01M	Nalidixic acid	15	5	18
J01X	Colistin	0	0	0
Number of isolates		27	554	99

samples. Due to differences in the sampling schemes, the proportion of DT104 in the samples does not necessarily reflect the true prevalence of DT104 in the population. Therefore we compared resistance levels among *S. Typhimurium* phage types other than DT104 and related phage types (DT104b, DTU302) from food animals and human cases acquired in Denmark; the results are presented in Table 20. The comparison shows that the resistance levels are quite similar, although there are differences. Gentamicin resistance was present in the human isolates, but not in the isolates from animals. This indicates that there are other sources of human *S. Typhimurium* infection than Danish animals. The Annual Report on Zoonosis estimated that imported food products are associated with a considerable proportion of human *Salmonella* infections. In addition, the number of samples from poultry and cattle was small, which might have contributed to the variations observed.

Table 19. Comparison of resistance (%) among *Salmonella Typhimurium* from food animals and human cases acquired domestically and abroad, Denmark

		DANMAP 2000			
ATC-group	Compound	Poultry	Cattle	Pigs	Humans
		Danish	Danish	Danish	Domestic Acquired abroad
J01A	Tetracycline	7	43	25	27 47
J01B	Chloramphenicol	0	32	9	17 34
J01C	Ampicillin	0	36	12	23 41
J01D	Ceftiofur	0	0	0	0 0
J01E	Sulfonamide	7	47	29	29 50
	Trimethoprim	0	0	7	8 6
J01G	Apramycin	0	0	0	3 0
	Gentamicin	0	0	0	7 6
	Spectinomycin	0	32	14	18 41
	Streptomycin	14	43	23	24 34
J01M	Nalidixic acid	0	0	1	2 6
J01X	Colistin	0	0	0	0 0
Number of isolates		14	53	216	300 32

Table 20. Comparison of resistance (%) among *Salmonella Typhimurium* other than DT104, DT104b and DTU302 from food animals and human cases acquired domestically, Denmark

		DANMAP 2000			
ATC-group	Compound	Poultry	Cattle	Pigs	Humans
		Danish %	Danish %	Danish %	Domestic %
J01A	Tetracycline	7	17	21	17
J01B	Chloramphenicol	0	0	3	5
J01C	Ampicillin	0	3	7	12
J01D	Ceftiofur	0	0	0	0
J01E	Sulfonamide	7	20	24	19
	Trimethoprim	0	0	7	5
J01G	Apramycin	0	0	0	<1
	Gentamicin	0	0	0	4
	Spectinomycin	0	0	9	6
	Streptomycin	14	17	19	14
J01M	Nalidixic acid	0	0	1	2
J01X	Colistin	0	0	0	0
Number of isolates		14	35	202	259

## Campylobacter

With 82 cases per 100,000 inhabitants, campylobacteriosis is the most common foodborne zoonosis in Denmark. *Campylobacter jejuni* was responsible for 90-95% of the human *Campylobacter* infections while *C. coli* was the second most common

species. Approximately 80% of the human cases are acquired in Denmark.

### Campylobacter from food animals

In 2000, thermophilic *Campylobacter* were isolated from 39% of fecal samples from broilers, 57% of fecal samples from cattle and 60% of fecal samples from

Table 21. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in fecal samples collected at slaughter of broilers, cattle and pigs, Denmark

Origin	No. of samples	<i>C. jejuni</i>			<i>C. coli</i>		
		No. positive	% positive	No. tested for antimicrobial susceptibility	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	1,217	438	36	76	40	3	20
Cattle	89	50	56	48	1	1	NT a)
Pigs	277	11	4	4	154	56	91

a) NT, not tested

Table 22. Susceptibility and occurrence of resistance (%) *Campylobacter jejuni* and *Campylobacter coli* from food animals, Denmark

ATC-group	Compound	<i>C. jejuni</i>							
		Broilers				Cattle			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5 - 4	0.5	0.5	0	0.5 - 16	0.5	1	2
J01B	Chloramphenicol	2 - 16	2	4	0	1 - 8	2	4	0
J01E	Sulfonamide	8 - 512	32	256	3	8 - 256	32	128	0
J01F	Erythromycin	0.5 - 64	1	4	5	0.25 - 2	1	2	0
J01G	Gentamicin	0.5 - 1	1	1	0	0.5 - 2	0.5	1	0
	Neomycin	1 - 128	2	2	1	1 - 4	1	2	0
	Streptomycin	1 - 128	2	128	12	1 - 128	2	4	2
J01M	Ciprofloxacin	0.06 - 16	0.125	0.5	8	0.03 - 32	0.125	16	15
	Nalidixic acid	4 - 256	8	16	7	4 - 256	8	128	15
J01X	Colistin	2 - 128	16	32	1	1 - 32	8	16	0
Number of isolates					76		48		

ATC-group	Compound	<i>C. coli</i>							
		Broilers				Pigs			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5 - 64	0.5	0.5	5	0.5 - 4	0.5	1	0
J01B	Chloramphenicol	2 - 8	4	8	0	1 - 32	4	8	1
J01E	Sulfonamide	8 - 512	32	512	15	8 - 1,024	16	64	1
J01F	Erythromycin	0.25 - 64	4	64	40	0.25 - 64	4	64	38
J01G	Gentamicin	0.5 - 2	1	1	0	0.5 - 2	1	2	0
	Neomycin	1 - 4	2	4	0	1 - 16	2	4	1
	Streptomycin	2 - 128	4	128	25	1 - 128	8	128	48
J01M	Ciprofloxacin	0.03 - 16	0.125	0.5	10	0.03 - 32	0.25	1	8
	Nalidixic acid	4 - 64	16	16	10	2 - 256	16	32	10
J01X	Colistin	0.5 - 128	2	8	5	0.5 - 32	2	8	0
Number of isolates					20		91		

pigs (Table 21). Among these, a random subsample was tested for antimicrobial susceptibility and included in this report.

Table 22 presents the occurrence of antimicrobial resistance among *C. jejuni* from cattle and broilers and *C. coli* from pigs and broilers in 2000. From 1999 to 2000, resistance to nalidixic acid in *C. coli* from pigs decreased significantly from 23% to 10%. This decrease coincides with the withdrawal from the market of an oral fluoroquinolone formulation for pigs.

The occurrence of resistance to selected antimicrobials from 1996-2000 is presented in Figure

11 and Figure 12. In recent years, the most widely used antimicrobial growth promoter (AGP) has been the macrolide tylosin. It was mainly used in pigs weighing above 35 kg. From 1998 to 1999, the use of tylosin for growth promotion decreased considerably resulting in a sharp decrease in erythromycin resistance among *C. coli* from pigs (Figure 12). In 2000, tylosin was used only for treatment of disease. However, the total consumption of macrolides in food animals increased from 5,000 kg in 1999 to 8,900 kg in 2000. This is the most likely explanation for the increase in resistance to macrolides in *C. coli* from pigs in 2000. Among *C. coli* from broilers, the sharp decrease in erythromycin resistance in 1999 was

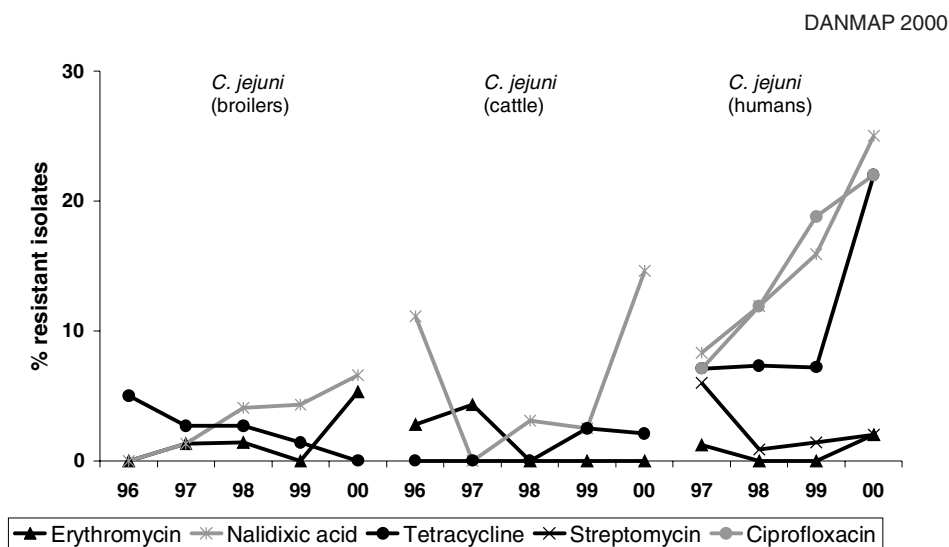


Figure 11. Trends in resistance to selected antimicrobials among *Campylobacter jejuni* from broilers, cattle and domestically acquired human cases, Denmark

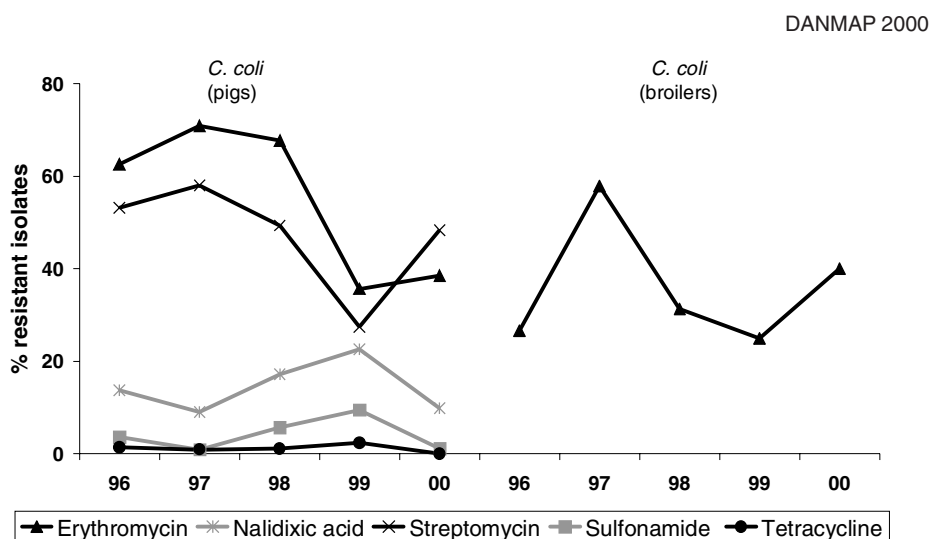


Figure 12. Trends in resistance to selected antimicrobials among *Campylobacter coli* from pigs and broilers, Denmark

most likely the result of the Danish ban on use of virginiamycin which started in January 1998. However, we have presently no explanation for the increase in erythromycin resistance observed in 2000. Finally, while nalidixic resistance is still low in *C. jejuni* from broilers, the regular increase observed during the past years is a cause for concern.

