DANMAP 99

DANMAP 99 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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Danish Veterinary & Food Administration
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Sammendrag

Forbrug af antibiotika

Forbruget af antibiotika til produktionsdyr er faldet fra lidt over 163 tons aktivt stof i 1997 til lige under 74 tons i 1999. Disse tal omfatter dog ikke forbruget i dambrug og en del af forbruget til kæledyr. Faldet skyldes landbrugets beslutning om at ophøre med brug af antibiotiske vækstfremmere. Denne beslutning trådte i kraft i første kvartal 1998 for fjerkræ, kvæg og svin over 35 kg og i sidste halvdel af 1999 for så vidt angår svin under 35 kg.

Det kan konstateres, at den første fase af ophøret med brug af vækstfremmere ikke førte til nogen øgning i forbruget af antibiotika til behandling. I 1997 blev der brugt hvad der svarer til 28 mg antibiotika til behandling pr. kg. dyr slagtet, sammenlignet med lige knapt 27 mg i 1998. Ikke alle produktionsopgørelser for 1999 forelå ved redaktionens slutning, men det kan konstateres at forbruget af tetracyklin til indgift gennem munden steg med 4 tons fra 1998 til 1999. I følge informationer fra branchen skyldes denne stigning diarreproblemer hos fravænningsgrise. Hvis dette er korrekt tyder det på, at de nuværende produktionsmetoder i nogle besætninger ikke er bæredygtige uden en mere eller mindre konstant antibiotikatildeling. Stigningen bringer det samlede forbrug af tetracyklin op på godt 16 tons, svarende til 26% af det samlede terapeutiske forbrug.

Der har været udtrykt bekymring i forbindelse med anvendelsen af fluorokinoloner til produktionsdyr, fordi denne gruppe stoffer repræsenterer førstevalgs antibiotika til mennesker med behandlingskrævende diarre. Myndighederne har anbefalet dyrlægerne at være tilbageholdende i brugen af fluorokinoloner. Et medicinalfirma, der markedsfører et fluorokinolonpræparat med en meget stor markedsandel reagerede på bekymringerne vedrørende resistens ved at tage et af de markedsførte præparater af markedet. Som resultat heraf faldt det samlede fluorokinolonforbrug med 2/3 fra 1998 til 1999. Det er stadig for tidligt at udtale sig med sikkerhed om effekten på resistens, men vi kan dog konstatere, at andelen af kinolonresistente bakterier har været faldende i 1999, både for indikator *E. coli* og for nogle af de dyrepatogene bakterier, medens der endnu ikke et tegn på fald blandt de zoonotiske bakterier.

Det samlede forbrug af coccidiostatika i fjerkræproduktionen er steget noget, skønt en stor del af stigningen skyldes overgang til at bruge de mindre potente coccidiostatika, som skal tilsættes foderet i højere koncentrationer. Disse coccidiostatika, ionophorerne, har indgået i resistensovervågningen siden programmets start, og der har indtil videre ikke været tegn på, at de er problematiske fra et resistenssynspunkt.

Den del af antibiotikaforbruget til mennesker, der ordineres af de praktiserende læger faldt med 5% fra 1998 til 1999. Til gengæld steg forbruget på hospitaler en smule, lidt over 1%. I begge dele af sundhedssystemet tegner penicilliner og makrolider sig for den største del af det samlede forbrug. De nye typer antibiotika, såsom fluorokinoloner og cephalosporiner udgør kun en meget lille del af forbruget.

Selv om det samlede antibiotikaforbrug regnet som DDD pr. 1000 indbyggere er meget lavt sammenlignet med andre lande, er der alligevel en forskel i forbruget mellem amterne på ca. 20%. Denne forskel har været ret konstant de seneste år. Det er usandsynligt at den afspejler en forskel i sundhedstilstanden, idet antallet af sengedage på hospitaler er proportionalt med indbyggertallet i amterne. På det foreliggende grundlag er det ikke muligt at sige, om forskellene i antibiotikaforbrug repræsenterer over- eller underforbrug i de enkelte amter.

I vinteren 1998-99 oplevede Danmark en langvarig stigning i forbruget af makrolider til behandling af luftvejsinfektioner hos mennesker. Denne rapport beskriver, hvorledes bekendtgørelsen om epidemien af *Mycoplasma pneumoniae* lungebetændelser medførte en omgående stigning i antallet af udstedte recepter på makrolider, og hvordan forbruget ikke vendte tilbage til det normale niveau før adskillige måneder efter at epidemien var ovre.

Udvikling i resistens

Det er et af DANMAPs hovedformål at sammenligne resistens hos bakterier fra produktionsdyr, levnedsmidler af animalsk oprindelse og mennesker. Vi har måttet konstatere, at dette formål var meget vanskeligt at opfylde i 1999, fordi de isolater, der var til rådighed ikke var tilstrækkeligt repræsentative. Et sådant eksempel udgøres af *Salmonella* Enteritidis, hvor resistensforekomsten blandt isolater fra fjerkræ var noget afvigende fra det billede, der kunne observeres blandt isolater fra mennesker. Tilbagesporing viste at forskel-

len skyldtes, at de ret få isolater fra fjerkræ sandsynligvis repræsenterede samme klon, som havde spredt sig mellem nogle besætninger.

Et tilsvarende problem eksisterer for *Salmonella* Typhimurium, hvor et program med henblik på aktiv tilbagesporing af kilder til smitte med DT104 medfører at denne pentaresistente fagtype kommer til at udgøre en uforholdsmæssig høj andel af *S.* Typhimurium isolaterne fra kvæg. Den tilsvarende humane opgørelse lider af tilsvarende skævhed på grund to fødevarebårne udbrud med DT104 i 1999. Alle tilfælde fra disse udbrud bidrager med hver et isolat, hvilket indebærer en skævhed, når man forsøger at sammenligne resistensbilledet for mennesker med billedet i de mulige salmonellasmittekilder. Det samme problem i en lidt mere generel form ses med bakterier fra levnedsmidler hvor det er vores erfaring, at det ofte kan være meget vanskeligt at fastslå om en given prøve er af dansk eller udenlandsk oprindelse.

Disse erfaringer viser, at skal man drage konklusioner om smitteveje for resistente bakterier på grundlag af overvågningsdata er der behov for detaljeret kendskab til de stikprøveplaner, hvorpå overvågningen er baseret.

For *Salmonella* gælder generelt, at når der ses bort fra den skævhed, som skyldes *S.* Typhimurium DT104, er resistensforekomsten beskeden, hvilket blandt andet fremgår af resultaterne for fjerkræ og svin. Resistensforekomsten er noget højere blandt isolater fra importerede levnedsmidler. Blandt disse var en tredjedel af isolaterne fra kategorien ”andet fjerkrækød” resistente overfor kinoloner (nalidixan syre).

I DANMAP overvåges også resistens hos bakterier fra normalfloraen hos dyr. En opgørelse af resistensudviklingen hos *Enterococcus faecium* siden programmets etablering i 1995 viser, at de markante ændringer i forbruget af vækstfremmere afspejler sig i resistensforekomsten. Dette gælder for resistens overfor avoparcin, der blev forbudt i 1995, ligesåvel som resistens overfor makrolider, virginiamycin og avilamycin. Resultaterne viser således, at den indgriben, der har fundet sted til begrænsning af forbruget af vækstfremmere har haft den ønskede effekt. Men – med avoparcin som eksempel – er det vigtigt at være opmærksom på, at selv om resistente *E. faecium* (VRE) ser ud til at være forholdsvis sjældent forekommende fire år efter forbuddet mod avoparcin, skyldes dette at den anvendte metode til isolation af VRE ikke har nogen særligt høj følsomhed. Undersøgelser foretaget af Statens Veterinære Serumlaboratorium under anvendelse af følsomme, VRE-selektive metoder viser, at lavgradig forekomst af VRE er almindelig i slagtekyllingeflokke, selv om smittepresset på fødevarekæden er reduceret meget betydeligt.

I DANMAP overvåges også resistens hos bakterier isoleret fra syge dyr og mennesker. Selv om der er meget udbredt resistens blandt *E. coli* fra diagnostiske indsendelser fra kvæg og svin afspejler det sig ikke i en høj resistensforekomst hos *E. coli* fra prøver udtaget i forbindelse med slagtning. Det betyder, at resistens hos de sygdomsfremkaldende *E. coli* skal ses som et resistens reservoir, snarere end som en umiddelbar sundhedsrisiko for mennesker.

Hvad angår sygdomsfremkaldende bakterier fra mennesker er resistensforholdene gennemgående gunstige. Resistensudviklingen hos *Streptococcus pneumoniae* er dog årsag til bekymring. Penicillinresistens hos *S. pneumoniae* er et stigende problem verden over. I Danmark har den været sjælden indtil 1995, men har nu nået 4% blandt isolater fra i øvrigt sterile væv. Resistens overfor makrolider er steget parallelt hermed. En analyse af forbruget af antibiotika har ledt til den interessante konstatering at mange års brug af penicilliner og makrolider ikke medførte resistensproblemer, men at fremkomsten af penicillin- og makrolidresistens tidsmæssigt er faldet sammen med introduktionen af makrolidet azithromycin og er steget parallelt med stigningen i forbruget af dette stof i lægepraksis.

Overordnet set blev resistenssituationen i Danmark forbedret i 1999. Dette gælder for dyr såvel som for mennesker. Kombinationen af resistensovervågning og overvågning af antibiotikaforbrug giver et solidt grundlag for indgriben i de tilfælde, hvor dette måtte være nødvendigt. Forsat kontrol med resistenssituationen kræver dog, at alle de involverede parter overholder retningslinier for hensigtsmæssig brug af antibiotika.

Summary

Consumption of antimicrobials

The consumption of antimicrobials in food animals has declined from a total of just over 163 tonnes active compound in 1997 to just under 74 tonnes in 1999. These figures do not include antimicrobials used in aquaculture or in companion animals. The decline is due to the decision by Danish animal producers to discontinue all use of antimicrobials for growth promotion. This decision became effective in poultry, cattle and pigs over 35 kg live weight during the first quarter of 1998 and in pigs under 35 kg during the last half of 1999.

Interestingly, the first stage of the withdrawal of growth promoters did not lead to any increase in the consumption of therapeutics. In 1997, 28 mg antimicrobial per kg meat produced was used for therapy, compared with just under 27 mg per kg in 1998. Production figures for 1999 were not available at the time of writing; however, the consumption of tetracyclines for oral use increased by 4 tonnes between 1998 and 1999. According to industry sources the increase was caused by problems with gastrointestinal disease in weaner pigs following the discontinued use of growth promoters. Should this turn out to be the case, it would indicate that the current management practices in some pig herds cannot be sustained without more or less constant supply of antimicrobials. The increase brings the total consumption of tetracyclines to just over 16 tonnes or 26% of the total consumption of antimicrobials for treatment.

There has been concern about the consumption of fluoroquinonolones in food animals, because this group of compounds represents first choice antimicrobials when patients are hospitalised with gastro-enteritis. The authorities have recommended to veterinarians that they prescribe fluoroquinolones only sparingly. The manufacturer of the most prominent fluoroquinolone for veterinary use responded to concerns about resistance and withdrew one of the main preparations from the market. As a result, the total consumption has declined by 2/3 from 1998 to 1999. It is still too early to draw conclusions about the effects on resistance. However, this report shows that for indicator *E. coli* and for some of the animal pathogens, there was a decline in quinolone resistance in 1999, while there is not yet any evidence of such decline among zoonotic bacteria.

The total consumption of coccidiostats in the poultry sector has increased slightly, although much of the increase is caused by a change towards increased use of less potent coccidiostats that are included in the feed in high concentrations. These coccidiostats, the ionophores, have been included in the resistance-monitoring programme since its start and they do not appear to be problematic from a resistance point of view.

In human medicine, the consumption of antimicrobials in the primary sector has declined by almost 5% from 1998 to 1999. In contrast, the consumption in hospitals has increased slightly, by just over 1%. In both health sectors, penicillins and macrolides account for the main part of the total consumption. Newer types of antibiotics such as fluoroquinolones and cephalosporins only account for a very small part of the total consumption.

While the total consumption in DDD per 1,000 inhabitants is very low as compared with other countries, the consumption of antimicrobials between Danish counties differs by about 20%. This difference has been fairly constant in recent years. It is unlikely that it reflects differences in health status between the counties, if the number of hospital bed days in relation to the size of population represents an indicator of health status. We do not know whether the differences in consumption represent under- or overuse of antimicrobials.

During the winter 1998-99 Denmark experienced a sustained increase in the consumption of macrolides to treat respiratory tract infections. The present report describes how the announcement of this epidemic of *Mycoplasma pneumoniae* to doctors caused an immediate increase in the number of prescriptions for macrolides, an increase that only returned to normal levels several months after the epidemic was over.

Trends in resistance

It is a main objective of the DANMAP programme to compare the occurrence of resistance in like bacteria from the 3 reservoirs: food animals, food of animal origin and humans. However, we have found that this objective was very difficult to fulfil, because the isolates included in the monitoring programme did not represent

unbiased random samples. One such example is *Salmonella* Enteritidis, where we found a somewhat different resistance pattern in isolates from poultry than we saw in isolates from humans. Traceback showed that the reason for the disparity between results was caused by the fact that a number of the rather few isolates from poultry were epidemiologically linked.

A similar problem exists with *Salmonella* Typhimurium DT104 where a programme for active traceback means that this pentaresistant *Salmonella* subtype accounts for a disproportionally high percentage of *S.* Typhimurium isolates from cattle. The human data present a similar problem, due to two recognised food borne outbreaks in 1999. All cases from these outbreaks are included in the data shown which does imply that there is a bias, when comparison with the potential sources of infection is attempted. The problem in a more general form exists with the bacteria isolated from foods. With food isolates our experience shows that it is often very difficult to determine whether a particular food sample is of domestic or imported origin.