#### **Campylobacter from humans**

For the first time, the data presented in this report have been obtained by linking the Danish register on gastro-intestinal infections with the resistance database maintained by the Department of Gastrointestinal Infections at the Statens Serum Institut. This allowed for a separate estimation of the prevalence of resistance among *C. jejuni* acquired in Denmark and those acquired abroad. This showed that resistance was generally much higher among *C. jejuni* acquired abroad (Table 23). Additionally, this allowed for analysis of resistance trends among human *C. jejuni* isolates acquired in Denmark. This analysis showed an increase in quinolone resistance from 1997 to 2000 and a sharp increase in tetracycline resistance from about 7% to 22% between 1999 and 2000, respectively (Figure 11). For 1999 and 2000, there was enough data on susceptibility to nalidixic acid and erythromycin in 9

Danish counties to perform an analysis at the county level describing the relationship between antimicrobial use in humans and resistance in *C. jejuni*. Depending on the county, resistance to nalidixic acid and to erythromycin varied from 2.9 to 23.1% and from 0 to 8.6%, respectively. In these counties, we found no correlation between a) the level of human consumption of quinolones and the percentage of nalidixic acid-resistant isolates among *C. jejuni* acquired in Denmark, or b) the level of human consumption of macrolides and the percentage of erythromycin-resistant isolates among *C. jejuni* acquired in Denmark. These results suggest that antimicrobial resistance in zoonotic pathogens such as *C. jejuni* is unrelated to antimicrobial pressure in humans. Other possible reasons for differences in resistance levels among counties must be investigated. For example, we have previously reported that resistance was more widespread in *Campylobacter* from imported foods and part of the difference observed among counties could result from differences in consumption of imported foods. Susceptibility data on *C. coli* isolated from humans are not presented because of the much lower frequency of occurrence of this species in human *Campylobacter* infections as compared to *C. jejuni*.

Table 23. Comparison of resistance (%) among *Campylobacter jejuni* from Danish food animals and human cases acquired domestically or abroad, Denmark

DANMAP 2000

ATC-group	Compound	Cattle	Broilers	Humans	
		Danish	Danish	Domestic	Acquired abroad
J01A	Tetracycline	2	0	22	57
J01B	Chloramphenicol	0	0	2	7
J01F	Erythromycin	0	5	2	7
J01G	Gentamicin	0	0	0	0
	Streptomycin	2	12	2	0
J01M	Ciprofloxacin	15	8	22	43
	Nalidixic acid	15	7	25	43
Number of isolates		48	76	49	14

## Resistance in indicator bacteria

### Enterococci from food animals

The indicator bacteria from food animals were isolated from faecal samples from cattle and pigs and cloacal swabs from broilers. The samples were collected at slaughter.

The prevalence of *Enterococcus faecium* and *Enterococcus faecalis* is shown in Table 24. The occurrence of resistance among enterococci is shown in Table 25 and 26.

Overall, resistance levels were lower in 2000 than before the use of antimicrobial growth promoters was discontinued. Among *E. faecium* from broilers there was an increase in penicillin resistance. In 1999 and 2000, penicillin resistance often occurred in combination with streptogramin resistance. This is in contrast to previous years when streptogramin resistance was mostly associated with erythromycin resistance. Virginiamycin has not been used in Denmark since January 1998. This resulted in a decrease in virginiamycin resistance in *E. faecium* from broilers that levelled out in 2000. (Figure 13). Analyses indicate that a likely explanation is that isolates with macrolide/streptogramin resistance decreased following the discontinued use of growth promoters in 1998. Instead, the use of penicillins for treatment of disease may have selected penicillin/streptogramin resistant isolates resulting in no further decrease in virginiamycin resistance from 1999 to 2000. Resistance to the two other growth promoters: tylosin and avilamycin decreased further from 1999 to 2000 (Figures 14 and 15). From 1998 to 2000, resistance to avoparcin among *E. faecium* from broilers has remained below 10% (Figure 16).

Among *E. faecium* isolates from cattle, resistance to tetracycline, penicillin, erythromycin, kanamycin, streptomycin, virginiamycin, Synercid® and nitrofurantoin all increased between 1999 and 2000. Further examinations showed that this was caused by the emergence of a particular clone in 2000. Strains belonging to the clone were resistant to erythromycin, kanamycin, streptomycin, Synercid®, virginiamycin and tetracycline. Variations included strains lacking streptomycin resistance while some had additional resistance to penicillin and/or nitrofurantoin.

This clone was also observed in pigs in 2000. A comparison of the isolates with this particular resistance profile showed that a majority of the pig isolates from 2000 belonged to this one clone but that it differed from isolates with a similar resistance profile included in previous DANMAP reports. Further investigations are needed to clarify the reason for the emergence of this *E. faecium* clone in cattle and pigs.

Among *E. faecium* isolates from pigs, resistance to virginiamycin increased from 8% in 1999 to 23% in 2000 (Figure 17). This increase may be explained by the spread of the clone described above. Similarly, resistance to tetracycline increased from 53% to 68%. In contrast, erythromycin resistance remained unchanged (Figure 18). In 2000, there was a marked increase in the consumption of tetracycline and macrolides in pigs (Table 4, page 11). It is likely that this may have facilitated the spread of the resistant clone. From 1999 to 2000, resistance to avoparcin has remained unchanged (Figure 19).

Table 24. Prevalence of *Enterococcus faecium* and *Enterococcus faecalis* in samples from broilers, cattle and pigs at slaughter, Denmark

Origin	No. of Samples	<i>E. faecium</i>			<i>E. faecalis</i>		
		No. positive	% positive	No. tested for antimicrobial susceptibility	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	1,174	328	28	189	467	40	93
Cattle	281	57	20	48	33	12	33
Pigs	966	277	29	182	251	26	196

DANMAP 2000

Table 25. Susceptibility and occurrence of resistance (%) among *Enterococcus faecium* from food animals, Denmark DANMAP 2000

ATC-group	Compound	<i>E. faecium</i>												
		Broilers						Cattle						
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	
J01A	Tetracycline	1 - >32	1	1	6	1 - >32	1	>32	46	1 - >32	>32	>32	68	
J01B	Chloramphenicol	2 - 32	8	8	1	2 - 8	8	8	0	2 - 64	8	8	1	
	Florfenicol	2 - 4	4	4	0	2 - 4	4	4	0	2 - 4	4	4	0	
J01C	Penicillin	2 - >128	32	32	67	2 - 16	4	16	29	2 - 16	8	16	45	
J01F	Erythromycin	1 - >32	1	32	13	1 - >32	2	>32	38	1 - >32	4	>32	47	
J01G	Gentamicin	128 - 128	128	128	0	128 - 128	128	128	0	128 - 128	128	128	0	
	Kanamycin	128 - >2,048	256	1,024	5	128 - >2,048	512	>2,048	35	128 - >2,048	256	>2,048	28	
	Streptomycin	128 - >2,048	128	128	3	128 - >2,048	128	2,048	23	128 - >2,048	128	>2,048	27	
J01X	Vancomycin	1 - >32	1	2	6	1 - 8	2	2	0	1 - >32	1	2	6	
	Dalopristin / quinupristin	0.5 - 16	2	8	37	0.5 - 32	2	32	42	0.5 - >32	2	16	24	
	Virginiamycin	0.5 - 32	1	8	34	0.5 - >32	1	>32	38	0.5 - >32	1	32	23	
	Avilamycin	1 - >32	4	8	5	1 - 4	2	4	0	1 - 4	2	2	0	
	Bacitracin	8 - >256	>256	>256	88	32 - >256	128	>256	83	8 - >256	128	>256	56	
	Nitrofurantoin	64 - 256	64	64	8	64 - 128	64	128	27	64 - 256	64	128	24	
	Salinomycin	1 - 8	8	8	0	1 - 1	1	1	0	1 - 2	1	1	0	
Number of isolates		189						48						182

Table 26. Susceptibility and occurrence of resistance (%) among *Enterococcus faecalis* from food animals, Denmark DANMAP 2000

ATC-group	Compound	<i>E. faecalis</i>												
		Broilers						Cattle						
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	
J01A	Tetracycline	1 - >32	1	>32	48	1 - >32	16	>32	70	1 - >32	32	>32	77	
J01B	Chloramphenicol	2 - 64	8	8	1	4 - >64	8	64	15	2 - 64	8	8	2	
	Florfenicol	2 - 4	4	4	0	2 - 4	2	4	0	2 - 4	4	4	0	
J01C	Penicillin	2 - 32	2	4	1	2 - 16	2	2	3	2 - 8	2	4	0	
J01F	Erythromycin	1 - >32	1	>32	26	1 - >32	1	>32	18	1 - >32	1	>32	28	
J01G	Gentamicin	128 - 128	128	128	0	128 - 256	128	128	0	128 - >2,048	128	128	2	
	Kanamycin	128 - >2,048	128	128	2	128 - >2,048	128	>2,048	18	128 - >2,048	128	>2,048	12	
	Streptomycin	128 - >2,048	128	256	5	128 - >2,048	128	>2,048	18	128 - >2,048	128	>2,048	22	
J01X	Vancomycin	1 - 4	1	2	0	1 - 4	1	2	0	1 - 4	1	2	0	
	Avilamycin	1 - 4	2	2	0	1 - 2	1	2	0	1 - >32	2	2	1	
	Bacitracin	8 - >256	>256	>256	72	8 - >256	64	>256	24	8 - >256	64	128	24	
	Flavomycin	0.5 - >32	1	4	1	0.5 - >32	1	2	3	0.5 - >32	1	2	2	
	Nitrofurantoin	64 - 64	64	64	0	64 - 128	64	64	3	64 - 64	64	64	0	
	Salinomycin	1 - 8	1	4	0	1 - 1	1	1	0	1 - 8	1	1	0	
Number of isolates		93						33						196

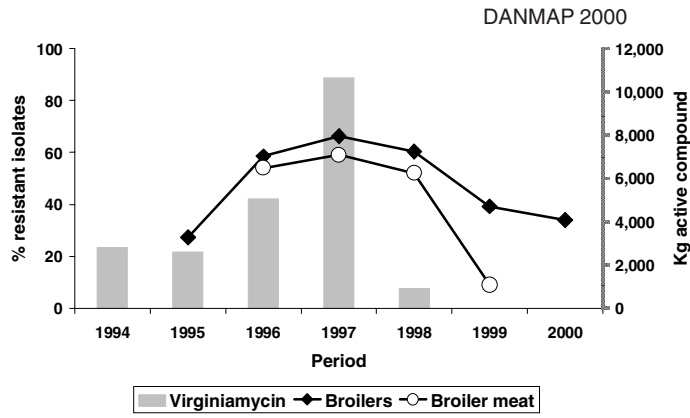


Figure 13. Trend in virginiamycin resistance among *Enterococcus faecium* from broilers and broiler meat and the consumption of the growth promoter virginiamycin, Denmark

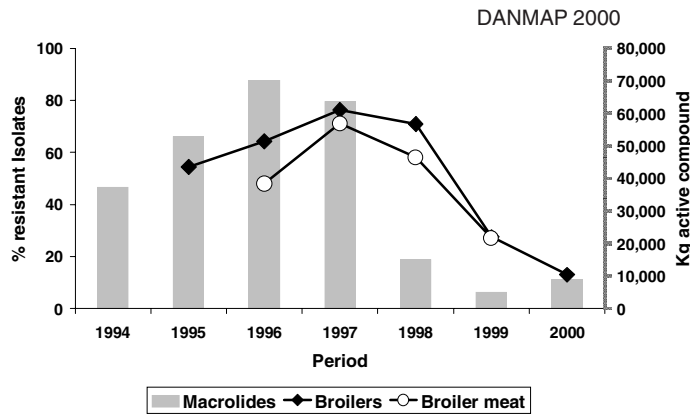


Figure 14. Trend in erythromycin resistance among *Enterococcus faecium* from broilers and broiler meat and the total consumption of macrolides, both as growth promoters and therapeutics, Denmark

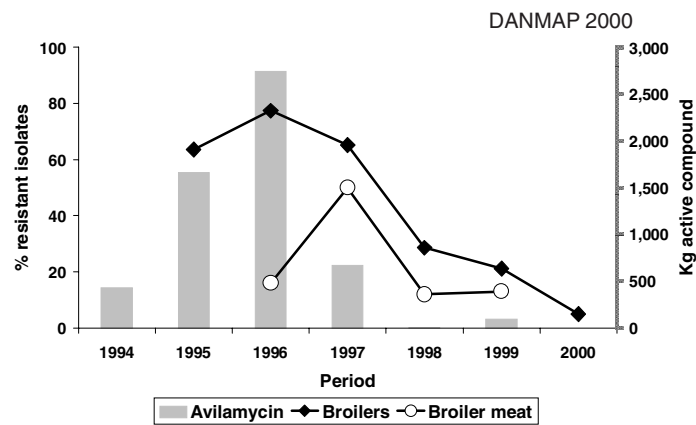


Figure 15. Trend in avilamycin resistance among *Enterococcus faecium* from broilers and broiler meat and the consumption of the growth promoter avilamycin, Denmark

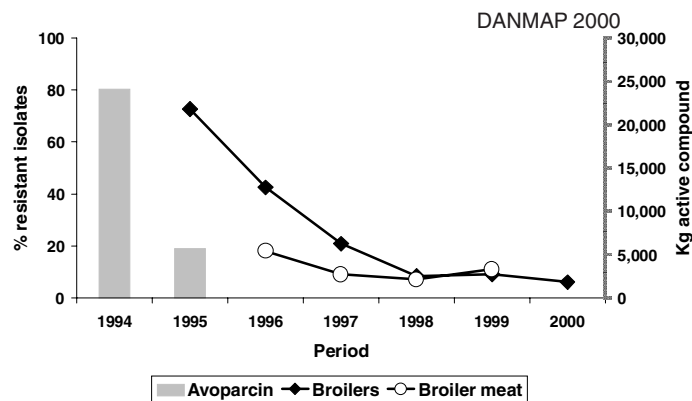


Figure 16. Trend in avoparcin resistance among *Enterococcus faecium* from broilers and broiler meat and the consumption of the growth promoter avoparcin, Denmark

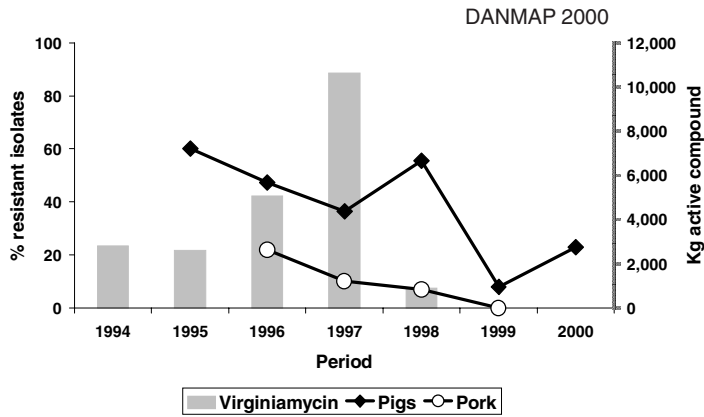


Figure 17. Trend in virginiamycin resistance among *Enterococcus faecium* from pigs and pork and the consumption of the growth promoter virginiamycin, Denmark

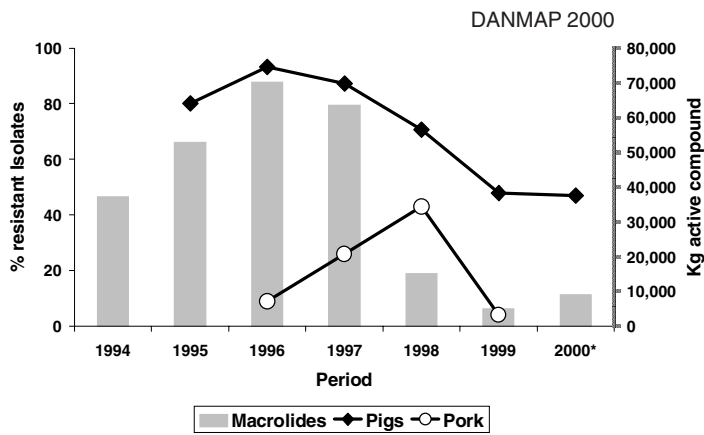


Figure 18. Trend in erythromycin resistance among *Enterococcus faecium* from pigs and pork and the total consumption of macrolides, both as growth promoters and therapeutics, Denmark

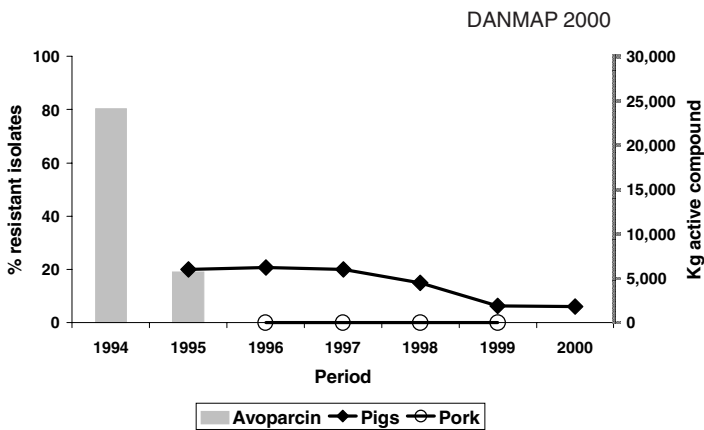


Figure 19. Trend in avoparcin resistance among *Enterococcus faecium* from pigs and pork and the consumption of the growth promoter avoparcin, Denmark



Among *E. faecalis* from pigs, we found a decrease in macrolide resistance from 48% in 1999 to 28% in 2000. This is interesting, considering the increased used of macrolides in pig herds.

#### Farm to table

Figure 13-19 presents trends in resistance to the

most commonly used growth promoters, avoparcin, virginiamycin, avilamycin and macrolides among *E. faecium* from food animals (1995-2000) and food (1996-1999). A sharp decrease in resistance to tylosin, virginiamycin, avoparcin and avilamycin was observed among *E. faecium* after the withdrawal of growth promoters. The decline was observed both in bacteria from animals and from meat products.

## Gene transfer in the mammalian gastrointestinal tract

Gene transfer plays an essential role in evolution and in assuring diversity in nature. Horizontal gene transfer is the transfer of genes between different organisms. Horizontal transfer of genes encoding antimicrobial resistance has been subject of major interest in relation to the potential impact on human health of antimicrobials used in agriculture. The streptogramin<sup>a)</sup> Synercid<sup>®</sup> was approved for human treatment in 1999. The streptogramin virginiamycin has been used for growth promotion in production animals in Europe for more than 25 years and virginiamycin resistant *Enterococcus faecium* from food animals were reported by the first DANMAP report. Streptogramin resistant *E. faecium* have also been observed in healthy humans (Jensen LB, et al. Antimicrob Agent Chemother 1998; 42:3330-3331). Putting these observations together lead to great concern about the possible impact on the future use of streptogramins in human therapy.