These experiences show that drawing conclusions about routes of spread of resistant bacteria on the basis of surveillance data requires a great deal of information about the sampling procedures on which the surveillance is based.

An overall assessment of the occurrence of resistance in *Salmonella enterica* shows that apart from the bias caused by *S.* Typhimurium DT104 the level is modest, as illustrated by the results for poultry and pigs. The occurrence is somewhat higher among isolates from foods known to be imported. In particular, almost one third of the isolates from the category “other poultry meat” which mainly represent imported foods were resistant to nalidixic acid.

DANMAP monitors resistance among commensal bacteria from food animals. A comparison of the resistance trends in *Enterococcus faecium* since 1995 when the programme was initiated shows that the marked changes in consumption of antimicrobial growth promoters is reflected in the occurrence of resistance. This applies to resistance to avoparcin, which was banned in 1995, as well as to macrolides, streptogramins and avilamycin. These results show that interventions such as those, which have been carried out, do have an effect on resistance. However, taking avoparcin as an example it is important to realize that while resistance (VRE) appears to occur fairly infrequently four years after the ban was imposed, this result has been obtained with a rather insensitive method. Studies at the Danish Veterinary Laboratory using selective methods show that VRE occur commonly in low numbers in broiler flocks, although the infective pressure on the food chain with VRE has been reduced dramatically.

DANMAP also monitors resistance in bacteria from diagnostic submissions from food animals and from humans. While there is very widespread resistance and potential treatment difficulties among *Escherichia coli* isolated from diseased cattle and pigs, this is not reflected in the isolates from animals at slaughter, where the occurrence is low. This means that resistance in the *E. coli* serotypes causing disease in animals should be seen as a resistance reservoir, rather than be considered an immediate public health hazard.

For human pathogens the resistance situation is generally favourable. However, resistance in *Streptococcus pneumoniae* isolates does represent an area of concern. Penicillin resistance in *S. pneumoniae* is an increasing problem worldwide. In Denmark, it was rare until 1995 and has now reached about 4% in samples from sterile body sites. This increase has been paralleled by an increase in macrolide resistance. Analysis of antimicrobial consumption data has led to the interesting observation that use for many years of penicillin and erythromycin did not seem to result in resistance. The emergence of resistance to penicillin and macrolides in *S. pneumoniae* coincided with the introduction of azithromycin and has paralleled its use in the primary health care sector.

Overall, the resistance situation in Denmark is improving. This applies equally to the situation in food animals and in humans. The combination of resistance monitoring and monitoring of antimicrobial consumption provides a strong basis for intervention, should this become necessary. However, successful control of antimicrobial resistance requires that all parties involved adhere to adequate guidelines for prudent use of antimicrobial agents. Our experience so far indicates that this can indeed be accomplished.

Demographic data

Table 1 shows the production of food animals. From 1998 to 1999, the number of cattle and pigs slaughtered decreased by 4.5% and 1.5%, respectively, while the production of poultry meat increased by 6.5%. The proportion of imported meat consumed has been estimated at 40% for beef and 25-35% for poultry, compared with less than 5% for pork. Table 2 shows detailed information on the distribution of the human population and on the health care system. Figure 1 shows the correlation between bed-days and inhabitant-days in Danish counties.

Table 1. Production of food animals (1,000's) and the production of meat (mio. kg), Denmark DANMAP 99

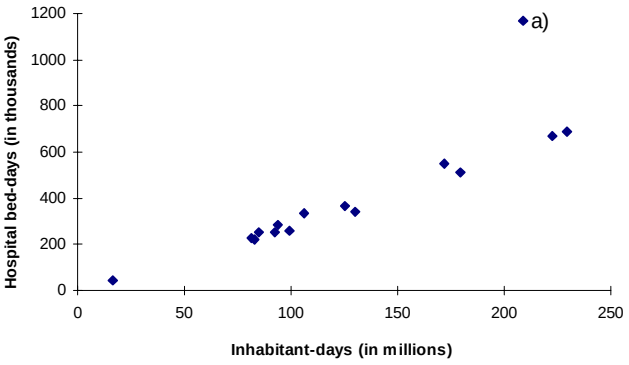
Year	Poultry		Cattle		Pigs	
	1,000 heads	mio. kg	1,000 heads	mio. kg	1,000 heads	mio. kg
1990	98,686	126	789	219	16,427	1,260
1992	111,536	150	862	236	18,559	1,442
1994	120,349	167	813	210	20,760	1,604
1996	111,495	165	789	198	20,530	1,592
1998	129,334	185	733	179	22,873	1,770
1999	140,116	197	700	a)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

a) Data on the production of meat in 1999 was not available

Table 2. Distribution of the human population by county and the health care structure, Denmark 1997 and 1999 DANMAP 99

County no.	County name a)	No. inhabitants (1/1/1999)	No. inh./km2 (1999)	No. Inh. /GP b) (1999)	No. bed-days c) (1997)	No. hospitals (1997)
1	Copenhagen Municipality	491,082	5,565	1,376	1,166,094 d)	5
2	Frederiksberg Municipality	90,227	10,288	1,611	-	1
3	Copenhagen	612,053	1,164	1,598	667,118	4
4	Frederiksborg	363,098	269	1,650	341,076	3
5	Roskilde	229,794	258	1,677	224,061	2
6	West Zealand	293,709	98	1,579	333,797	6
7	Storstroem	258,761	76	1,540	281,767	5
8	Bornholm	44,529	76	1,310	44,967	1
9	Funen	471,732	135	1,547	547,682	10
10	South Jutland	253,771	64	1,566	250,343	4
11	Ribe	224,348	72	1,580	226,494	5
12	Vejle	346,182	116	1,595	368,270	6
13	Ringkoebing	272,644	56	1,623	261,496	5
14	Aarhus	634,435	139	1,582	690,284	10
15	Viborg	233,396	57	1,577	251,148	5
16	North Jutland	493,816	80	1,543	509,482	7
All	Denmark	5,313,577	123	1,561	6,164,079	79

a) The municipalities of Copenhagen and Frederiksberg both have status of county
b) GP, general practitioner
c) Excl. psychiatry and private hospitals
d) Public hospitals in Copenhagen and Frederiksberg municipalities are under the same administration



a) Copenhagen and Frederiksberg municipalities combined. The Copenhagen Municipality includes one university hospital which recruits complicated cases nationwide and patients from other counties, Greenland and the Faroe Islands.

Figure 1. Correlation between the the number of hospital bed-days and the number of inhabitant-days by county, Denmark, 1997

Consumption of antimicrobials

Consumption in food animals

Therapeutics

Data on consumption on antimicrobials for therapy are collected by the Danish Medicines Agency. Please see Appendix 1 for details. Table 3 compares the consumption of antimicrobials in food animals in 1998 and 1999 by ATC group and route of administration. The total consumption has increased by 8.0%. The change has mainly been in the consumption of oral tetracyclines, which has increased by about 4,000 kg active compound or 40% from 1998 to 1999. The increase coincides with the voluntary withdrawal of antimicrobial growth promoters in pigs under 35 kg live weight. Whether the association is causal is not clear but according to industry sources the increase was caused by problems with gastrointestinal disease in weaner pigs following the discontinued use of growth promoters.

The consumption of fluoroquinolones has decreased markedly, about 65%. The decline has occurred in oral as well as injectable formulations and may be a response to the prudent use recommendations mentioned in the DANMAP 98 report, in addition to the withdrawal from the market of an oral formulation of one of the most widely used fluoroquinolones, enrofloxacin. In addition to the use of fluoroquinolones, the quinolone oxolinic acid is used in aquaculture. The consumption in 1999 amounted to 1,200 kg active compound. Aquaculture also used 2,800 kg sulfonamides + trimethoprim. Table 4 shows the trend in consumption of antimicrobials between 1986 and 1999.

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

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ATC-group	Compound	Oral		Injection		Intramammary		Intrauterine		Total	
		1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
J01A	Tetracyclines	9,600	13,600	2,400	2,600	< 25	<25	< 25	< 25	12,100	16,200
J01C	Extended Spectrum Penicillins	4,000	3,700	2,500	2,700	150	150	0	0	6,600	6,600
	Narrow Spectrum Penicillins	0	0	14,100	14,500	150	150	50	50	14,300	14,700
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	4,400	3,400	3,300	3,400	0	< 25	0	0	7,700 a	6,800 a)
J01F	Macrolides + lincosamides	2,100	3,400	1,800	1,900	0	< 25	0	0	4,000	5,300
J01G	Aminoglycosides	4,600	4,500	2,900	2,800	150	150	100	100	7,800	7,500
J01M	Quinolones	250	50	150	100	0	0	0	0	400 b)	150 b)
J01X	Others	3,100	3,300	250	300	< 25	< 25	0	0	3,400	3,600
J01	Total	28,100	32,000	27,500	28,400	450	500	1,200	1,200	57,300	61,900

a) Does not include consumption in aquaculture

Table 4. Trends in the estimated total consumption 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 1996-1999: Danish Medicines Agency

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

a) Does not include consumption in aquaculture

b) The macrolides also includes: tiamulin

Growth promoters and coccidiostats

Information on the consumption of antimicrobial growth promoters and coccidiostats is collected by the Danish Plant Directorate. The consumption of antimicrobial growth promoters has declined markedly from 49 tonnes active compound in 1998 to 12 tonnes in 1999 (Table 5). Of the total consumption in 1999, 10.9 tonnes were used during the first half of the year. This means that the consumption of antimicrobials for growth promotion has declined by almost 90% since 1997. This trend is a result of the decision in early 1998 by Danish farming organisations to phase out the use of growth promoters and of the EU ban on those growth promoters that confer cross resistance to antimicrobials used in human medicine.

In contrast, the use of coccidiostats in poultry has increased by almost 40% (Table 6). The increase is mainly in the ionophore group of compounds, mainly monensin and narasin, which are used in the broiler and turkey production. According to the industry, a factor contributing to the increase may be that in recent years coccidiostats tend to be used in the maximum permitted concentrations. It must also be borne in mind that the dosage range for the compounds shown in Table 6 vary widely and that comparisons based on the total consumption are not very meaningful. The ionophores belong to compounds that are used in high concentrations, over 100 ppm.

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

DANMAP 99

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

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

DANMAP 99

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

Few antimicrobial residues in slaughtered livestock animals and milk

Since 1987, the Danish Veterinary and Food Administration has organised a continuous surveillance of antimicrobial residues in slaughtered livestock animals. The sampling scheme is stratified, which means that the sampling rate is higher in for example sows and boars than in slaughterpigs, because residue violations are expected to occur more often in the former group. The applied analytical method was a microbiological test, which is sufficiently sensitive for most but not all of the relevant substances.

Every year more than 16,000 slaughterpigs (0.1% of the production) were examined for antimicrobial residues at the time of slaughter. From 1994 to 1999, a total of 102,680 samples were collected from slaughterpigs, 19 (0.02%) were positives, which is extremely low. In 1999 alone, antimicrobial residues were detected in 2 out of 18,126 samples from slaughterpigs (0.01%).

In 1995, the sampling frequency was increased among sows from 0.5% to 1% of the number of animals slaughtered. From 1993 to 1999, a total of 26,100 samples were collected from sows, 34 (0.13%) were positive. In 1999, antimicrobial residues were detected in 3 out of 4,737 samples from sows (0.06%).

Furthermore, in recent years specific methods of analysis have been used for those substances which are not detected with suficient sensitivity by the microbiological method. In 1999, the specific methods were applied on 1,497 samples from pigs. Of these, 701 samples were examined for tylosin and in 3 samples (0.43%) tylosin residues were detected. In 1999, a total of 27,314 samples from pigs (including sows and boars) were examined and antimicrobial residues were found in 0.03%.

The sampling scheme for adult cattle and fattening calves was 0.5% and 0.2% of the production, respectively. From 1997 to 1999, a total of 6,805 samples were analysed and 4 (0.06%) were positive. In 1999, 2 out of 1,914 samples were positive (0.10%).

Milk samples are collected on all dairy farms 13 times a year. Since 1993, antimicrobial residues have been detected in 0.04% of the samples per year. In 1998, a total of 147,915 samples were analysed with 62 positives, corresponding to 0.04%.

Comparison to other European Member States should be done with great caution. For example when comparing test results from pigs information often is lacking on risk groups (the proportion of sows and boars versus fattening pigs) and the applied analytical method. In Denmark kidney tissue is analysed, because most of the antibiotics are concentrated here, whereas other countries analyse muscle tissue.

However, it can be concluded that Sweden, Finland and the UK were in line with Denmark. In these countries, antimicrobial residues were detected in around 0.03% of the samples from pigs. Regarding cattle slaughtered, Finland, the UK and the Netherlands were in line with Denmark. Here, antimicrobial residues were found in 0.06% of the samples. Finally when regarding milk samples, the UK, Portugal, the Netherlands, Sweden, Finland and Austria were in line with Denmark with 0.04% positive samples.

Further information on the monitoring of residues in Denmark and Europe can be obtained from Senior Scientific Adviser Flemming Kæreby (FK@FDIR.DK).
Questions concerning antibiotic resistance in relation to residues should be addressed to Sigrid Andersen (M.Sc., Ph.D.) at SRA@FDIR.DK.

Denmark initiates monitoring of antimicrobial consumption in animals

One of the key recommendations at the WHO meeting held in 1997 on the medical impact of the use of antimicrobials in food animals and on the EU conference in Copenhagen in 1998 on “The Microbial Threat”, was that countries should establish programmes to monitor the use of antimicrobials in humans and animals. Since 1996, all consumption of antimicrobials for humans has been recorded on the basis of prescriptions redeemed at pharmacies. There has however, been no similar monitoring of the use in animals at a similar level of detail.