Earlier studies conducted within the DANMAP research programme had demonstrated that the *vat(D)* (formerly called *satA*) gene encoding streptogramin A resistance in *E. faecium* could be transferred between isogenic (identical) strains of *E. faecium* with a frequency of  $2.3 \times 10^{-4}$  transconjugants per donor *in vitro* (Hammerum AM et al. FEMS Microb Lett 1998; 168:145-51). Later, it has been demonstrated that this specific type of horizontal transfer of the *vat(D)* gene also takes place *in vivo* in the gastrointestinal tract using an advanced animal model, the gnotobiotic rat (Jacobsen BL et al. Microb Ecol Health Dis 1999; 11:241-247). Faecal samples were taken from gnotobiotic rats fed a recipient *E. faecium* followed by a *E. faecium* donor carrying the *vat(D)* gene. When analysing the *E. faecium* isolates obtained from the faecal samples for their antibiotic resistance patterns we observed high numbers of transconjugants, showing horizontal transfer of the *vat(D)* gene followed by persistence and proliferation of the transconjugants *in vivo*. This very important finding has lead to further studies of the *in vivo* transfer of the *vat(D)* gene between non-isogenic strains of *E. faecium* under similar experimental conditions. Preliminary results indicate that transfer of the *vat(D)* gene does take place *in vivo* between non-isogenic (different) strains of *E. faecium*.

a) The streptogramins include virginiamycin, pristinamycin and Synercid<sup>®</sup> (quinupristin/dalfopristin).

### *Escherichia coli* from food animals

The prevalence of *E. coli* in samples from animals at slaughter is shown in Table 27. Table 28 presents the results of the susceptibility testing.

Comparing the results from Table 28 with the corresponding results from DANMAP 99, the resistance level among indicator *E. coli* has changed little. There are however a few exceptions among the *E. coli* isolates from pigs. From 1999 to 2000, resistance to tetracycline decreased from 34% to 24%, the resistance to sulfonamides decreased from 40% to 26% and resistance to streptomycin decreased from 55% to 43% (Figure 20). The resistance combination of tetracycline, streptomycin and sulfonamide is very common among indicator *E. coli*. Because there was a marked increase in the consumption of tetracycline from 1999 to 2000 (Table

Table 27. Prevalence of *Escherichia coli* in samples from broilers, cattle and pigs at slaughter, Denmark

DANMAP 2000				
Origin	No. of samples	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	1,163	732	63	194
Cattle	102	98	96	98
Pigs	312	308	99	299

Table 28. Susceptibility and occurrence of resistance (%) among *Escherichia coli* from food animals, Denmark

		DANMAP 2000													
ATC-group	Compound	<i>E. coli</i> (from healthy animals)													
		Broilers				Cattle				Pigs					
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant		
J01A	Tetracyclines	2 - >32	2	>32	14	2 - >32	2	2	2	2 - >32	2	>32	24		
J01B	Chloramphenicol	2 - 8	4	8	0	2 - 8	4	8	0	2 - >64	4	8	4		
	Florfenicol	2 - 8	4	8	0	2 - 8	4	8	0	2 - 32	4	8	0		
J01C	Ampicillin	1 - >32	2	>32	14	1 - >32	2	4	3	1 - >32	2	4	8		
J01D	Ceftiofur	0.5 - 4	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0		
J01E	Sulfonamid	32 - >512	32	>512	20	32 - >512	32	32	1	32 - >512	32	>512	26		
	Trimethoprim	4 - >32	4	4	2	4 - >32	4	4	1	4 - >32	4	4	7		
J01G	Apramycin	4 - 8	4	8	0	4 - 16	4	8	1	4 - 16	4	8	0		
	Gentamicin	1 - 2	1	2	0	1 - 1	1	1	0	1 - 2	1	1	0		
	Neomycin	2 - >32	2	2	1	2 - 4	2	2	0	2 - >32	2	2	3		
	Spectinomycin	8 - 128	16	32	2	4 - 32	16	16	0	8 - >128	16	>128	34		
	Streptomycin	4 - 64	8	16	6	4 - 64	8	16	5	4 - 64	8	64	43		
J01M	Ciprofloxacin	0.03 - 1	0.03	0.125	0	0.03 - 0.06	0.03	0.03	0	0.03 - 0.125	0.03	0.03	0		
	Nalidixic acid	4 - >128	4	32	11	4 - 4	4	4	0	4 - 16	4	4	0		
J01X	Colistin	4 - 4	4	4	0	4 - 4	4	4	0	4 - 4	4	4	0		
Number of isolates					194					98					299

4, page 11), it was surprising to observe a decrease in these three particular resistance phenotypes. While the increased tetracycline consumption is likely to be responsible for the increase in tetracycline resistance in *E. coli* O149 (see page 35), apparently it does not appear to have yet had an effect in *E. coli* serotypes prevalent in the older age groups of animals. When comparing resistance profiles from 1999 and 2000, the proportion of *E. coli* isolates resistant to one or more antibiotics in the test panel has decreased for the pig and broiler isolates while it has remained unchanged for the cattle isolates.

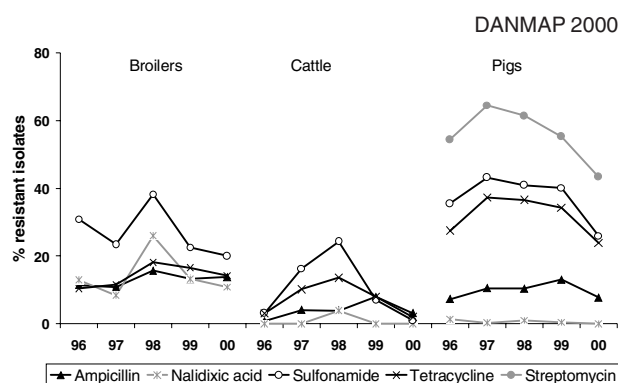


Figure 20. Trends in resistance to some selected antimicrobials among *Escherichia coli* from food animals, Denmark

## Resistance in bacteria from diagnostic submissions

### Bacteria from food animals

The DANMAP programme monitors resistance in the following bacterial species from diagnostic submissions from food animals: *Escherichia coli* from poultry, cattle and pigs, coagulase negative staphylococci (CNS) and *Staphylococcus aureus* from cattle, and *Staphylococcus hyicus* from pigs.

#### *Escherichia coli*

We have included the serotypes O2 and O78 from poultry, F5 from cattle and serotype O149 from young pigs.

The results are presented in Table 29. When comparing the results from 2000 with those from 1999, the resistance levels were almost unchanged. However, we did find a few exceptions to this general trend. Among *E. coli* F5 from cattle we observed a decline in resistance to nalidixic acid and ciprofloxacin from 18% and 6% in 1999 to 7% and 0% in 2000, respectively. This decrease was observed despite a 20 kg increase in fluoroquinolone consumption between 1999 and 2000. Among *E. coli* O149 from pigs, tetracycline resistance increased from 54% in 1999 to 67% in 2000 (Figure 21). During the same period, we observed a substantial increase in tetracycline consumption in pigs (Table 4, page 11). Figure 21 presents the resistance trend for selected antimicrobials from 1996 to 2000. From 1998 to 1999, a simultaneous decrease in resistance to nalidixic acid, streptomycin and ampicillin was observed among pathogenic *E. coli* from broilers. In 1998, twenty isolates (30%) from broilers had an identical resistance pattern including resistance to ampicillin, nalidixic acid, streptomycin sulfonamide and tetracycline. Nineteen of these isolates were serotype O78 and the remaining isolate was serotype O2. In 1999, only 3 (6%, all serotype O78) of the isolates had the above mentioned resistance pattern, which might explain the simultaneous decrease in resistance.

### Staphylococci

The isolates of CNS and *S. aureus* originated from cases of bovine mastitis. The results are presented in Table 30. In general, CNS and *S. aureus* were susceptible to most of the antimicrobials with little change from 1999 to 2000. However, when the resistance levels were compared over a 5 year period (1996-2000) some resistance trends appeared (Figure 22). Since 1997, we have observed a marked decrease in sulfonamide resistance among CNS and *S. aureus*. For CNS this decrease coincided with a decrease in trimethoprim resistance. The proportion of *S. aureus* isolates resistant to penicillin also decreased between 1996 and 2000.

In general, *S. hyicus* from pigs were more often resistant to the antimicrobials in the test panel than were staphylococci from cattle (Table 30). When comparing the results from 2000 with the corresponding results from DANMAP 99, the resistance levels of *S. hyicus* were almost unchanged, with only a decrease in penicillin resistance. When comparing resistance levels over a 5-year period (1996-2000) a decrease in erythromycin resistance was observed after tylosin was withdrawn as a growth promoter from the slaughter pig production in March 1998. However, from 1999 to 2000, no further decrease in erythromycin resistance was observed. Tylosin remains used for treatment of disease with an increased consumption noted in 2000 (Table 4, page 11). Therefore, some selective pressure from macrolides was maintained. In addition, resistance to erythromycin often occurs in combination with other resistance phenotypes, and use of other antibiotics will inevitably co-select for erythromycin resistance. Like CNS and *S. aureus*, a decrease in sulfonamide and trimethoprim resistance was observed among *S. hyicus*. The consumption of tetracycline in pigs increased markedly in 2000, but there is not yet evidence of an increase in tetracycline resistance among *S. hyicus*.