In Denmark, all antibiotics are prescription-only medicines and with very few exceptions, veterinary medicines are distributed exclusively through pharmacies. VETSTAT (Veterinary Medicine Statistic) is a programme designed to monitor the use of all prescription medicines in animals, in Denmark. It will monitor all therapeutic use of medicines in production animals, including sera and vaccines, as well as all use of coccidiostats at farm level. The term “production animals” in this context includes pigs, cattle, poultry, sheep, fish and mink. Medicine used for companion animals including horses will be monitored as well but will be less detailed.

The proportion of medicines sold from the pharmacy directly to the farmers based on prescriptions from veterinarians accounts for about 85% of the total turnover, a proportion which is increasing. Therefore, the pharmacy will play a major role in the recording of sales. When issuing a prescription for use in production animals the veterinarian will include information on the identity of the farm, the species and the age group of the animals and the reason for prescribing, in addition to the name and quantity of drug.

Medicines sold or used by veterinarians must be reported by the practitioners themselves to VETSTAT at a similar level of detail.

Similarly, feed mills will report all sales of medicated animal feed and feed containing coccidiostats. Data from pharmacies, veterinarians and feed mills are collected in a central database and forwarded to the Danish Zoonosis Centre.

The pharmacies and the veterinarians started to collect data on May 1, 2000. The feed mills will be ready to start reporting on July 1, 2000. It is expected that the data quality will be sufficiently robust at the end of year 2000. Hereafter, it will be possible for farmers and veterinarians to compare their own data with statistical information so that consumption data can be used as a farm management tool.

The register will form the basis of pharmaco-epidemiological studies, including the analysis of prescription habits, on the consumption of medicine in relation to animal species, age of animals and diagnosis.
Further details may be obtained by contacting Erik Jacobsen, M. Sc. Pharm. The Danish Zoonosis Centre, Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark. E-mail: eja@svs.dk

Consumption in humans

Primary health care sector

Table 7 presents the consumption of antimicrobials for systemic use in primary health care from 1994 to 1999. After the slight increase observed in 1998, there was an overall decrease in 1999. With 11.3 DDD/1,000 inhabitant-days, antimicrobial use in Danish primary health care is now back to its 1997 level. This general decrease was observed in all Danish counties, with the exception of Bornholm county where the consumption already was one of the lowest in Denmark (Figure 2). Most of this overall decrease can be explained by a decrease in the use of beta-lactamase sensitive penicillins and penicillins with extended spectrum, which has not been compensated by the use of other antimicrobials (Table 7). Additionally, there has been a 13.7% decrease in the consumption of fluoroquinolones — a decrease related to cessation of their subsidisation in May 1999.

Although antimicrobial use in primary health care in Denmark is still one of the lowest in the world, differences among counties remain. With less than 10 DDD/1,000 inhabitant-days in 1999, Aarhus and Bornholm counties had the lowest consumption while 5 other counties used more than 12 DDD/1,000 inhabitant-days (Figure 3). Although there have been variations in the level of antimicrobial use in primary health care since 1994, the ranking of Danish counties by their antimicrobial use has somewhat remained the same (Figure 2) and there is presently no explanation for these differences.

In 1999, beta-lactamase sensitive penicillins 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, each representing approximately 20% of the total use. In comparison, fluoroquinolones, combinations of penicillins including beta-lactamase inhibitors (essentially amoxicillin-clavulanate) and cephalosporins only represented 1.8%, 0.2% and less than 0.1% of total use in primary health care, respectively.

Despite a 4.1% decrease in 1999, macrolide use remains high and above the 1.0-1.5 DDD/1,000 inhabitant-days level recommended in the DANMAP 98 report following the Finnish experience (Seppälä H., et al. NEJM 1997; 337:441-446). Additionally, the consumption of individual drugs among macrolides has changed during the past years (Figure 4). Azithromycin is now the second most frequently used macrolide after erythromycin. Finally, in the DANMAP 98 report, we mentioned a sharp increase in macrolide consumption during the last two months of 1998. It was found that the increase was related to a *Mycoplasma pneumoniae* outbreak. During 1999, this phenomenon has been explored in detail and the results are presented in a separate box, page 18.

Hospitals

Table 8 presents the consumption of antimicrobials for systemic use in hospitals from 1997 to 1999. Overall, there has been an increase of 1.3% between 1998 and 1999. However, this increase was not observed in all counties and some counties even showed a decrease in hospital antimicrobial use from 1998 to 1999 as shown in Figure 5. There has been no national initiative in 1999 to promote control of antimicrobial use in

Table 7. Consumption of antimicrobials for systemic use in human primary health care (DDD/1,000 inhabitant-days), Denmark

DANMAP 99

ATC group	Therapeutic group	Year						% Change
		1994	1995	1996	1997	1998	1999	(1998-1999)
J01AA	Tetracyclines	1.72	1.58	1.07	0.98	0.98	0.93	- 6.1
J01CA	Penicillins with extended spectrum	2.95	2.83	2.44	2.39	2.39	2.29	- 4.2
J01CE	Beta-lactamase sensitive penicillins	4.46	4.63	4.46	4.57	4.81	4.48	- 6.9
J01CF	Beta-lactamase resistant penicillins	0.24	0.28	0.29	0.34	0.40	0.48	+ 20.2
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.03	0.03	0.02	0.02	0.03	0.02	- 8.9
J01DA	Cephalosporins and related substances	0.02	0.02	0.02	0.02	0.02	0.01	- 11.1
J01EA	Trimethoprim and derivatives	0.31	0.31	0.31	0.30	0.32	0.32	+ 1.1
J01EB	Short-acting sulfonamides	0.45	0.44	0.42	0.41	0.41	0.38	- 6.2
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.09	0.08	0.07	0.08	0.04	0.03	- 23.6
J01FA	Macrolides	2.07	2.11	1.88	1.96	2.20	2.11	- 4.1
J01FF	Lincosamides	0.00	0.00	0.00	0.01	0.01	0.01	- 9.9
J01MA	Fluoroquinolones	0.36	0.31	0.24	0.22	0.23	0.20	- 13.7
J01XB	Polymyxins	0.03	0.03	0.03	0.03	0.02	0.03	+ 4.4
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.02	0.02	0.02	+ 9.1
J01	Antimicrobials for systemic use (Total)	12.75	12.66	11.26	11.35	11.87	11.31	- 4.7

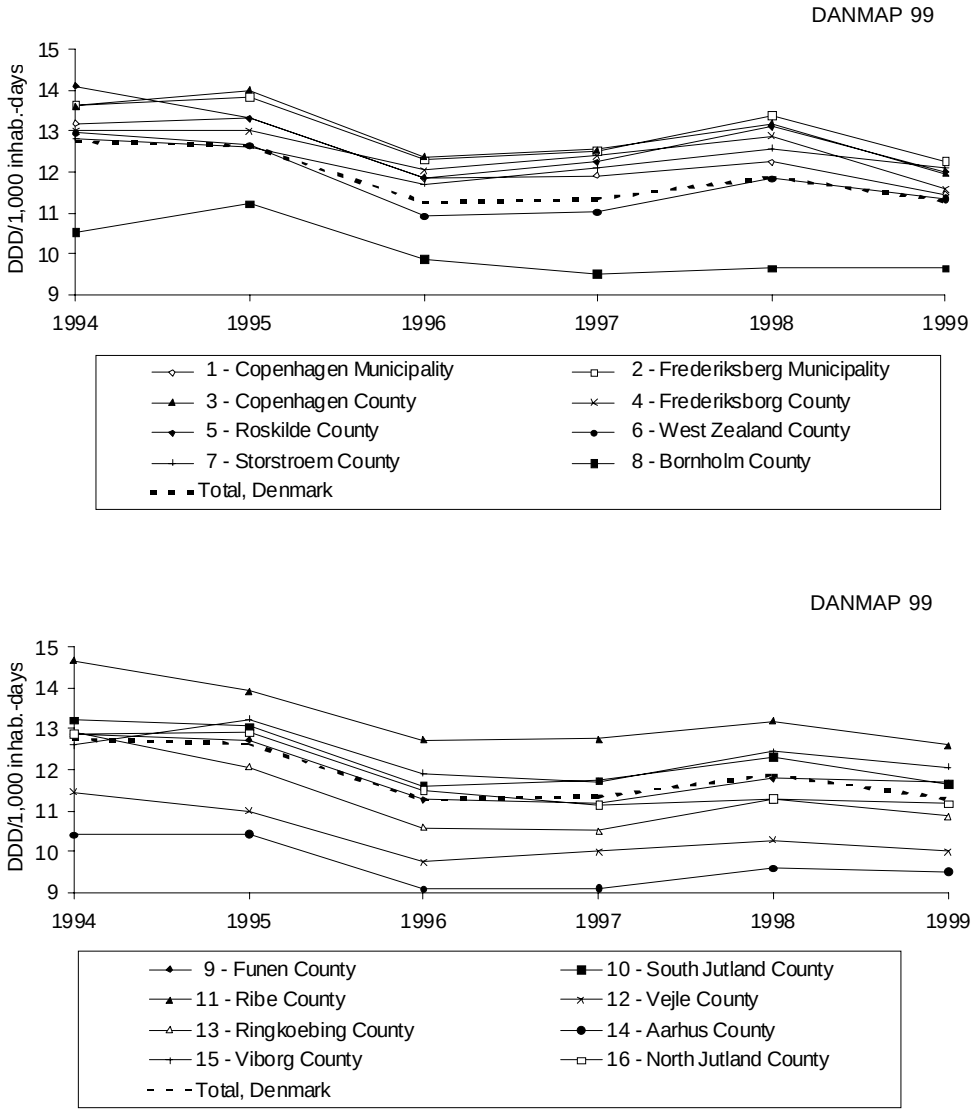


Figure 2. Consumption of antimicrobials for systemic use (J01) in human primary health care by county, Denmark

hospitals and little is known about the initiatives of single counties or hospitals in this field, which would explain a decrease of consumption in some counties. Finally, data from the Copenhagen and Frederiksberg municipalities (counties 1 and 2, indicated by a grey shade in Figure 5) are not readily comparable over time because of changes in the health care structure in 1997 and 1998.

Differences were observed among counties in 1999. However, hospital consumption in a county represents the pooled consumption of all hospitals in this county and there are certainly differences among hospitals within the same county. This aspect has not yet been explored except in the Copenhagen Municipality

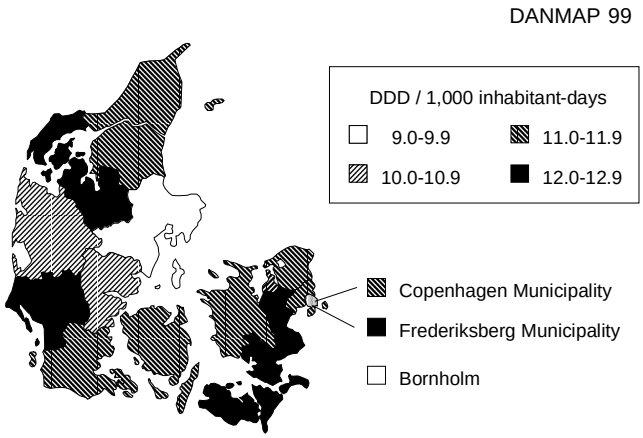


Figure 3. Consumption of antimicrobials for systemic use (J01) in human primary health care by county, Denmark, 1999

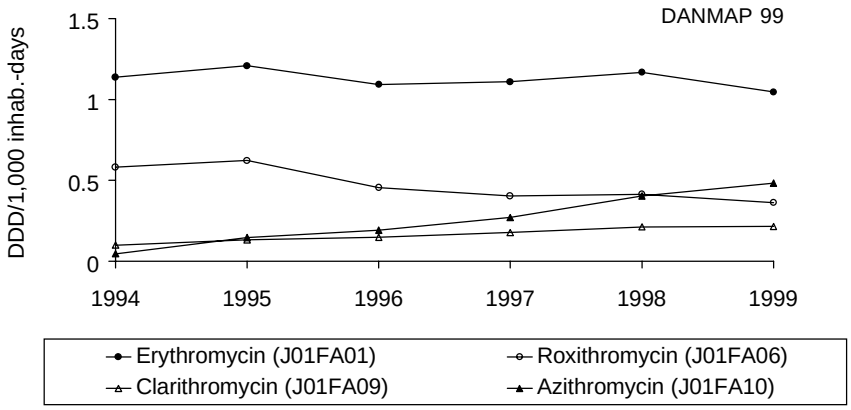


Figure 4. Consumption of macrolides (J01FA) for systemic use in human primary health care, Denmark

(county 1). In 1999, this county had a hospital consumption approximately twice the national average (Figure 5). However, hospitals in the Copenhagen Municipality include one university hospital, which recruits patients from all other counties, from Greenland and from the Faroe Islands. The denominator used in this report, i.e. the number of inhabitant-days in the Copenhagen Municipality (because of unavailability of the number of patient-days, see Appendix 1), is therefore underestimated. The patient population in this hospital is also different and represents more complicated cases.

Although Figure 5 shows differences in the overall level of hospital antimicrobial use among counties, one should be cautious when trying to make such comparisons. First, because the number of bed-days for 1999 was not available, consumption is expressed as a number of DDD per 1,000 inhabitant-days. In 1997, the number of bed-days was correlated with the number of inhabitant-days in Danish counties, with exception of the Copenhagen and Frederiksberg municipalities (Figure 1 page 9); however, this measurement unit is far from being ideal.

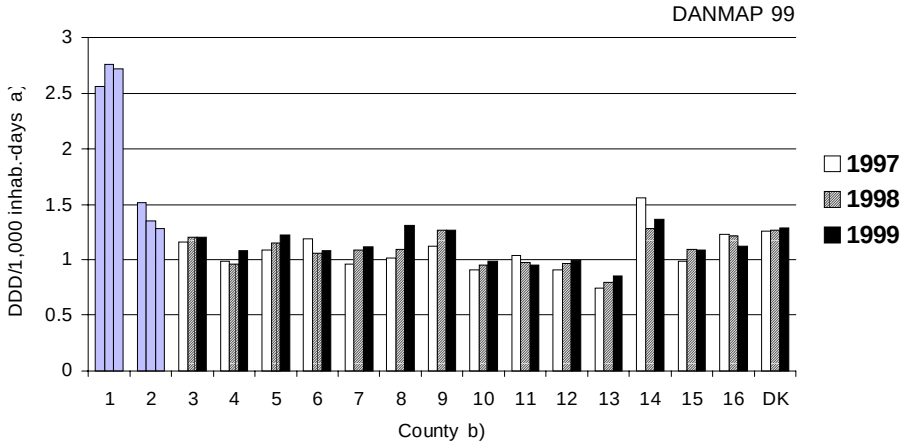
The distribution of hospital antimicrobial use by class is presented in Figure 6. In 1999, all penicillins combined represented more than 60% of hospital antimicrobial use in Denmark. Cephalosporins (mainly cefuroxime) and fluoroquinolones (mainly ciprofloxacin) only

Table 8. Consumption of antimicrobials for systemic use in hospitals (DDD/1,000 inhabitant-days), Denmark a)

DANMAP 99

ATC group	Therapeutic group	Year			% Change
		1997	1998	1999	(1998-1999)
J01AA	Tetracyclines	0.01	0.01	0.01	- 13.1
J01CA	Penicillins with extended spectrum	0.35	0.35	0.34	- 3.2
J01CE	Beta-lactamase sensitive penicillins	0.26	0.28	0.29	+ 4.0
J01CF	Beta-lactamase resistant penicillins	0.14	0.14	0.15	+ 3.2
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	< 0.01	< 0.01	< 0.01	+ 18.6
J01DA	Cephalosporins and related substances	0.10	0.10	0.11	+ 8.6
J01DH	Carbapenems	0.01	0.01	0.01	+ 28.2
J01EA	Trimethoprim and derivatives	0.01	0.01	0.01	- 14.9
J01EB	Short-acting sulfonamides	0.04	0.04	0.04	- 5.3
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.01	0.04	0.04	- 2.8
J01FA	Macrolides	0.11	0.11	0.10	- 7.1
J01FF	Lincosamides	< 0.01	0.01	< 0.01	- 18.0
J01GB	Aminoglycosides (other than streptomycins)	0.10	0.06	0.06	+ 1.9
J01MA	Fluoroquinolones	0.05	0.05	0.06	+ 18.7
J01XA	Glycopeptides	0.01	0.01	0.01	+ 18.0
J01XB	Polymyxins	< 0.01	< 0.01	< 0.01	+ 31.7
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	+ 3.8
J01XD	Imidazole derivatives	0.04	0.04	0.05	+ 10.3
J01	Antimicrobials for systemic use (Total)	1.26	1.27	1.29	+ 1.3

a) The no. of bed-days for 1999 was not known at the time of writing of the DANMAP report and could not be used to express hospital antimicrobial use. In 1997, the no. of hospital bed-days was correlated to the no. of inhabitant-days, with the exception of the Copenhagen and Frederiksberg municipalities combined (Figure 1)



a) The no. of bed-days for 1999 was not available at the time of writing of this report and could not be used to express hospital antimicrobial use. In 1997, the no. of hospital bed-days was correlated to the no. of inhabitant-days, with the exception of the Copenhagen (1) and Frederiksberg (2) municipalities (in grey on this figure)
b) For correspondence between county code and name, please see Table 2.

Figure 5. Consumption of antimicrobials for systemic use (J01) in hospitals by county, Denmark

represented 8.5% and 4.7% of total hospital use, respectively. The very low consumption of first-generation cephalosporins (less than 1% of total use) is probably explained by the fact that this class of antimicrobials is very rarely used for surgical prophylaxis. Tetracyclines, combinations of penicillins including beta-lactamase inhibitors, carbapenems and glycopeptides each represented less than 1% of total hospital use. Although these broad-spectrum antimicrobials still represent a limited fraction of use in Danish hospitals, their use increased between 1998 and 1999. Hospital consumption of carbapenems increased by 28% and hospital consumption of combinations of penicillins including beta-lactamase inhibitors, fluoroquinolones and glycopeptides increased by approximately 18% each (Table 8). This recent increase is of concern and deserves a close monitoring in the future.

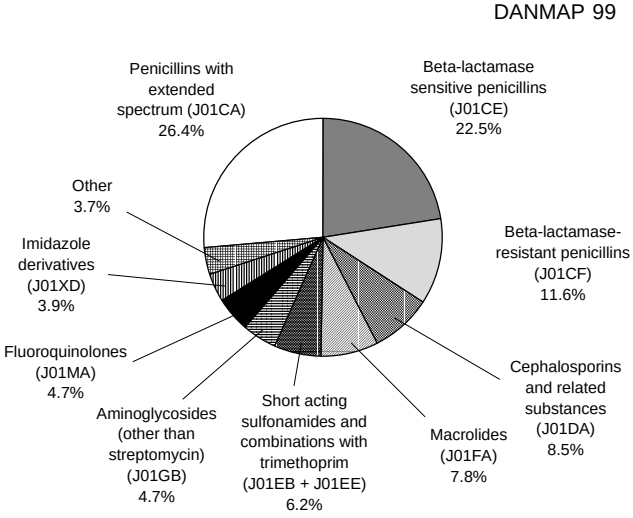


Figure 6. Distribution of the consumption of antimicrobials for systemic use (J01) in hospitals, Denmark, 1999

Announcement of a *Mycoplasma pneumoniae* epidemic in the Danish community and recommendation of macrolides for treatment leads to prolonged consumption increase

An epidemic of *Mycoplasma pneumoniae* respiratory tract infections was observed in Denmark during the second half of 1998. The fraction of positive PCR tests was used as a surrogate marker for incidence. Starting in May, 1998, there was an increase in both the number and the fraction of positive PCR tests which reached 25.3% positive tests in October, 1998 (Figure 7).

In mid-November 1998, i.e. in week 46, the *M. pneumoniae* outbreak was announced in the Danish weekly infectious disease prevention newsletter EPI-NEWS (Available from: URL: http://www.ssi.dk/en/epi-nyt.uk/1998/week46.htm). The article mentioned that the antibiotics of choice for treating this type of infection were macrolides.

The announcement resulted in a 10-fold increase in the number of specimens submitted for *M. pneumoniae* PCR testing and a 2-fold increase in *M. pneumoniae* serology tests in December, 1998 as compared to November, 1998. However, the fraction of positive results for both tests started to decrease in November (Figure 7). The announcement also resulted in an immediate increase in the number of macrolide prescriptions redeemed each week (Figure 8). The monthly number of redeemed macrolide prescriptions reached 89,810 in December, 1998, nearly 2-fold higher than the average number observed in December 1994-1997 (Figure 7). Although it cannot be ruled out that increased awareness and more frequent prescribing of macrolides contributed to control of the outbreak, the increase in macrolide consumption was delayed when compared to the peak of the outbreak. The fraction of positive *M. pneumoniae* PCR and serology tests was back to normal by February 1999, when there was still a 1.6-fold excess of redeemed macrolide prescriptions compared with February 1994-1997. The monthly number of macrolide prescriptions only returned to its average level in April 1999.

Our experience with this epidemic stresses the necessity of timely reporting to guide empirical treatment by general practitioners. However, reports should emphasize the need for proper diagnostic procedures in order to avoid unnecessary testing and excessive antimicrobial use.

Further details may be obtained by contacting Thomas Lund Sørensen, MD, Department of Microbiological Research and Development, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark. E-mail: tls@ssi.dk

DANMAP 99

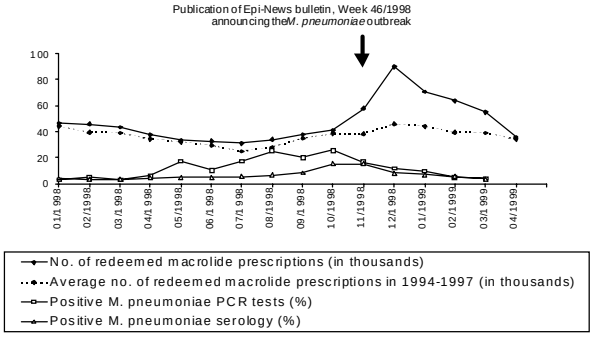


Figure 7. Monthly macrolide prescriptions and positive Mycoplasma pneumoniae *serology and PCR tests, Denmark*

DANMAP 99

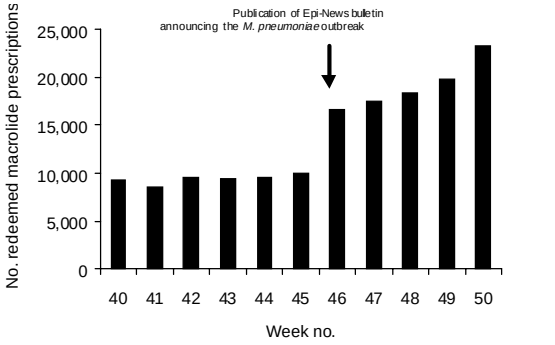


Figure 8. Weekly redeemed macrolide prescriptions, Denmark. weeks 40-50, 1998

Resistance in zoonotic bacteria

Salmonella

In 1999, salmonellosis was the second most common foodborne zoonosis in Denmark. The majority of all human S*almonella* cases were caused by *Salmonella* Enteritidis (62%). *S.* Enteritidis was the predominant serotype in egg layers while the prevalence in other animal species was low (Annual Report on Zoonoses in Denmark 1999 - Available from The Danish Zoonosis Centre at: http://www.svs.dk). The second most frequent serotype was *Salmonella* Typhimurium which accounted for 18% of all human cases. This serotype predominated in pig herds but was also prevalent in both broiler flocks and cattle herds.

The levels of antimicrobial resistance varied between different *Salmonella* serotypes. Resistance to one or more antibiotics was infrequent among *S.* Enteritidis isolates but common among *S.* Typhimurium. Similar differences existed between phage types of *S.* Typhimurium where DT104 represented a highly resistant phage type. Therefore the distribution of serotypes and phage types must be considered when the occurrence of resistance is compared between isolates from different animal species.

Salmonella from food animals

Tables 9 and 10 show the *Salmonella* serotype and *S.* Typhimurium phage type distributions of isolates from food animals included in this report. The *Salmonella* isolates from pigs and poultry were mainly from subclinical infections, while the majority of isolates from cattle were from clinical salmonellosis. *S.* Typhimurium DT104 account for many of the isolates from cattle

Table 10. Distribution (%) of Salmonella *Typhimurium phage types from animals, food and humans, Denmark, 1999*

DANMAP 99

Phage type	Poultry	0ther poultry meat a)	Cattle	Pigs	Humans
2		11			<1
8		6			<1
10				2	<1
12	14	6	45	45	20
15a		6		1	
17	3			9	4
41	22		5		<1
66	5		3	14	5
104		28	33	2	24
107				2	1
110	22		3	2	2
120	3	28		<1	4
135	19		3		2
170			5	3	5
193				3	4
195				<1	<1
204			3	<1	
208				1	<1
u288				2	8
u302				1	1
Others incl. not typable	14	17	3	12	19
Number of isolates	37	18	40	182	559

a) Other poultry meat is mainly turkey and duck and no broiler meat is included. Ten out of 150 isolates from poultry meat (Table 9) are from Danish products. None of the *S.* Typhimurium DT104 isolates were of Danish origin.

Table 9. Distribution (%) of Salmonella *serotypes isolated from animals, food and humans, Denmark, 1999*

DANMAP 99

Serotypes	Poultry	Broiler meat a)	Other poultry meat b)	Cattle	Beef c)	Pigs	Pork c)	Humans d)
4.12:b:-	11	0	0	0	0	<1	0	0
Derby	3	0	3	6	5	12	2	<1
Dublin	0	0	0	53	60	0	4	<1
Enteritidis	28	8	7	2	0	<1	2	62
Hadar	<1	18	14	0	0	0	0	2
Heidelberg	1	34 e)	6	0	0	<1	2	<1
Indiana	4	6	11	0	10	0	2	<1
Infantis	13	8	1	0	0	4	16	<1
Saintpaul	0	0	5	0	0	0	4	<1
Typhimurium	26	3	12	34	15	72	29	18
Virchow	0	6	8	0	0	<1	0	2
Others incl. not typable	13	16	35	5	10	10	39	13
Number of isolates	142	99	150	118	20	252	49	3,268

a) 8 out of 99 isolates were of Danish origin
b) 10 out of 150 isolates of Danish origin
c) Include samples of imported products
d) Include all isolates typed in 1999 (excl. *Salmonella* Typhi, *Salmonella* Paratyphi A, and *Salmonella* Paratyphi B)
e) All imported

herds (Table 10). This results from trace-back efforts when DT104 is detected in a herd. Therefore, the figures in Table 10 are biased and do not reflect the *S. Typhimurium* DT104 prevalence among cattle herds.

Table 11 shows the occurrence of resistance among all *Salmonella* serotypes from animals in 1999 and Table 12 the results for *S.* Enteritidis from broilers and layers and *S.* Typhimurium from broilers and layers, cattle and pigs.

The proportion of *S.* Typhimurium isolates resistant to nalidixic acid or ciprofloxacin has remained unchanged from 1998 to 1999 for all food animals. When comparing the nalidixic acid MIC distributions from 1998 and 1999 there has been a slight shift towards increased susceptibility while the ciprofloxacin distribution has remained unchanged (data is not shown).