Table 29. Susceptibility and occurrence of resistance (%) among *Escherichia coli* from diagnostic submissions from animals, Denmark

DANMAP 2000

ATC-group	Compound	<i>E. coli</i> (from diagnostic submissions)											
		Poultry				Cattle				Pigs			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	2 - >32	>32	>32	58	2 - >32	>32	>32	72	2 - >32	>32	>32	67
J01B	Chloramphenicol	2 - 8	4	8	0	2 - >64	8	64	11	2 - >64	4	>64	32
	Florfenicol	2 - 8	4	8	0	2 - 32	4	8	1	2 - 32	4	8	0
J01C	Ampicillin	2 - >32	2	4	4	1 - >32	>32	>32	83	1 - >32	2	>32	27
J01D	Ceftiofur	0.5 - 0.5	0.5	0.5	0	0.5 - 2	0.5	0.5	0	0.5 - 1	0.5	0.5	0
J01E	Sulfonamide	32 - >512	32	>512	46	32 - >512	>512	>512	83	32 - >512	>512	>512	76
	Trimethoprim	4 - >32	4	>32	27	4 - >32	>32	>32	64	4 - >32	4	>32	35
J01G	Apramycin	4 - 16	4	8	4	4 - 16	4	8	2	4 - >64	4	4	3
	Gentamicin	1 - 2	1	1	0	1 - >32	1	16	12	1 - >32	1	1	2
	Neomycin	2 - 4	2	2	0	2 - >32	2	>32	17	2 - >32	2	>32	28
	Spectinomycin	16 - 32	16	32	0	2 - >128	16	>128	18	8 - >128	128	>128	61
	Streptomycin	4 - 16	8	16	0	4 - >64	>64	>64	81	4 - >64	64	>64	72
J01M	Ciprofloxacin	0.03 - 0.03	0.03	0.03	0	0.03 - 0.25	0.03	0.03	0	0.03 - >4	0.03	0.25	2
	Nalidixic acid	4 - 4	4	4	0	4 - >128	4	4	7	4 - >128	4	128	21
J01X	Colistin	4 - 4	4	4	0	4 - 4	4	4	0	4 - 8	4	4	0
Number of isolates					26	121				390			

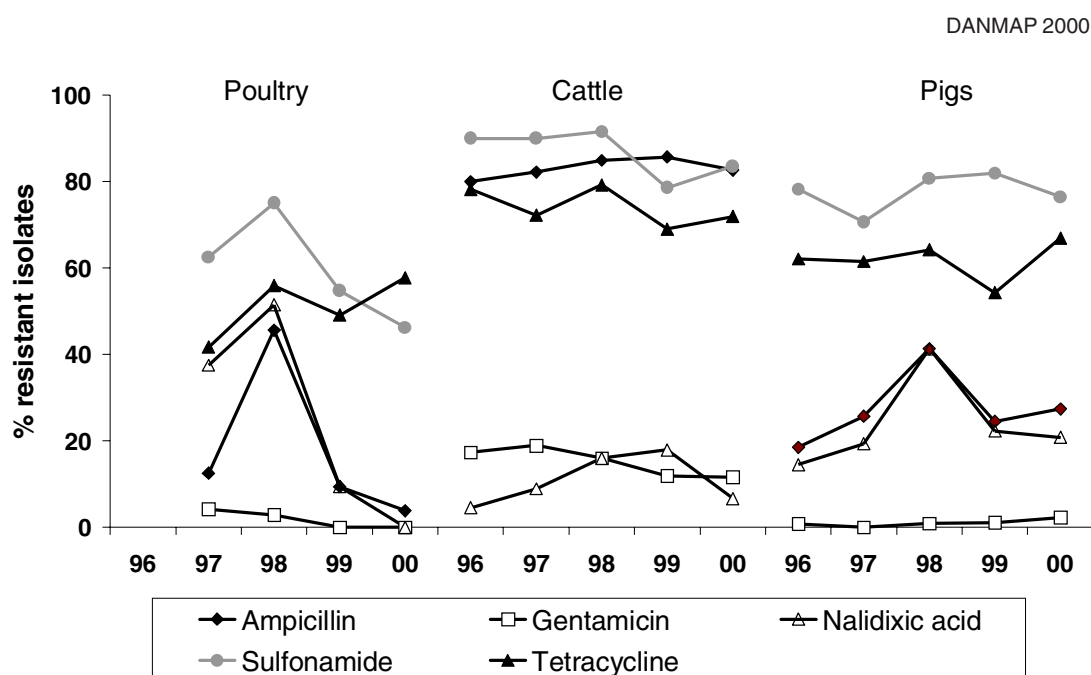


Figure 21. Trends in resistance to some selected antimicrobials among *Escherichia coli* from diagnostic submissions from animals, Denmark

Table 30. Susceptibility and occurrence of resistance (%) among staphylococci from diagnostic submissions from animals, Denmark

DANMAP 2000

ATC-group	Compound	Staphylococci											
		Cattle								Pigs			
		CNS				<i>S. aureus</i>				<i>S. hyicus</i>			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5 - >32	0.5	0.5	2	0.5 - 1	0.5	0.5	0	0.5 - >32	0.5	32	21
J01B	Chloramphenicol	2 - 8	4	8	0	4 - 16	8	8	0	4 - 8	8	8	0
	Florfenicol	1 - 4	2	4	0	2 - 4	4	4	0	1 - 4	2	4	0
J01C	Oxacillin	0.5 - 0.5	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - 2	0.5	0.5	0
	Penicillin	0.06 - >16	0.06	1	25	0.06 - >16	0.06	2	14	0.06 - >16	2	16	54
J01D	Ceftiofur	0.125 - 2	0.5	1	0	0.125 - 2	0.5	1	0	0.25 - 1	1	1	0
J01E	Sulfonamide	8 - >512	32	128	6	8 - >512	32	128	3	8 - 256	32	64	0
	Trimethoprim	1 - >32	2	8	7	1 - 4	1	4	0	1 - >32	4	>32	15
J01F	Erythromycin	0.125 - 0.5	0.25	0.5	0	0.25 - 0.5	0.25	0.5	0	0.25 - >16	0.5	>16	17
J01G	Gentamicin	1 - 1	1	1	0	1 - 2	1	1	0	1 - 4	1	1	0
	Kanamycin	4 - 8	4	4	0	4 - 16	4	4	0	4 - >128	4	4	2
	Spectinomycin	16 - 128	64	64	1	64 - 128	64	128	13	8 - >256	64	>256	11
	Streptomycin	2 - 128	2	8	5	2 - >128	8	16	1	2 - >128	8	>128	40
	Ciprofloxacin	0.125 - 0.5	0.125	0.25	0	0.125 - 2	0.25	0.5	0	0.125 - 8	0.125	0.25	5
J01X	Vancomycin	1 - 4	1	2	0	1 - 4	1	1	0	1 - 4	1	2	0
	Virginiamycin	1 - 1	1	1	0	1 - 1	1	1	0	1 - 32	1	2	2
	Quinupristin/Dalfopristin	1 - 1	1	1	0	1 - 1	1	1	0	1 - 4	1	1	2
	Avilamycin	2 - 16	4	8	2	2 - 16	4	8	4	2 - 8	4	8	0
	Bacitracin	16 - >256	64	128	36	16 - 128	16	64	6	16 - 128	64	128	15
Number of isolates				83					70				81

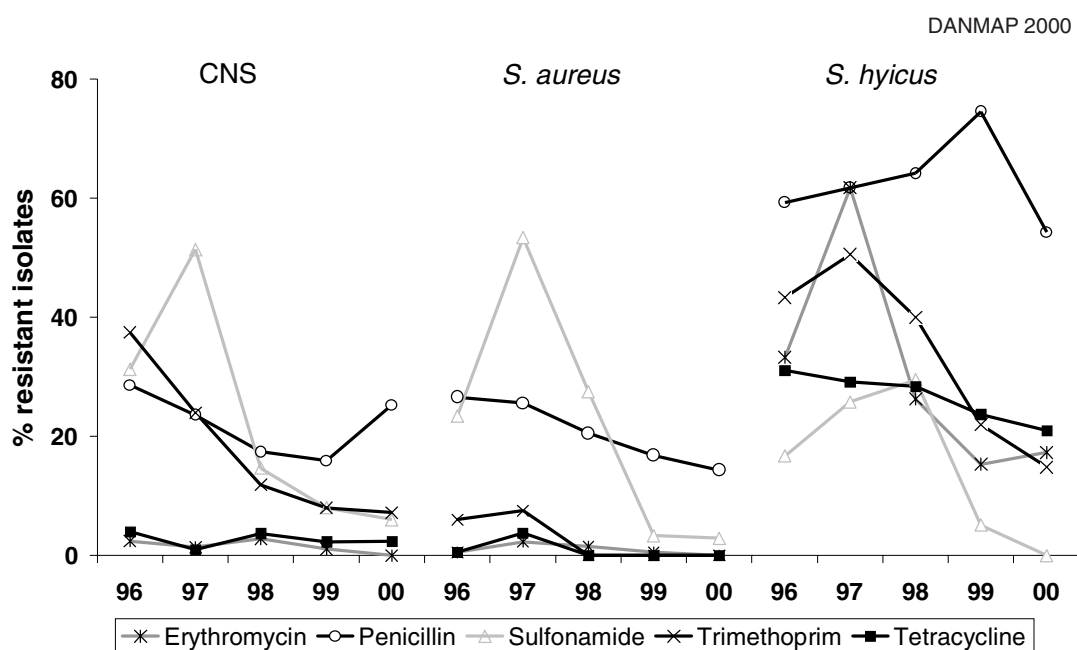


Figure 22. Trends in resistance to some selected antimicrobials among staphylococci diagnostic submissions from animals, Denmark

## Bacteria from humans

For *Salmonella* spp., *Campylobacter* spp., *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 county, and the counties of Copenhagen, Roskilde, West Zealand, Storstroem, Aarhus, Viborg and North Jutland, which represent more than 60% of the Danish population. More information on demographics is presented in Table 2, page 10.