Resistance to nalidixic acid among *S.* Enteritidis isolates from poultry was not found in any of the isolates in 1997 but in 7% in 1998. In 1999, 8 out of 40 isolates (20%) were resistant to nalidixic acid, which constitutes a significant increase. A detailed investigation showed that the isolates originated from the table egg sector, Two out of 8 isolates originated from layer flocks, 5 isolates were from rearing flocks and 1 from a hatchery. All isolates were phage type 8 and all 8 farms had trade contacts to the same hatchery.

Consequently the increase in nalidixic acid resistance among *S.* Enteritidis was most likely due to clonal spread caused by trade with day old chickens harbouring strains resistant to nalidixic acid.

All isolates resistant to nalidixic acid showed decreased susceptibility to fluoroquinolones. Several reports have indicated that decreased susceptibility to fluoroquinolones is associated with lack of clinical efficacy.

Salmonella from foods

329 strains of *Salmonella* were obtained from the Municipal Food and Environmental Laboratories. The strains were isolated as part of the routine inspection conducted by these laboratories at wholesale and retail outlets. The isolates originated from beef (6%), pork (15%), broiler meat (30%), other poultry meat (46%), and foods of other origin (3%). The group “other poultry meat” included 43% isolates from turkey meat and 18% from duck meat. Please refer to “Annual Report on Zoonoses in Denmark 1999“ for information about prevalences.

The distribution of *Salmonella* serotypes is shown in Table 9. In pork *S.* Typhimurium was represented by 14 isolates out of 49, including 4 isolates of *S.* Typhimurium DT104. Due to the low number of isolates these data are not shown in Table 10.

Table 13 shows the occurrence of resistance among all *Salmonella* serotypes from food. As opposed to previous years the susceptibility of isolates from foods was tested by MIC-determinations (Sensititre).

In 1999, the sampling scheme for food products was changed. As a result more than 90% of the *Salmonella* strains isolated from broiler meat and other poultry originated from imported products. The exact distribution between isolates of Danish and imported origin was not known for pork and beef. Therefore, the occurrence of resistance among *Salmonella* isolated from food products could not be compared to the results obtained in previous years.

Table 11. Susceptibility and occurrence of resistance (%) among Salmonella *from food animals, Denmark, 1999* DANMAP 99

ATC-group	Compound	*Salmonella enterica*											
		Poultry				Cattle				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	2	2	4	1 - >32	2	32	15	1 - >32	2	>32	13
J01B	Chloramphenicol	2 - >64	8	8	1	2 - >64	4	>64	14	1 - >64	8	8	2
	Florfenicol	2 - 16	4	8	0	2 - >32	4	32	14	2 - 32	4	8	1
J01C	Ampicillin	0.5 - >32	2	2	4	0.5 - >32	1	>32	14	0.5 - >32	1	2	7
J01E	Sulfonamide	8 - >512	64	128	8	16 - >512	32	>512	19	8 - >512	64	>512	17
	Trimethoprim	0.5 - >32	0.5	0.5	1	0.5 - >32	0.5	0.5	1	0.5 - >32	0.5	0.5	6
J01G	Gentamicin	0.5 - >32	0.5	0.5	1	0.5 - 0.5	0.5	0.5	0	0.5 - 32	0.5	0.5	0
	Kanamycin	1 - >64	2	4	1	1 - 64	2	2	1	1 - >64	2	4	4
	Streptomycin	2 - >128	8	16	5	2 - >128	8	64	18	4 - >128	8	128	17
J01M	Ciprofloxacin	0.03 - 1	0.03	0.06	0	0.03 - 0.5	0.03	0.03	0	0.03 - 0.5	0.03	0.03	0
	Nalidixic acid	2 - >128	4	8	10	2 - >128	4	8	3	4 - >128	4	8	1
J01X	Colistin	2 - 4	2	2	0	2 - 4	2	4	0	2 - 4	2	2	0
	Carbadox	8 - 32	8	16	0	8 - 64	16	16	0	8 - 128	8	16	0
	Nitrofurantoin	32 - 128	32	64	1	32 - 64	32	64	0	32 - 128	32	64	1
Number of isolates					142				118				252

Table 12. Susceptibility and occurrence of resistance (%) among Salmonella *Enteritidis and* Salmonella *Typhimurium from food animals, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*S.* Enteritidis				*S.* Typhimurium											
		Poultry				Poultry				Cattle				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	2	2	5	1 - >32	2	2	5	1 - >32	2	>32	38	1 - >32	2	>32	14
J01B	Chloramphenicol	4 - 32	8	8	3	4 - 8	8	8	0	2 - >64	8	>64	33	1 - >64	8	8	3
	Florfenicol	2 - 16	4	4	0	2 - 8	4	8	0	2 - >32	4	>32	33	2 - 32	4	8	2
J01C	Ampicillin	1 - 2	2	2	0	1 - >32	2	2	5	1 - >32	1	>32	33	0.5 - >32	1	4	8
J01E	Sulfonamid	8 - >512	64	64	3	32 - >512	64	>512	14	32 - >512	64	>512	43	8 - >512	64	>512	19
	Trimethoprim	0.5 - 2	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - >32	0.5	0.5	6
J01G	Gentamicin	0.5 - 32	0.5	0.5	3	0.5 - >32	0.5	1	3	0.5 - 0.5	0.5	0.5	0	0.5 - 1	0.5	0.5	0
	Kanamycin	1 - >64	2	2	3	1 - 16	2	4	0	1 - 4	2	4	0	1 - >64	2	4	6
	Streptomycin	2 - 64	4	8	3	8 - >128	8	16	5	8 - >128	16	128	43	4 - >128	8	128	20
J01M	Ciprofloxacin	0.03 - 1	0.03	0.25	0	0.03 - 0.03	0.03	0.03	0	0.03 - 0.03	0.03	0.03	0	0.03 - 0.5	0.03	0.03	0
	Nalidixic acid	4 - >128	4	>128	20	4 - 8	4	8	0	4 - 8	4	4	0	4 - >128	4	8	1
J01X	Colistin	2 - 4	2	2	0	2 - 2	2	2	0	2 - 4	2	2	0	2 - 2	2	2	0
	Carbadox	8 - 16	8	16	0	8 - 32	8	16	0	8 - 32	8	16	0	8 - 128	8	16	<1
	Nitrofurantoin	32 - 128	32	64	3	32 - 64	32	32	0	32 - 64	32	64	0	32 - 128	32	64	2
Number of isolates					40				37				40				182

Table 13. Susceptibility and occurrence of resistance (%) among Salmonella *from food, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*Salmonella*															
		Broiler meat				Other poultry meat				Beef				Pork			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	2	>32	27	1 - 32	2	32	45	1 - 32	1	2	5	0.5 - >32	2	>32	18
J01B	Chloramphenicol	1- >64	4	8	4	1 - >64	4	>64	13	1- >64	4	8	5	4 - 64	4	16	10
	Florfenicol	1 - 32	4	4	1	0.5 - 32	4	4	4	1 - 32	4	8	5	2 - >32	4	8	8
J01C	Ampicillin	0.5 - >32	1	>32	28	0.5 - >32	1	>32	31	0.5 - >32	0.5	1	5	0.5 - >32	1	>32	20
J01E	Sulfonamid	16 - >512	64	>512	16	8 - >512	128	>512	34	8 - >512	64	>512	20	32 - >512	64	>512	39
	Trimethoprim	0.5 - >32	0.5	>32	10	0.5 - >32	0.5	>32	23	0.5 - >32	0.5	0.5	5	0.5 - >32	0.5	1	10
J01G	Gentamicin	0.5 - 8	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - 16	0.5	0.5	2
	Kanamycin	1 - 64	2	2	1	1 - >64	2	4	7	1 - 2	2	2	0	1 - >64	1	2	2
	Streptomycin	2 - >128	16	64	31	2 - 128	16	128	39	4 - 64	8	16	5	4 - 128	8	32	12
J01M	Ciprofloxacin	0.03 - 0.25	0.03	0.25	0	0.03 - 0.5	0.03	0.25	0	0.03 - 0.06	0.03	0.03	0	0.03 - 0.12	0.03	0.03	0
	Nalidixic acid	2 - >128	4	>128	24	2 - >128	4	>128	33	2 - 32	4	4	5	2 - 16	4	4	0
J01X	Carbadox	8 - 32	8	16	0	8 - 32	8	16	0	8 - 32	8	16	0	8 - 64	8	16	0
	Colistin	2 - 2	2	2	0	2 - 8	2	2	0	2 - 4	2	4	0	2 - 2	2	2	0
	Nitrofurantoin	32 - >128	32	128	12	32 - 128	32	128	13	32 - >128	32	64	5	32 - 128	32	64	6
Number of isolates					99				150				20				49

Salmonella in humans

In 1999, 3,268 human infections with zoonotic *Salmonella* serotypes were registered in Denmark. The serotype and *S.* Typhimurium phage type distributions are shown in Table 9 and in Table 10, respectively.

Following the increase observed in 1998, there has been a further increase in the level of antimicrobial resistance among *S.* Typhimurium isolates from humans in 1999 (Table 14). This increase can be partly explained by an increase in the percentage of *S.* Typhimurium DT104. Indeed, after the first domestic outbreak of *S.* Typhimurium DT104 reported in 1998, there were 2 additional outbreaks in 1999 (see box page 24).

Farm to table

A direct comparison of the *Salmonella* serotype distributions between the animal species, food categories and humans is not possible due to differences in sampling schemes. In 1999, the sampling scheme for food products was changed in favour of imported products and therefore the distribution between *Salmonella* isolates of imported and Danish origin included in

Table 14. Occurrence of resistance (%) among Salmonella *Enteritidis and* Salmonella *Typhimurium isolated from humans, Denmark*

DANMAP 99

ATC group	Compound	*S.* Enteritidis					*S.* Typhimurium			
		1995	1996	1997	1998	1999 a)	1995	1997	1998	1999 a)
J01A	Tetracycline	1	1	1	1	2	19	18	22	32
J01B	Chloramphenicol	1	0	0	0	<1	8	8	18	27
J01C	Ampicillin	3	1	2	2	2	10	11	24	31
J01D	Ceftiofur					0				<1
J01E	Sulfamethizol	3	1	1	1	2	18	19	30	37
	Trimethoprim	1	1	1	1	1	3	3	5	1
J01G	Apramycin	0	0	0	0	0	1	1	2	1
	Gentamicin	1	0	1	1	<1	1	1	1	1
	Kanamycin		0	1	0	1		1	1	<1
	Spectinomycin	2	1	1	1	1	9	10	20	29
	Streptomycin	3	0	1	1	<1	19	19	32	32
J01M	Ciprofloxacin	0	0	0	0	b)	0	0	1	b)
	Nalidixic acid	1	1	1	2	5	1	1	9	3
J01X	Colistin	0	0	1	0	0	0	1	0	0
Number of isolates		384	206	658	446	489	398	644	624	557

a) Includes imported cases
b) From 1999 onwards, resistance to quinolones is based on susceptibilty results for nalidixic acid

Table 15. Comparison of resistance (%) and percent change as compared to 1998 among Salmonella *Typhimurium from food animals and humans, Denmark*

DANMAP 99

ATC-group	Compound	Poultry		Cattle		Pigs		Humans	
		1999 %	% Change 98-99	1999 %	% Change 98-99	1999 %	% Change 98-99	1999 a) %	% Change 98-99
J01A	Tetracyclines	5	- 1	38	+ 15	14	- 2	32	+ 10
J01B	Chloramphenicol	0	0	33	+ 14	3	- 3	27	+ 9
J01C	Ampicillin	5	- 4	33	+ 14	8	+ 1	31	+ 7
J01E	Sulfonamid	14	- 5	43	+ 5	19	- 10	37	+ 7
	Trimethoprim	0	- 9	0	0	6	- 5	1	- 4
J01G	Gentamicin	3	+ 3	0	0	0	0	1	0
	Kanamycin	0	0	0	- 4	6	0	<1	0
	Streptomycin	5	- 17	43	+ 16	20	+ 3	32	0
J01M	Ciprofloxacin	0	0	0	0	0	0	b)	
	Nalidixic acid	0	- 6	0	- 8	1	- 1	3	- 6
J01X	Colistin	0	0	0	0	0	0	0	0
Number of isolates		37		40		182		557	

a) Imported cases included
b) From 1999 onwards, resistance to quinolones is based on susceptibility results for nalidixic acid

this DANMAP report did not reflect the distribution to which the consumers were exposed.

The *S*. Typhimurium isolates from humans and cattle seem to have similar resistance patterns. This does not necessarily identify cattle as the source of human *S*. Typhimurium infections but it reflects that *S.* Typhimurium DT104 is frequent in both samples from cattle and humans (Table 15).

A tentative comparison of resistance among *S*. Enteritidis isolated from poultry and humans showed that the resistance levels were quite similar except for the nalidixic acid resistance (Table 16). The comprehensive *Salmonella* surveillance and control programme provides data to explain these differences. All 8 nalidixic acid resistant *S*. Enteritidis isolates were from the layer production where parent flocks, rearing flocks and layer flocks are tested continuously for all *Salmonella* serotypes. Parent and rearing flocks infected with *Salmonella* are slaughtered while eggs intended for consumption are diverted to pasteurisation. The spread of nalidixic acid resistant *S*. Enteritidis was in most cases stopped before the infection reached commercial layer flocks (only 2 out of 8 isolates were from layer flocks). Therefore the sample of *S*. Enteritidis isolates from poultry does not correctly reflect the infective pressure on consumers.