### *Escherichia coli*

The results for the period 1995-2000 are presented in Tables 31 and 32 and Figure 23. The left-hand side of the figure shows the level of resistance to selected antimicrobials among *E. coli* blood isolates. Although one should be cautious about comparisons of resistance levels among the nine counties, the general increase in ampicillin resistance in *E. coli* blood isolates observed during the past years

Table 31. Occurrence of resistance (%) among *Escherichia coli* isolates from human clinical blood samples in 9 counties, Denmark

ATC group	Compound	DANMAP 2000									
		Blood isolates									
		Hospitals in county no.: a)									
		1+2	3	5	6	7	14	15	16		
J01C	Ampicillin	42	41	30	37	33	45	45	39		
J01D	Cefuroxime	1	4	0	2	0	5	2	1		
J01E	Sulfamethizol										
J01G	Gentamicin	1	1	1	<1	<1	<1	1	1		
J01M	Ciprofloxacin										
Number of isolates		377	501	71	207	217	548	140	363		

a) County no.: 1+2, Copenhagen+Frederiksberg Municipalities; 3, Copenhagen County; 5, Roskilde County; 6, West Zealand County; 7, Storstroem County; 14, Aarhus County; 15, Viborg County; 16, North Jutland County

seemed to have stopped in 2000 where it remained at approximately 40%. Similarly to what has been reported during the past years, gentamicin and cefuroxime resistance in *E. coli* blood isolates remained low in 2000. The right hand side of Figure 23 shows the level of resistance to selected antimicrobials among *E. coli* urine isolates. The results are presented separately for isolates from primary health care and from hospitals. Despite a resistance level of 30 to 40%, sulfamethizol is 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 is submitted to the laboratory because of treatment failure and therefore represents a selected population. 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, sulfamethizol 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 9, page 18). Ampicillin resistance in *E. coli* urine isolates was approximately 35%, which seems to correspond to a decrease as compared to 1999. Whether this decrease is real remains to be determined. As for ampicillin resistance in *E. coli* blood isolates, it can not be excluded that this decrease is merely the result of normal variations from year to year or due to a change in our population sample because of the participation of several new counties in 2000. Finally, ciprofloxacin resistance in *E. coli* urine isolates remained very low in 2000.

### Coagulase-negative staphylococci

Figure 24 shows the level of resistance to selected antimicrobials among coagulase-negative staphylococci blood isolates from nine counties. Penicillin resistance was about 80%. Depending on the county, methicillin resistance varied from less

Table 32. Occurrence of resistance (%) among *Escherichia coli* isolates from human clinical urine samples in 9 counties, Denmark

ATC group	Compound	DANMAP 2000														
		Hospitals in county no.:									Primary health care in county no.:					
		1+2	3	5	6	7	14	15	16	1+2	3	6	7	14	15	16
J01C	Ampicillin	37	37	35	33	34	40	37	38	39	38	44	36	47	47	39
J01D	Cefuroxime															
J01E	Sulfamethizol	33	35	34	30	31	32	32	34	39	38	40	36	32	43	38
J01G	Gentamicin															
J01M	Ciprofloxacin	1			1		2	1	1	2		2		2	3	1
Number of isolates		6,432	6,669	1,535	1,661	1,810	4,176	1,300	3,280	4,309	1,666	180	808	3,849	285	2,506

a) County no.: 1+2, Copenhagen+Frederiksberg Municipalities; 3, Copenhagen County; 5, Roskilde County; 6, West Zealand County; 7, Storstroem County; 14, Aarhus County; 15, Viborg County; 16, North Jutland County

than 10% to approximately 50%. However, it is possible that differences in the level of resistance merely were the consequences of the procedure for selection of isolates that are submitted to 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 was approximately 30%.

### ***Streptococcus pneumoniae***

As 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 2000, susceptibility testing was performed on 825 non duplicate isolates from blood or spinal fluid samples. Resistance to penicillin in *Streptococcus pneumoniae* isolates is an increasing problem worldwide. In Denmark, this type of resistance was rare until 1995 when it started to increase to reach approximately 4% in *S. pneumoniae* blood and spinal fluid isolates in 1999. In 2000, there was a slight decrease in the percentage of penicillin-resistant or intermediate *S. pneumoniae* (Figure 25); although it is too early to determine whether this decrease is real or only due to normal variations in the percentage of resistance. Similarly, resistance to erythromycin has been increasing since 1992 and increased again in 2000 to reach 5% of isolates (Figure 25). To better examine this increase, we plotted the monthly percentage of erythromycin-resistant/intermediate isolates among *S. pneumoniae* from blood and spinal fluid (Figure 26). This figure shows that a sharp increase occurred during the winter 1997-1998 when resistance rapidly moved from nearly 0% to almost 3% over a few months. Preliminary data from DNA fingerprinting show that this sharp increase and most of erythromycin-resistant/intermediate *S. pneumoniae* observed in Denmark since this date correspond to a single clone (Margit Kaltoft, personal communication). Additionally, Figure 26 shows short term changes resembling seasonal variations. With the exception of the sharp increase in macrolide consumption observed in November 1998, there has been no major change in the yearly macrolide consumption which has remained over 2 DDD/1,000 inhabitant-days during the past years (Table 8, page

15). However, macrolide consumption in primary health care shows seasonal variations with a peak during winters and it is remarkable that these variations match similar short term variations in the percentage of erythromycin-resistant/intermediate *S. pneumoniae*. These results suggest that the increase of erythromycin-resistant *S. pneumoniae* in Denmark is due to both person-to-person transmission of a successful clone and pressure due to macrolide consumption in primary health care. The possible differential effect of pressure due to erythromycin consumption versus azithromycin consumption, and possibly consumption of other antimicrobials is presently under investigation.

### ***Staphylococcus aureus***

In the DANMAP 99 report, we described the gradual disappearance of methicillin-resistant *Staphylococcus aureus* (MRSA) from Denmark at the end of the 1970s. Since then MRSA have represented a very low percentage (less than 0.5%) of *S. aureus* blood isolates and more than one half of these MRSA strains have been acquired outside Denmark. However, a recent analysis of the MRSA register maintained by the *Staphylococcus* Laboratory at the Statens Serum Institut showed that Denmark is facing a slow but definite increase in MRSA cases. From 1996 to 2000, there has been a steady increase from 34 to 97 cases of MRSA infection per year. Although MRSA from primary care corresponded to 20-30% cases, the majority of cases was reported by hospitals. From 1996 to 2000, the number of hospitals that reported at least one MRSA increased from 16 to 28. In 2000, 6 hospitals reported four or more MRSA and were responsible for approximately one half of all hospital cases. One single hospital reported 13 MRSA cases in 2000. It is presently not known whether this increase is related to an increase in the number of MRSA cases imported from other countries or to an increase in domestic cases. Preliminary results from typing suggest that a substantial number of hospital cases corresponded to outbreaks, although generally limited to a few cases, and that transmission of MRSA has occurred among hospitals that are likely to exchange patients because they belong to the same hospital administration or because of geographical proximity. This increase in MRSA is worrying and close monitoring is warranted. More information on MRSA surveillance in Denmark can be found in the newsletter of the Danish National Centre for Hospital Hygiene at: <http://www.ssi.dk/dk/cas-nyt/1.htm>).

DANMAP 2000

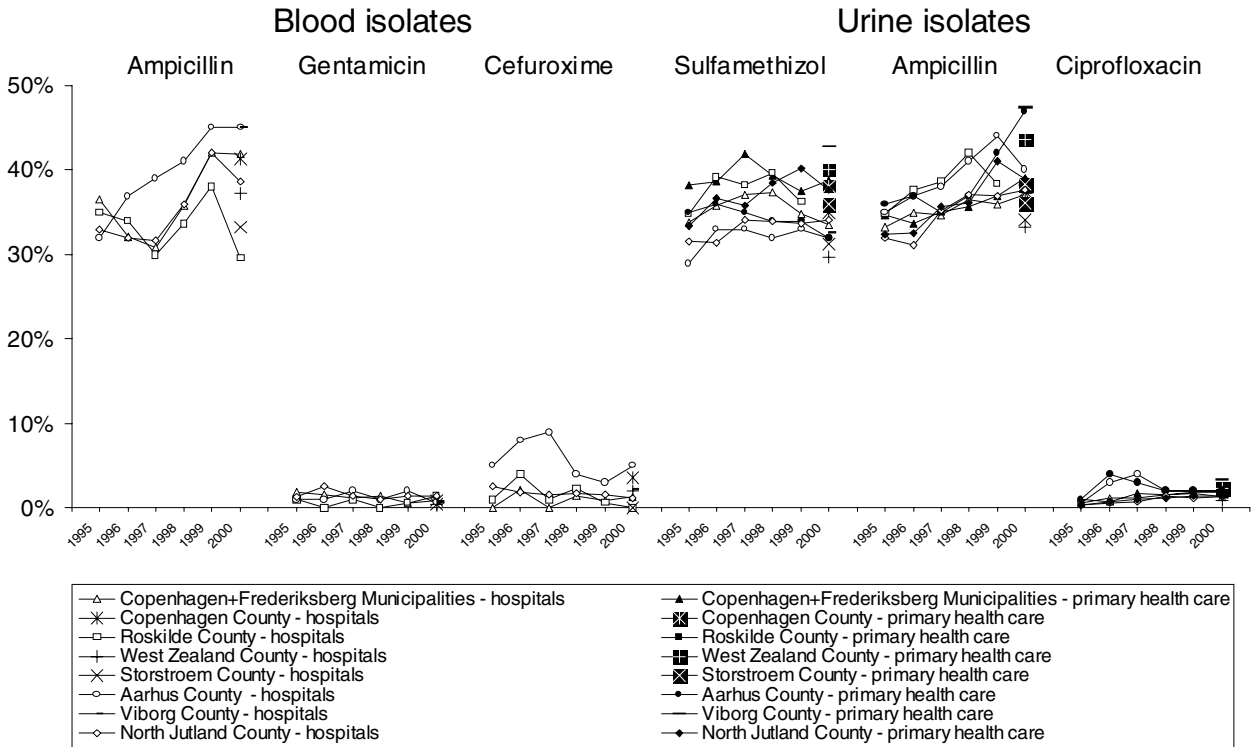


Figure 23. Resistance (%) to selected antimicrobials among Escherichia coli blood and urine isolates from humans, Denmark