Table 16. Comparison of resistance (%) and percent change as compared to 1998 among Salmonella *Enteritidis from poultry and humans, Denmark*

DANMAP 99

ATC-group	Compound	Poultry		Humans	
		1999 %	% Change 98-99	1999 a) %	% Change 98-99
J01A	Tetracyclines	5	+ 3	2	+ 1
J01B	Chloramphenicol	3	+ 3	<1	0
J01C	Ampicillin	0	- 4	2	0
J01E	Sulfonamide	3	0	2	+ 1
	Trimethoprim	0	- 3	1	0
J01G	Gentamicin	3	+ 3	<1	0
	Kanamycin	3	+ 3	1	+ 1
	Streptomycin	3	+ 1	<1	0
J01M	Ciprofloxacin	0	0	b)	
	Nalidixic acid	20	+ 13	5	- 3
J01X	Colistin	0	0	0	0
Number of isolates		40		489	

a) Includes imported cases

b) From 1999 onwards, resistance to quinolones is based on susceptibility results for nalidixic acid

Multidrug-resistant *S.* Typhimurium DT104 outbreaks in Denmark

The first Danish outbreak of multidrug-resistant *S.* Typhimurium DT104 caused by food of domestic origin occurred in June and July 1998 and included 25 culture-confirmed cases. The outbreak strain exhibited the classical penta-resistance pattern (ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline), but was also resistant to nalidixic acid. This resistance profile had not been detected in Danish food animals or food previously, and only rarely in humans. The investigation identified pork meat of Danish origin as the source. The outbreak started during the first week of June 1998, one week after the identification of *S.* Typhimurium with the same rare resistance pattern at a slaughterhouse in Zealand. Subsequent investigations confirmed that all isolates were of phage type DT104 and had the identical DNA-fingerprinting pattern (Weekly Epidemiological Record 1998;73:327-8; N Engl J Med 1999;341:1420-5).

Two additional outbreaks were observed in 1999. The first outbreak was a general outbreak that occurred in Ribe county in June 1999. It included a total of 26 culture-confirmed cases. All cases had eaten food from one restaurant in Esbjerg. However, investigation of the outbreak could not identify any exposure to a specific food item that was associated with the disease. During inspection of the premises, it was noted that cross-contamination from raw meat to lettuce had occurred. The restaurant had received more than 500 kg of imported pork during the preceding week and this may have been the source of *S.* Typhimurium DT104 in this outbreak.

The other outbreak due to *S.* Typhimurium DT104 occurred in Aalborg in September 1999 and included 32 culture-confirmed cases from two different parties. Food for both parties was delivered from a local butcher. A cohort study associated infection with roast beef and bacteriological examination of remaining food showed that the roast beef was heavily contaminated with *S.* Typhimurium DT104. However, bacteriological examination of the raw meat remaining from the same batch did not show growth of *Salmonella.* Hence, it may be possible that this incident was caused by cross-contamination by other products during preparation of the roast beef rather than by contaminated raw beef.

Further details about these outbreaks may be obtained by contacting Kåre Mølbak, MD, Department of Gastrointestinal Infections, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark. E-mail: krm@ssi.dk

Campylobacter

With 78 cases per 100,000 inhabitants, campylobacteriosis was the most frequent foodborne zoonosis in Denmark. *Campylobacter jejuni* was responsible for 90-95% of all the cases while *Campylobacter coli* was the second most common species. Approximately 80% of the Danish human cases of campylobacteriosis are of domestic origin (Annual Report on Zoonoses in Denmark 1999).

Campylobacter from food animals

Thermophilic *Campylobacter* were detected in approximately 32% of fecal samples from broilers, 49% of fecal samples from cattle and 46% of fecal samples from pigs (Table 17). *C. jejuni* was the predominant

Table 17. Prevalence of Campylobacter jejuni *and* C. coli *in fecal samples collected at slaughter of broilers, cattle and pigs, Denmark, 1999*

DANMAP 99

Origin	No. of samples	*C. jejuni*			*C. coli*		
		No. positive	% positive	No. tested for antimicrobial susceptibility	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	1,039	304	29	69	31	3	20
Cattle	83	40	48	40	1	1	NT a)
Pigs	244	11	5	11	99	41	84

a) NT, not tested.

species in broilers and cattle, whereas *C. coli* was the most prevalent species in pigs. Among all *C. jejuni* and *C. coli* isolates a random subsample was included in the DANMAP report.

In 1999, the breakpoints used for the determination of susceptibility of *Campylobacter* to ciprofloxacin, nalidixic acid, gentamicin, streptomycin and tetracycline have been adjusted (Table A1). All figures shown in this report are based on these new breakpoints, including data from previous years, which have been re-interpreted to allow year-to-year comparisons. Therefore results published in previous DANMAP reports are not immediately comparable with the figures in this report.

Table 18 presents the occurrence of antimicrobial resistance in *C. jejuni* from broilers and cattle and in *C. coli* isolated from broilers and pigs. In 1999, less than 5% of all *C. jejuni* isolates from cattle and broilers were resistant to ciprofloxacin and nalidixic acid, a level comparable to the one observed in 1998. Resistance to ciprofloxacin and nalidixic acid in *C. coli* isolates from pigs in 1999 was 11% and 23%, respectively, which is not different from the levels observed in 1998. A more detailed analysis of resistance to these two antimicrobials is presented in Figure 9 with the distributions of nalidixic acid and ciprofloxacin MIC values for both *C. jejuni* and *C. coli* from food animals. While the distribution of ciprofloxacin MIC values has remained unchanged from 1998 to 1999 for both

Table 18. Susceptibility and occurrence of resistance (%) in Campylobacter *from food animals, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*C. jejuni*							
		Broilers				Cattle			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - 16	0.5	1	1	0.5 - 16	0.5	0.5	3
J01B	Chloramphenicol	0.5 - 16	2	4	0	1 - 8	2	4	0
J01E	Sulfonamide	8 - 256	32	256	0	8 - 512	64	512	13
J01F	Erythromycin	0.25 - 2	0.5	1	0	0.125 - 1	0.5	1	0
J01G	Gentamicin	0.5 - 0.5	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0
	Neomycin	1 - 1	1	1	0	1 - 1	1	1	0
	Streptomycin	1 - 128	1	1	4	1 - 128	1	1	5
J01M	Ciprofloxacin	0.03 - 16	0.125	0.25	3	0.03 - 8	0.125	0.125	3
	Nalidixic acid	1 - 256	8	16	4	4 - 128	16	16	3
J01X	Colistin	0.5 - 32	4	16	0	0.5 - 32	8	16	0
	Carbadox	0.06 - 1	0.06	0.125	0	0.06 - 0.125	0.06	0.125	0
Number of isolates					69				40

DANMAP 99

ATC-group	Compound	*C. coli*							
		Broilers				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - 1	0.5	1	0	0.5 - 32	0.5	1	2
J01B	Chloramphenicol	0.5 - 16	2	8	0	1 - 32	4	16	2
J01E	Sulfonamide	8 - 512	128	256	10	8 - 1,024	64	256	10
J01F	Erythromycin	0.25 - 64	1	64	25	0.25 - 64	2	64	36
J01G	Gentamicin	0.5 - 0.5	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0
	Neomycin	1 - 1	1	1	0	1 - 1	1	1	0
	Streptomycin	1 - 128	1	16	15	1 - 128	1	64	27
J01M	Ciprofloxacin	0.03 - 8	0.06	0.25	5	0.03 - 16	0.125	2	11
	Nalidixic acid	1 - 256	16	32	5	1 - 256	16	128	23
J01X	Colistin	0.5 - 32	2	32	0	0.5 - 32	2	8	0
	Carbadox	0.06 - 1	0.125	0.5	0	0.06 - 2	0.25	2	0
Number of isolates					20				84

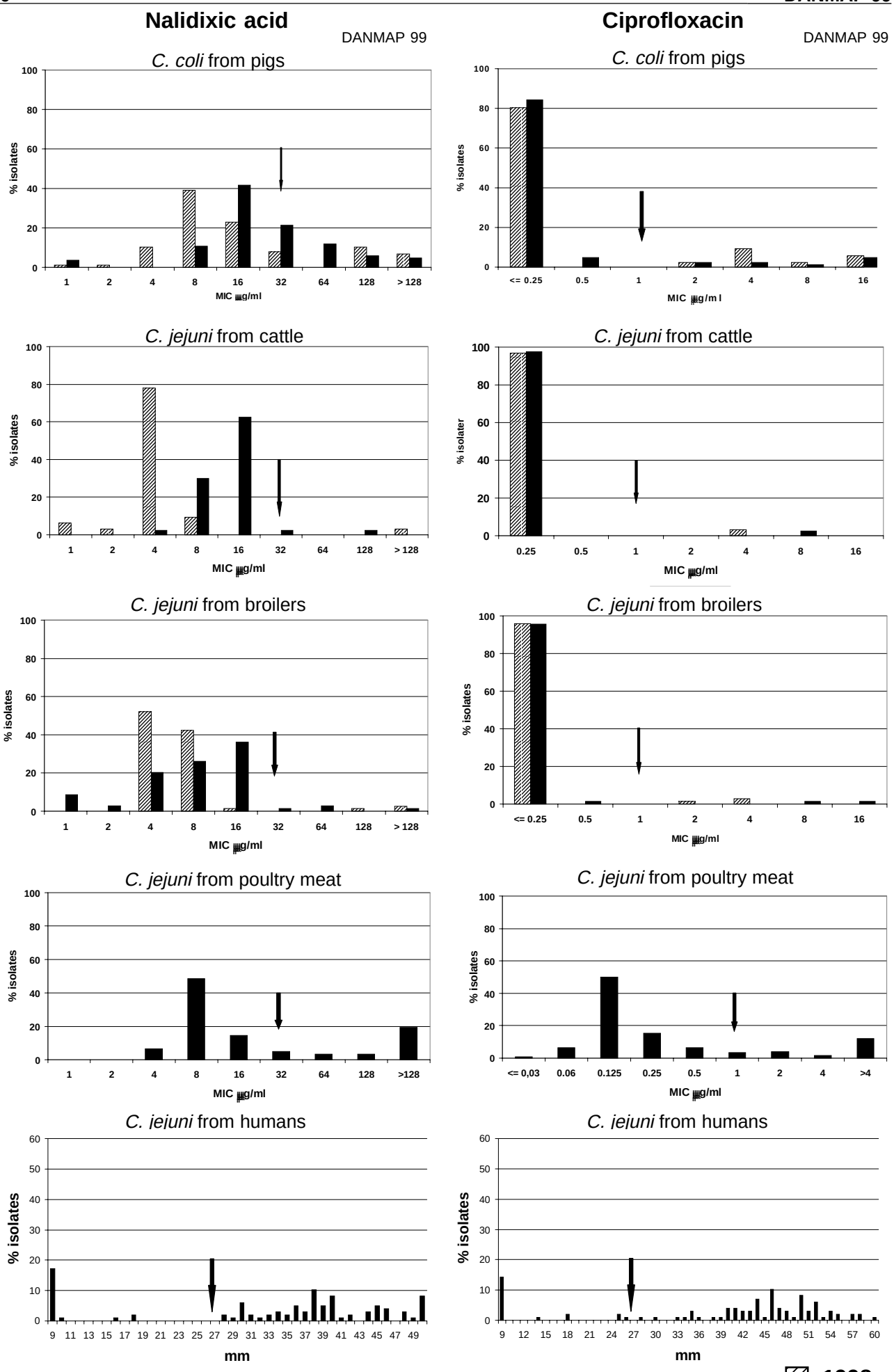


Figure 9. Distribution of nalidixic acid and ciprofloxacin MIC values and inhibition zones for Campylobacter *isolates in Denmark, 1998-1999. Arrows show breakpoints*

Table 19. Prevalence of Campylobacter jejuni *in samples of raw poultry collected from retail outlets, Denmark, 1999*

DANMAP 99

Origin	No. of samples	No. positive	% positive	No. tested for antimicrobial susceptibility
Broiler meat	994	339	34	93
Other poultry meat (mainly turkey and duck)	742	130	18	31

Campylobacter species, the distribution of nalidixic acid MIC values for both *Campylobacter* species has shifted toward decreased susceptibility during the same period.

Resistance to erythromycin among *C. coli* isolates from pigs showed a decrease of almost 50% from 1998 to 1999. The most likely explanation for this is the withdrawal of tylosin as antimicrobial growth promoter in Danish pig production. Finally, streptomycin resistance among *C. coli* from pigs decreased from 49% in 1998 to 27% in 1999. There is presently no obvious explanation for this decrease.

***Campylobacter* from food**

Campylobacter were isolated from Danish and imported poultry meat samples collected from retail outlets. The prevalence of thermophilic *Campylobacter* in poultry meat is shown in Table 19. A subsample with sufficiently detailed description of origin and sampling circumstances was selected for speciation and resistance dertermination. Of 150 isolates selected 124 were *C. jejuni*, 24 were *C. coli*, and 2 isolates were not culturable.

Susceptibility testing of the food isolates was performed by plate dilution, as opposed to previously where a modified tablet diffusion method was used. The results of the susceptibiliy tests are presented in Table 20. The most significant antimicrobials with regard to resistance in *C. jejuni* were tetracycline, ciprofloxacin and nalidixic acid. The distributions of nalidixic acid and ciprofloxacin MIC values for *C. jejuni* isolates from poultry meat are shown in Figure 9.

Table 20. Susceptibility and occurrence of resistance (%) among Campylobacter jejuni *isolates from retail poultry meat, Denmark, 1999*

DANMAP 99

ATC group	Compound	Broiler meat				Other poultry meat a)			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.25 - >32	0.25	>32	13	0.25 - >32	8	>32	55
J01B	Chloramphenicol	0.25 - 16	2	8	0	0.25 - 16	2	8	0
J01F	Erythromycin	0.5 - 4	1	1	0	0.5 - 4	1	2	0
J01G	Gentamycin	0.25 - 8	0.25	0.25	0	0.25 - 1	0.25	0.5	0
	Kanamycin	0.5 - 8	1	4	0	0.5 - 8	1	8	0
	Streptomycin	1 - >128	1	2	4	1 - 32	1	16	13
J01M	Ciprofloxacin	0.06 - >4	0.125	2	11	0.03 - >4	1	>4	39
	Nalidixic acid	4 - >128	16	>128	20	4 - >128	16	>128	42
Number of isolates					93				31

a) Mainly turkey and duck.

Table 21. Occurrence of resistance (%) among Campylobacter jejuni *isolates from Danish versus imported broiler meat, Denmark, 1999*

DANMAP 99

ATC group	Compound	Danish broiler meat		Imported broiler meat		Test a)
		No. resistant	% resistant	No. resistant	% resistant	p-value
J01A	Tetracycline	3	5	9	38	0.0003
J01M	Ciprofloxacin	5	8	4	17	NS b)
	Nalidixic acid	13	20	5	21	NS
Number of isolates			65		24	

a) Fisher exact test
b) NS, non statistically significant.

A comparison of the numbers of resistant *C. jejuni* isolates in Danish and imported broiler meat samples is presented in Table 21. Tetracycline resistance was significantly more frequent in *C. jejuni* isolates from imported than Danish broiler meat (Fisher Exact test, p=0.003). Although ciprofloxacin resistance was more frequent in *C. jejuni* isolates from imported (16.7%) than Danish broiler meat (8.3%), this difference was not statistically significant. There was no difference in nalidixic acid resistance in *C. jejuni* broiler meat isolates from these two sources.

Campylobacter spp. from humans

The occurrence of resistance in *C. jejuni* isolates from human samples in 1999 and the distribution of the zone diameters measured for nalidixic acid and ciprofloxacin are presented in Table 22 and Figure 9, respectively. Only data from 1999 are presented. Unfortunately, comparison of resistance levels and distributions of zone diameters between 1998 and 1999 is impossible because the recruitment of human *Campylobacter* sp. isolates in 1999 was very different from the one in 1998 where only non-imported cases were reported and several isolates from outbreaks were included in the database.

Table 22 presents a comparison of resistance levels in *C. jejuni* isolates from broilers, broiler meat and humans. In 1999, tetracycline resistance was much higher in broiler meat and in humans than in broilers. This picture is consistent with the data presented in Table 21 showing that *C. jejuni* from Danish broiler meat are rarely resistant to tetracycline in comparison to isolates from imported broiler meat.

Table 22. Comparison of resistance (%) among Campylobacter jejuni *from broilers, broiler meat and humans, Denmark, 1999*

DANMAP 99

ATC-group	Compound	Broilers	Broiler meat a)	Humans b)
J01A	Tetracycline	1	13	10
J01B	Chloramphenicol	0	0	0
J01F	Erythromycin	0	0	0
J01G	Gentamicin	0	0	0
	Streptomycin	4	4	2
J01M	Ciprofloxacin	3	11	20
	Nalidixic acid	4	20	21
Number of isolates		69	93	98

a) Percent change from 1998 to 1999 is not shown due to a change in method from tablet diffusion to Sensititre. In addition breakpoints were changed for some antimicrobials

b) Percent change from 1998 to 1999 is not shown due to a change in the sampling scheme

Resistance in indicator bacteria

Escherichia coli, Enterococcus faecium and *Enterococcus faecalis* have been chosen as indicator bacteria because they may be readily isolated from the normal populations of food animals and humans as well as from food. In addition, they respond to selective pressure by antimicrobials. Accordingly, they provide a measure of the selective pressure on the population of enteric bacteria.

In 1999, enterococci and *E. coli* were not collected from healthy humans in the community.

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 and were representative of the normal population. As far as possible only one sample was collected from each herd or flock and the number of samples collected at each slaughterhouse was proportional to the number of slaughtered animals.

The prevalence of *E. faecium* and *E. faecalis* is shown in Table 23. The occurrence of resistance among enterococci is shown in Tables 24 and 25.

The occurrence of resistance to antimicrobial growth promoters among *E. faecium* from broilers and pigs has been monitored since DANMAP was initiated in the last quarter of 1995. In the same period data on the annual consumption of antimicrobial growth promoters have been available. In Figures 10-13 the annual consumption of 4 antimicrobial growth promoters is compared to the occurrence of resistance among *E. faecium* from broilers and pigs.

The growth promoter avoparcin was banned in 1995 (Figure 10). The ban in 1995 was followed immediately by a decrease in the occurrence of glycopeptide resistant *E. faecium* in broilers. However, the occurrence of resistance among isolates from pigs remained relatively constant until 1998. It was shown that all glycopeptide resistant *E. faecium* from pigs belonged to the same clone, and that the genes coding for resistance to glycopeptides and macrolides were located closely on the same plasmid (Aarestrup FM. J. Clin. Microbiol. (Accepted)). Therefore, glycopeptide resistant enterococci have probably been maintained as a consequence of co-selection by the use of the macrolide tylosin. Due to the decreased use of tylosin during 1998 and 1999 the occurrence of glycopeptide resistant enterococci in pigs decreased significantly (Figure 10).

In January 1998, the growth promoter virginiamycin was banned in Denmark.The ban was followed by a decrease in the occurrence of resistance to virginiamycin among *E. faecium* from both broilers and pigs (Figure 11).

The broiler producers have voluntarily abandoned all use of antimicrobial growth promoters in broiler flocks hatched after February 15, 1998. From March 1998, antimicrobial growth promoters were voluntarily withdrawn for pigs above 35 kg. The use of growth promoters in pigs below 35 kg was phased out during the last half of 1999.

Avilamycin was almost exclusively used in the broiler production. Avilamycin was voluntarily withdrawn on February 15, 1998, but the consumption was reduced already from 1996 to 1997, followed by a decrease in resistance (Figure 12).

The most widely used growth promoter in recent years has been the macrolide tylosin. It was used in pigs and mainly in those above 35 kg live weight. From 1995 to 1998 resistance to erythromycin has been frequent

Table 23. Prevalence of Enterococcus faecium *and* Enterococcus faecalis *in samples collected from broilers, cattle and pigs at slaughter, Denmark, 1999*

DANMAP 99

Origin	No. of Samples	*E. faecium*			*E. faecalis*		
		No. positive	% positive	No. tested for antimicrobial susceptibility	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	1,175	196	17	189	394	34	201
Cattle	245	33	13	32	4	2	NT a)
Pigs	910	258	28	202	274	30	200

a) Not tested

among *E. faecium* isolates from both broilers and pigs. However, a considerable decrease in the consumption of tylosin from 1997 to 1998 was followed by a decrease in the occurrence of resistance to erythromycin among *E. faecium* from both broilers and pigs (Figure 13). A similar decrease was observed among *E. faecalis* from both broilers and pigs. Macrolides are still used for treatment of pigs and this consumption increased from 1998 to 1999 (Table 3). Therefore, the selective pressure from macrolides is still present.

The occurrence of macrolide resistance among *E. faecium* from broilers was most likely due to selection by the B component of the streptogramin growth promoter virginiamycin, since macrolides are hardly used in Danish broiler production.

Resistance to tetracycline among *E. faecium* and *E. faecalis* from broilers decreased significantly from 1998 to 1999, while resistance to penicillin among *E. faecium* from both broilers and pigs increased significantly in the same period. There is no obvious explanation for these changes.

Enterococci from food

A total of 891 sample were collected from pork, beef, broiler meat, fish and vegetables. This resulted in 213 isolates of *E. faecium* and 127 isolates of *E. faecalis*. Prevalences are shown in Table 26.

Table 24. Susceptibility and occurrence of resistance (%) among Enterococcus faecium *from food animals, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*E. faecium*											
		Broilers				Cattle				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	1	4	10	1 - >32	1	1	6	1 - >32	32	>32	53
J01B	Chloramphenicol	4 - 32	8	8	1	4 - 16	8	8	0	4 - 64	8	16	0
J01C	Penicillin	2 - 128	16	32	53	2 - 16	4	8	3	2 - 32	4	16	39
J01F	Erythromycin	1 - >32	1	>32	28	1 - > 32	2	4	6	1 - > 32	4	>32	48
J01G	Gentamicin	128 - 256	128	128	0	128 - 128	128	128	0	128 - 512	128	128	0
	Kanamycin	128 - >2,048	512	2,048	13	128 - >2,048	512	1,024	6	128 - >2,048	512	>2,048	28
	Streptomycin	128 - >2,048	128	128	2	128 - >2,048	128	128	3	128 - >2,048	128	>2,048	27
J01X	Vancomycin	1 - >32	1	4	9	1 - 4	1	2	0	1 - >32	1	4	6
	Synercid a)	0.5 - 32	2	8	41	0.5 - 4	1	2	6	0.5 - 16	2	4	19
	Virginiamycin	0.5 - >32	2	16	39	0.5 - 4	1	2	0	0.5 - 32	2	4	8
	Avilamycin	1 - >32	8	>32	21	1 - 8	4	8	0	1 - 32	4	8	1
	Bacitracin	8 - >256	>256	>256	85	8 - 256	128	>256	63	8 - >256	128	>256	54
	Nitrofurantoin	64 - 256	64	128	16	64-128	64	64	3	64 - 256	64	128	24
	Salinomycin	1 - 8	8	8	0	1 - 4	1	2	0	1 - >32	1	2	1
Number of isolates					189				32				202

a) Synercid consists of dalfopristin and quinopristin

Table 25. Susceptibility and occurrence of resistance (%) among Enterococcus faecalis *from food animals, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*E. faecalis*							
		Broilers				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	>32	1	48	1- >32	>32	>32	83
J01B	Chloramphenicol	2 - 64	8	16	0	2 - 64	8	16	3
J01C	Penicillin	2 - 8	2	4	0	2 - 8	4	4	0
J01F	Erythromycin	1 - >32	2	>32	24	1 - >32	2	>32	48
J01G	Gentamicin	128 - 256	128	128	0	128 - 2,048	128	128	2
	Kanamycin	128 - >2,048	128	128	1	128 - >2,048	128	>2,048	20
	Streptomycin	128 - >2,048	128	256	5	128 - >2,048	128	>2,048	32
J01X	Vancomycin	1 - 4	1	2	0	1 - 2	1	2	0
	Avilamycin	1 - >32	2	4	4	1 - >32	2	4	3
	Bacitracin	8 - >256	256	>256	67	8 - >256	64	128	24
	Flavomycin	0.5 - >32	2	4	5	0.5 - 16	2	4	1
	Nitrofurantoin	64 - 128	64	64	2	64 - 64	64	64	0
	Salinomycin	1 - 8	1	4	0	1 - 2	1	2	0
Number of isolates					201				200

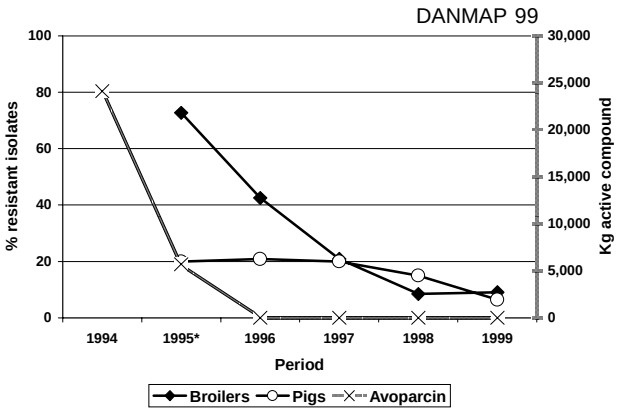


Figure 10. Trend in occurrence of resistance to avoparcin among Enterococcus faecium *from broilers and pigs and the consumption of the growth promoter avoparcin, Denmark*

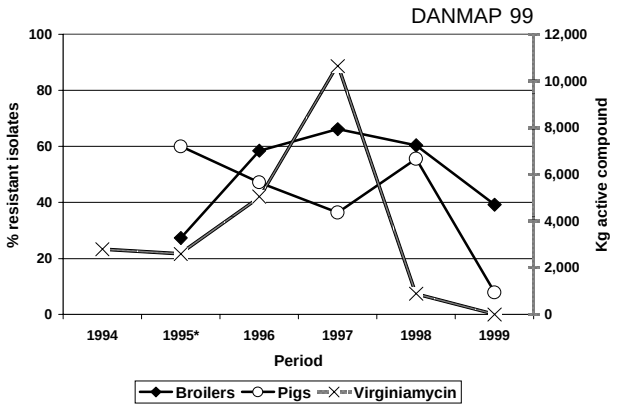


Figure 11. Trend in occurrence of resistance to virginiamycin among Enterococcus faecium *from broilers and pigs and the consumption of the growth promoter virginiamycin, Denmark*

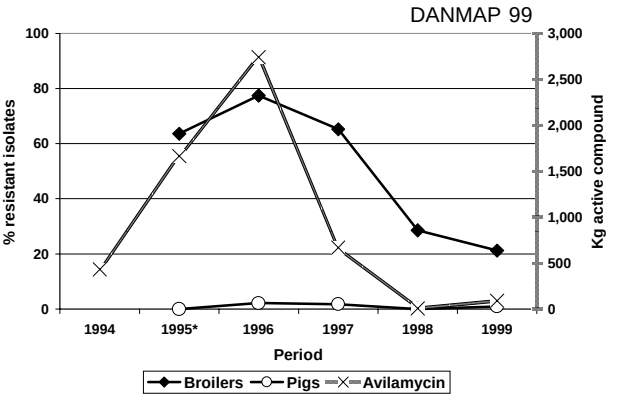


Figure 12. Trend in occurrence of resistance to avilamycin among Enterococcus faecium *from broilers and pigs and the consumption of the growth promoter avilamycin, Denmark*

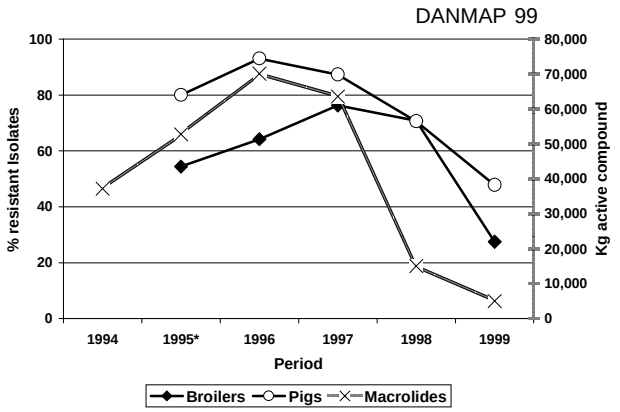


Figure 13. Trend in occurrence of resistance to erythromycin among Enterococcus faecium *from broilers and pigs and the total consumption of macrolides, both as growth promoters and therapeutics, Denmark*

Susceptibility testing was performed as MIC-determinations in microtitre wells (Sensititre). The results are shown in Table 27 and 28.