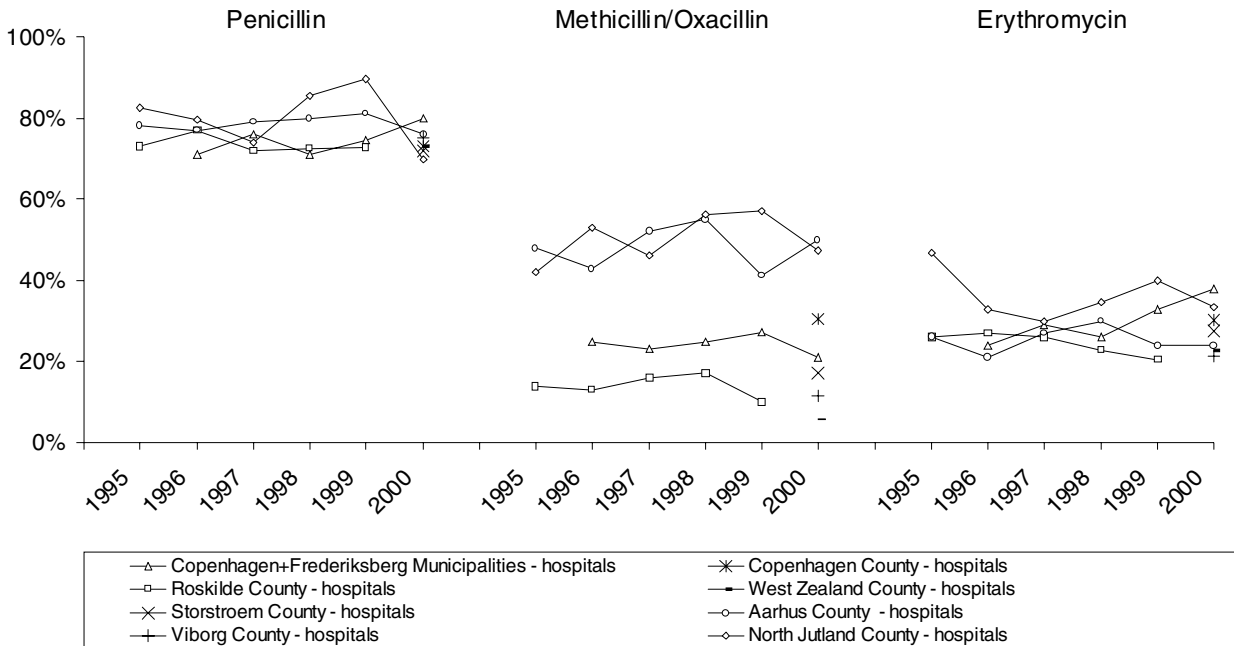


Figure 24. Resistance (%) to selected antimicrobials in coagulase-negative staphylococci blood isolates from humans, Denmark



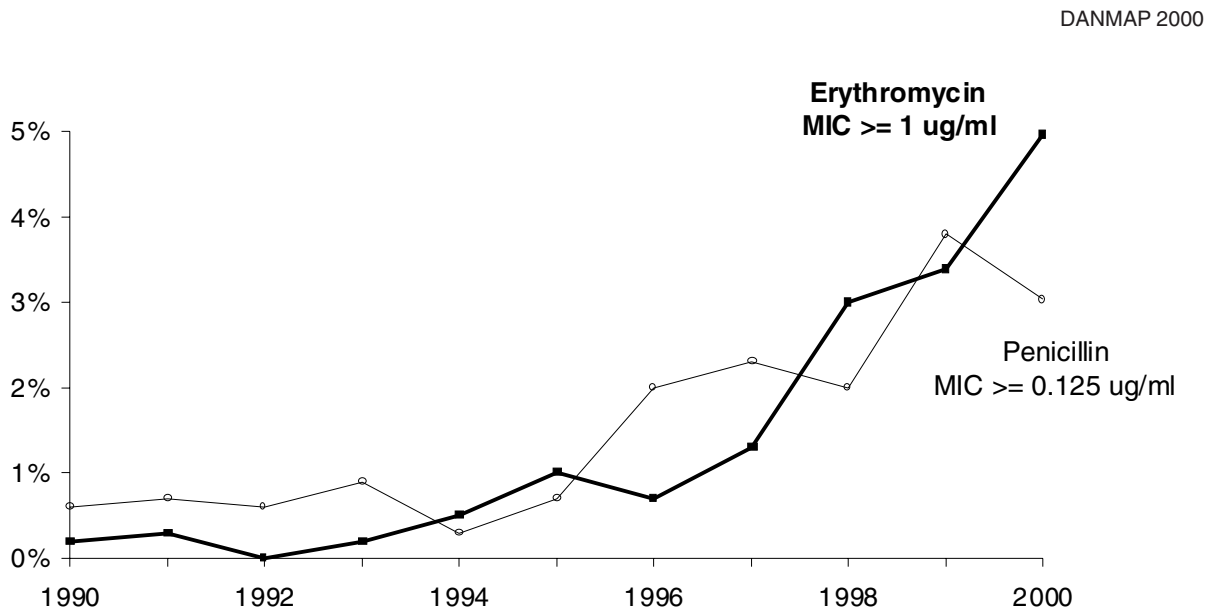


Figure 25. Resistance (%) to selected antimicrobials in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark

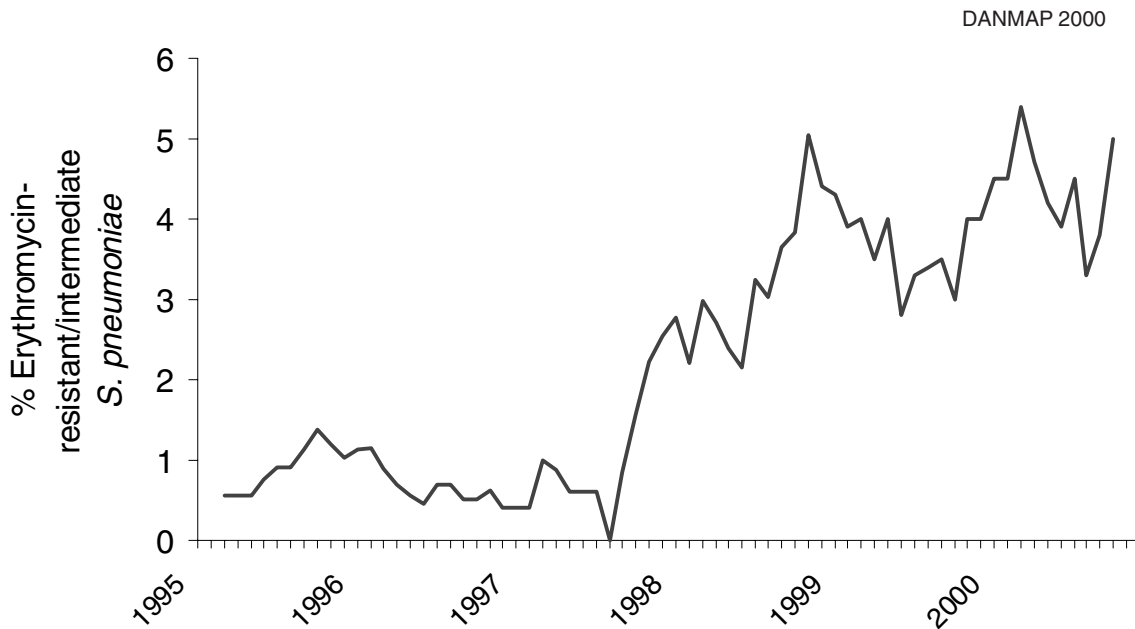


Figure 26. Monthly percentage of erythromycin-resistant/intermediate isolates among *Streptococcus pneumoniae* from blood and spinal fluid, Denmark, (5-month moving average). All isolates from blood and spinal fluid were tested for susceptibility to antimicrobials, which corresponded to at least 30 isolates each month. Duplicate isolates from the same patient were removed

## Acknowledgements

The Danish Veterinary Laboratory would like to thank the meat inspection staff and the company personnel for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin the results would be much less useful. We are also very grateful to the Cattle Health Laboratory at Ladelund and the Laboratory of the Danish Pig Producers and Slaughterhouses for making isolates of animal pathogens available to the programme. We would like to thank for the permission to use the data on the consumption of antimicrobials for therapy in animals prior to 1996. These data were collected by

Niels Erik Rønn from the Federation of Danish Pig Producers and Slaughterhouses in collaboration with Erik Jacobsen from the Danish Pharmacy Association (present address: Danish Veterinary Laboratory). Finally we would like to thank the laboratory technicians of the antimicrobial resistance group.

Statens Serum Institut would like to thank the Danish Medicines Agency for providing data on consumption of antimicrobials in humans, and the clinical microbiology laboratories for providing data on resistance in bacteria from human clinical samples.

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# 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 farmer on presentation of a prescription. By law, the profit that veterinarians can make on the sale of medicines is severely limited and they have little financial enticement to sell medicines. Accordingly, an estimated 80 percent of all antimicrobials used for therapy in food animals are sold to farmers by pharmacies.

All medicines must be approved by the Danish Medicines Agency (DMA), and importers and manufacturers are required to provide an annual report to the DMA on the quantities sold.

The data on the usage of therapeutics presented in this report originates from two sources. Data for the period until 1995 has been collected by section head N. E. Rønn, the Federation of Danish Pig Producers and Slaughterhouses and by E. Jacobsen, the Danish Pharmacy Association. These results are based on voluntary reporting to Danish Medical Statistics and may be incomplete. They should therefore be regarded as estimates although they probably reflect rather accurately the true trend in use. Results for 1996 onwards are based on reporting of quantities sold by the pharmaceutical industry to the DMA. These statistics will be affected by changes in the stocks held by wholesalers and pharmacies and provide little information on the food animal species in which the antimicrobials are used. Products and formulations obviously intended for use only in pets have been excluded from the statistics shown in this report.

The results shown in Table 3, page 11 were rounded, so that quantities between 1 and 25 are shown as "< 25"; quantities between 25 and 1,000 were rounded to the nearest 50, and quantities over 1,000 kg were rounded to the nearest 100.

The Danish Plant Directorate is responsible for the collection of data on the use of antimicrobials for

growth promotion and on the use of coccidiostats. The statistic is based on compulsory reporting by companies authorised to produce premixes containing antimicrobials. The system used for collection of data allows us to discriminate between the quantities of, for example tylosin, used for growth promotion and for therapy.

#### Antimicrobials in humans

By law, the Danish Medicines Agency (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 since 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 reimbursement of cost if applicable. Information on the indication for the prescription is not yet available. The data are transferred monthly to the DMA in an electronic format. On-line transfer of the transactions in real time is being established.

The present report includes data on the consumption of antibacterials for systemic use of group J01 of the 2001 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 coagulase negative staphylococci 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 Laboratory 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 percent, 80 percent and 95 percent, respectively, of the total production of these animal species in Denmark. Accordingly, the bacterial isolates may be regarded as representing a stratified random sample of the respective populations, so that the occurrence of resistance provides an estimate of the true occurrence in the populations.

The *Salmonella* isolates included in DANMAP are selected as a true random sample among isolates serotyped at the Danish Veterinary Laboratory. The DVL 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 DVL, the Cattle Health Laboratory in Ladelund 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 humans

With exception of *Salmonella* Typhimurium isolates which are all tested for susceptibility to

antimicrobials, *Salmonella* sp. and *Campylobacter* sp. from humans represent a random sample of isolates grown from faeces samples submitted for microbiological diagnostic to the Department of Gastrointestinal Infections at the Statens Serum Institut in 2000.

All *S. aureus* blood isolates nationwide are sent to the *Staphylococcus* reference laboratory at the Statens Serum Institut for confirmation of susceptibility testing and phage typing.

Similarly, all *S. pneumoniae* blood and spinal fluid isolates nationwide are sent to the *Streptococcus* Unit at the Statens Serum Institut for confirmation of susceptibility testing and typing.

*Escherichia coli* and coagulase negative staphylococci from humans represent all isolates grown from either blood or urine samples submitted for microbiological diagnostic at one of the eight participating laboratories serving the Copenhagen and Frederiksberg municipalities, Copenhagen county, Roskilde county, West Zealand county, Storstroem county, Aarhus county, Viborg county and North Jutland county, respectively.

## Isolation of bacteria

### Examination of samples from animals

***Salmonella*.** Examination of samples from cattle and pigs was done by non-selective pre-enrichment of 22 g material in 200 ml of BPW and incubation 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. Incubation overnight 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*.** 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 re-inoculated on Slanetz agar and incubated for 2 days at 37°C. The isolates were then sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified according to the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, 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. faecium* were identified to species level by using standard biochemical and physiological tests as described above. All isolates that were verified as *E. faecium* and *E. faecium* were subjected to antimicrobial susceptibility testing.

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

### Examination of samples from humans

*Salmonella* sp. 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* sp. were isolated from faeces samples using a modified CCDA medium (SSI Diagnostika).

Other clinical isolates were isolated on various common media used in clinical microbiology laboratories.

## Susceptibility testing

### Isolates from animals

Plate dilution was used to test the susceptibility of *Campylobacter* isolates to all antimicrobials 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 37°C 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 A1.

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

**Gastrointestinal pathogens.** Susceptibility testing for *Salmonella* sp. isolates was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco, Roskilde, Denmark) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined in Table A2.

Susceptibility testing for *Campylobacter* sp. isolates was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on 5% blood yeast extract-

Table A1 Breakpoints and range of dilutions used for testing bacteria from animals. Isolates with MIC higher than the figures shown were considered resistant

DANMAP 2000

Antimicrobial agent	<i>E. coli, Salmonella</i>		Staphylococci		Enterococci		<i>Campylobacter</i>	
	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range
Ampicillin	16	1-32					16	1-32
Apramycin	8	4-64						
Avilamycin			8	2-32	8	1-32		
Bacitracin			64	16-256	64	8-256		
Ceftiofur	4	0.5-8	4	0.12-16				
Chloramphenicol	16	2-64	16	2-64	16	2-64	16	1-64
Ciprofloxacin	2	0.03-4	2	0.12-8			1	0.03-16
Colistin	8	4-64					32	0.5-64
Erythromycin			4	0.12-16	4	1-32	16	0.25-32
Flavomycin					8	0.5-32		
Florfenicol	16	2-64	16	1-64	16	2-32		
Gentamicin	8	1-32	8	1-32	512	128-2,048	8	0.5-32
Kanamycin			32	4-128	1,024	128-2,048		
Nalidixic acid	16	4-128					32	1-128
Neomycin	8	2-32					8	1-64
Nitrofurantoin					64	64 - 256		
Oxacillin + 2% NaCl			2	0.5-8				
Penicillin			0.12	0.06-16	8	2-128		
Salinomycin					8	1-32		
Spectinomycin	64	2-128	64	8-256				
Streptomycin	16	4-64	16	2-128	1,024	128-2,048	8	1-64
Sulfamethoxazole	256	32-512	256	8-512			256	8-512
Quinupristin/dalfopristin a)			2	1-32	2	0.5-32		
Tetracycline	8	2-32	8	0.5-32	8	1-32	8	0.5-32
Trimethoprim	8	4-32	8	1-32				
Vancomycin			16	1-32	16	1-32		
Virginiamycin			4	1-32	4	0.5-32		

a) The trade name is Synercid®

supplemented agar (SSI Diagnostika) and the breakpoints defined in Table A2.

***Staphylococcus aureus.*** The *Staphylococcus* Unit at the Statens Serum Institut is using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by A/S Rosco.

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

***Escherichia coli* and coagulase-negative staphylococci.** In 2000, the clinical microbiology laboratories serving the Roskilde, Storstroem, Viborg and North Jutland counties were using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on

Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by A/S Rosco.

In 2000, the clinical microbiology laboratories serving the Copenhagen and Frederiksberg Municipalities, West Zealand county and Aarhus county (2nd seme-

Table A2. Breakpoints used for gastrointestinal pathogens from humans. Isolates were considered resistant if they had an inhibition zone less than shown in the table

DANMAP 2000

Antimicrobial agent	Species	
	<i>Salmonella enterica</i>	<i>Campylobacter</i>
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	19 mm	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

ster only) were using the disk diffusion method (Oxoid, Basingstoke, UK) on 5% horse blood Iso-Sensitest (ISA) medium (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>). During the first half of 2000, the laboratory serving Aarhus county used a pre-diffusion disk method previously described in the DANMAP 99 report and in the following publications: Schumacher H, et al. APMIS 1998;106:979-86; Schumacher H, et al. J Antimicrob Chemother 2000;46:215-21.

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

### Performance test

A performance test was carried out to ascertain the comparability of presented susceptibility results in four laboratories. The laboratory in Department of Gastrointestinal Infections and the Clinical Microbiology laboratory at the Statens Serum Institute and two departments at the Danish Veterinary Laboratory received 8 strains of *Salmonella* species and one *E. coli* strain (the *E. coli* reference strain ATCC 25922). The results of the performance test are presented in Table A3.

Among the *Salmonella* strains there was an overall agreement for 99.4% of the tests performed. The *E. coli* reference strain was inside the expected quality control range as given by either NCCLS guidelines or Rosco A/S in 100% of the tests.

### 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 human isolates

Data on *Salmonella* sp. and *Campylobacter* sp. infections were exported from the Danish registry on gastro-intestinal infections (Microsoft® Access) maintained by the Department of Gastrointestinal 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) in a Microsoft® Excel database at the same department. Using the isolate identification

Table A3 Results of performance testing (correct results/no. of tests) among laboratories participating in DANMAP

Antimicrobial agent	Salmonella			DANMAP 2000
	S+I a)	R	Total	<i>E. coli</i> Within range b)
Ampicillin	20/20	12/12	32/32	4/4
Chloramphenicol	24/24	8/8	32/32	4/4
Ciprofloxacin	32/32	0/0	32/32	4/4
Gentamicin	28/28	3/4	31/32	4/4
Kanamycin	21/21	3/3	24/24	3/3
Nalidixic acid	24/24	8/8	32/32	2/2
Streptomycin	16/16	16/16	32/32	4/4
Sulfonamide	12/12	19/20	31/32	4/4
Tetracycline	20/20	12/12	32/32	4/4
Trimethoprim (TMP)	28/28	4/4	32/32	4/4
TMP+Sulfonamide	21/21	3/3	24/24	4/4
Total	246/246	88/90	334/336	41/41

a) S+I, susceptible and intermediate; R, resistant

b) Results within range of the *E. coli* reference strain according to NCCLS guideline M7 January 2000 or Rosco A/S

number, this second database was linked to the Danish register on gastro-intestinal infections and data were analysed using Epi Info v. 6.04c.

Information on all methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from infection or colonisation cases in Denmark are stored in a Microsoft® Excel database at the *Staphylococcus* reference laboratory at the Statens Serum Institut. Only one MRSA isolate per patient nationwide was included in the present report.

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

The eight other clinical microbiology laboratories provided compiled data on resistance levels in *Escherichia coli* blood and urine isolates and in coagulase-negative staphylococci blood isolates. In seven of these laboratories, data were extracted from the laboratory information system, i.e. ADBakt (Autonik AB, Skoldinge, Sweden) for Copenhagen

Municipality (Hvidovre Hospital), Copenhagen county (Herlev Hospital), West Zealand county (Slagelse Hospital) and North Jutland county (Ålborg Hospital), and MADS (Clinical Microbiology Laboratory, Aarhus Kommunehospital, Aarhus, Denmark) for Aarhus county, Storstroem county (Næstved Hospital) and Viborg county (Viborg 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 the laboratories serving the Copenhagen and Frederiksberg municipalities and North Jutland county were able to comply with this rule. Other laboratories removed duplicate isolates using a window of 21 days (Copenhagen and West Zealand counties) or the whole study period, i.e. one year (Storstroem and Viborg counties). In the two remaining laboratories, removing duplicate isolates would have required too much additional work and was not performed.

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## Appendix 2

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