Resistance to tetracycline was mainly encountered among the isolates from broiler meat, a tendency similar to last year. Thus, 53% of the *E. faecalis* isolates from broiler meat were resistant to tetracycline. Resistance to vancomycin (which exhibits 100% cross-resistance to avoparcin) was only found in *E. faecium* from 6 poultry meat samples of both Danish and imported origin. Macrolide resistance for broiler meat was 27% and 29% for *E. faecium* and *E. faecalis*, respectively. In other food categories macrolide resistance was found at lower percentages. This is an overall decrease compared to 1998, where a relatively high frequency of resistance to the macrolide erythromycin was recorded for both species (Tables 29 and 30).

Table 26. Prevalence of enterococci in food samples collected from retail outlets, Denmark, 1999

DANMAP 99

Origin	No. of samples	*E. faecium*		*E. faecalis*	
		No. positive	% positive	No. positive	% positive
Beef	147	64	44	22	15
Pork	147	45	31	43	29
Broiler	147	45	31	17	12
Fish	210	47	22	35	17

Table 27. Susceptibility and occurrence of resistance (%) among Enterococcus faecium *from retail outlets, Denmark, 1999*

ATC group	Compound	*E. faecium*															
		Broiler meat				Beef				Pork				Fish			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	1	>32	18	1 - >32	1	1	5	1 - >32	1	1	7	1 - >32	1	>32	15
J01B	Chloramphenicol	2 - 16	4	8	0	2 - 16	4	8	0	2 - 16	4	8	0	2 - 16	4	8	0
	Florfenicol	2 - 4	2	2	0	2 - 4	2	4	0	2 - 4	2	2	0	2 - 4	2	2	0
J01C	Penicillin	2 - 16	2	16	16	2 - 2	2	2	0	2 - 2	2	2	0	2 - 2	2	2	0
J01F	Erythromycin	1 - >32	1	>32	27	1 - >32	1	8	9	1 - >32	1	4	4	1 - >32	2	8	11
J01G	Gentamicin	128 -128	128	128	0	128 - 128	128	128	0	128 - 128	128	128	0	128 - 128	128	128	0
	Kanamycin	128 - >2048	128	256	2	128 - >2048	128	256	2	128 - 512	128	256	0	128 - >2048	128	256	2
	Streptomycin	128 - 2048	128	128	2	128 - >2048	128	128	3	128 - 1024	128	128	0	128 - 1024	128	128	0
J01M	Nitrofurantoin	64 - 128	64	64	2	64 - 64	64	64	0	64 - 128	64	64	2	64 - 64	64	64	0
J01X	Vancomycin	1 - >32	1	>32	11	1 - 2	1	1	0	1 - 2	1	2	0	1- 2	1	2	0
	Virginiamycin	0.5 - 32	1	4	9	0.5 - 4	1	2	0	0,5 - 4	1	2	0	0.5 - 2	1	1	0
	Synercid a)	0.5 - 16	2	4	24	0.5 - 4	2	2	3	0.5 - 4	2	2	7	0.5 - 4	2	2	2
	Avilamycin	1 - >32	2	32	13	1 - 8	2	4	0	1 - 4	2	4	0	1 - 4	2	4	0
	Bacitracin	8 - >256	256	>256	67	8 - >256	128	64	22	8 - >256	64	64	4	8 - >256	64	128	15
	Salinomycin	1- 8	2	4	0	1 - 4	1	1	0	1 - 1	1	1	0	1 - 4	1	1	0
Number of isolates					45				64				45				47

a) Synercid consists of dalfopristin and quinopristin

Table 28. Susceptibility and occurrence of resistance (%) among Enterococcus faecalis *from retail outlets, Denmark, 1999*

ATC group	Compound	*E. faecalis*															
		Broiler meat				Beef				Pork				Fish			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	1	>32	53	1 - >32	1	>32	23	1 - >32	1	>32	14	1 - >32	1	>32	20
J01B	Chloramphenicol	2 - 8	4	8	0	2 - 8	4	8	0	2 - 32	4	8	2	2 - 8	4	8	0
	Florfenicol	2 - 4	2	4	0	2 - 4	2	2	0	2 - 4	2	4	0	2 - 4	2	4	0
J01C	Penicillin	2 - 4	2	2	0	2 - 2	2	2	0	2 - 4	2	2	0	2 - 2	2	2	0
J01F	Erythromycin	1 - >32	1	>32	29	1 - >32	1	2	5	1 - >32	1	2	2	1 - >32	1	2	3
J01G	Gentamicin	128 - 128	128	128	0	128 - 128	128	128	0	128 - 128	128	128	0	128 - 128	128	128	0
	Kanamycin	128 - >2048	128	128	6	128 - 128	128	128	0	128 - >2048	128	128	2	128 - >2048	128	128	3
	Streptomycin	128 - >2048	128	2048	18	128 - >2048	128	256	5	128 - >2048	128	128	2	128 - >2048	128	256	3
J01M	Nitrofurantoin	64 - 64	64	64	0	64 - 64	64	64	0	64 - 64	64	64	0	64 - 64	64	64	0
J01X	Vancomycin	1 - 2	1	2	0	1 - 2	1	2	0	1 - 8	1	2	0	1 - 2	1	2	0
	Avilamycin	1 - 4	2	2	0	1 - 2	2	2	0	1 - 32	2	2	2	1 - 4	2	2	0
	Bacitracin	8 - >256	>256	>256	65	8 - 64	16	32	0	8 - 64	16	64	0	8 - >256	32	32	6
	Flavomycin	0.5 - 8	1	4	0	0.5 - 2	1	1	0	0.5 - >32	1	2	2	0.5 - >32	1	16	14
	Salinomycin	1 - 4	1	4	0	1 - 1	1	1	0	1 - >32	1	1	2	1	1	1	0
Number of isolates					17				22				43				35

Virginiamycin resistance has decreased in all food categories and was observed only in 4 isolates of *E. faecium* from broiler meat of Danish origin (9%). Resistance to avilamycin was found among *E. faecium* isolates from broiler meat at the same level as in 1998, and in one isolate of *E. faecalis* from pork. Salinomycin resistance was only observed in one *E. faecalis* isolate from pork.

Enterococci from farm to table

A comparison of resistance in food animals and food samples is given in Tables 29 and 30. The number of enterococci isolates from foods of imported origin is 1 out of 45 isolates of *E. faecium* from pork, 4 out of 45 from broiler meat, and 5 out of 64 isolates from beef. For *E. faecalis* 5 out of 43 isolates from pork and 2 out of 17 isolates from broiler meat are of imported origin. For a few isolates there are no information available about country of origin. Since the vast majority of these food samples are of Danish origin a direct comparison may be relevant, even though the number of isolates from foods is somewhat smaller than from food animals. The occurrence of resistances in cattle/beef seems to be comparable. Also, to some extent, there is a similarity between resistance patterns in broilers/ broiler meat. However, in the comparison of pigs/pork the resistance patterns are very different. In pigs there is a higher occurence of resistance to several of the antibiotics. The reasons for these observations are yet unknown. However, the observations are in accordance with results from previous years.

***Escherichia coli* from food animals**

The prevalence of *E. coli* in samples from animals at slaughter is shown in Table 31 and Table 32 presents the result of the susceptibility testing.

Comparing the results from Table 32 with the corresponding results from DANMAP 98, the resistance level among indicator *E. coli* is almost unchanged, but there are a few exceptions. The proportion of *E. coli,* from broilers resistant to nalidixic acid decreased from

Table 29. Occurrence of resistance (%) among Enterococcus faecium *from food animals and foods and percent change as compared to 1998, Denmark*

DANMAP 99

ATC group	Compound	Broilers		Broiler meat		Cattle		Beef		Pigs		Pork	
		1999	% Change	1999	% Change	1999	% Change	1999	% Change	1999	% Change	1999	% Change
		%	98-99	%	98-99	%	98-99	%	98-99	%	98-99	%	98-99
J01A	Tetracycline	10	- 21	18	- 10	6	+ 2	6	+ 3	53	- 4	7	+ 3
J01B	Chloramphenicol	1	0	0	- 7	0	0	0	- 6	0	- 6	0	- 7
J01C	Penicillin	53	+ 37	16	+ 16	3	+ 3	0	0	39	+ 23	0	0
J01F	Erythromycin	28	- 43	27	- 31	6	- 11	9	-30	48	- 23	4	- 39
J01G	Gentamicin	0	0	0	0	0	0	0	0	0	0	0	0
	Kanamycin	13	+ 9	2	a)	6	+ 6	2	a)	28	+ 4	0	a)
	Streptomycin	2	- 3	2	+ 2	3	+ 3	3	+ 3	27	+ 3	0	- 4
J01X	Vancomycin	9	+ 1	11	+ 4	0	0	0	0	6	- 9	0	0
	Virginiamycin	39	- 21	9	- 43	0	- 43	0	- 16	8	- 47	0	- 7
	Avilamycin	21	- 8	13	+ 1	0	0	0	- 13	1	+ 1	0	0
	Bacitracin	85	+ 8	67	- 2	63	+ 15	22	+ 16	54	+ 11	4	- 3
	Nitrofurantoin	16	+ 5	2	a)	3	- 19	0	a)	24	+ 18	2	a)
	Salinomycin	0	0	0	- 12	0	0	0	- 10	1	+ 1	0	- 7
Number of isolates		189		45		32		64		202		45	

a) Not susceptibility tested in 1998

Table 30. Occurrence of resistance (%) among Enterococcus faecalis *from food animals and foods and percent change as compared to 1998, Denmark*

DANMAP 99

ATC group	Compound	Broilers		Broiler meat		Pigs		Pork	
		1999	% Change	1999	% Change	1999	% Change	1999	% Change
		%	98-99	%	98-99	%	98-99	%	98-99
J01A	Tetracycline	48	- 13	53	- 6	83	+ 2	14	+ 4
J01B	Chloramphenicol	0	- 3	0	- 35	3	- 1	2	- 18
J01C	Penicillin	0	- 1	0	0	0	0	0	0
J01F	Erythromycin	24	- 19	29	- 18	48	- 31	2	- 6
J01G	Gentamicin	0	- 1	0	0	2	+ 1	0	0
	Kanamycin	1	0	6	a)	20	- 7	2	a)
	Streptomycin	5	- 5	18	+ 6	32	- 5	2	2
J01X	Vancomycin	0	0	0	0	0	0	0	0
	Avilamycin	4	+ 4	0	- 12	3	+ 2	2	- 3
	Bacitracin	67	+ 10	65	0	24	+ 14	0	0
	Flavomycin	5	+ 4	0	0	1	- 2	2	+ 2
	Nitrofurantoin	2	- 2	0	a)	0	- 1	0	a)
	Salinomycin	0	0	0	- 12	0	0	2	- 3
Number of isolates		201		17		200		43	

a) Not susceptibility tested in 1998

26% in 1998 to 13% in 1999. During the same period, the proportion of isolates resistant to ciprofloxacin remained unchanged. In addition *E. coli* from broilers showed a decrease in resistance to sulfonamide and streptomycin and *E. coli* from cattle showed a decrease in resistance to sulfonamide. In the same period the consumption of quinolones and sulfonamide declined.

A comparison of resistance profiles from 1998 and 1999 showed the same tendency. The proportion of *E. coli* isolates resistant to one or more antimicrobials in the test panel has decreased from 1998 to 1999 and the trends were the same for broilers, cattle and pigs. In other words the indicator *E. coli* isolates tends to become more susceptible.

Table 31. Prevalence of Escherichia coli *in samples from broilers, cattle and pigs at slaughter, Denmark, 1999*

DANMAP 99

Origin	No. of samples	No. positive	% positive	No. tested for antimicrobial susceptibility
Broilers	989	545	55	182
Cattle	123	119	97	100
Pigs	302	287	95	284

Table 32. Susceptibility and occurrence of resistance (%) among Escherichia coli *from food animals, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*E. coli* from healthy animals											
		Broilers				Cattle				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracyclines	0.5 - >32	2	>32	16	0.5 - >32	2	2	8	0.5 - >32	2	>32	34
J01B	Chloramphenicol	1- 32	4	8	1	2 - 16	8	8	0	1 - 32	4	8	4
	Florfenicol	0.5 - 8	4	8	0	2 - 8	4	8	0	0.5 - 16	4	8	0
J01C	Ampicillin	0.5 - >32	2	>32	13	1 - >32	4	8	8	0.5 - >32	2	>32	13
J01E	Sulfonamide	8 - >512	16	>512	23	8 - >512	16	32	7	8 - >512	16	>512	40
	Trimethoprim	0.5 - >32	0.5	1	8	0.5 - >32	0.5	0.5	3	0.5 - >32	0.5	2	10
J01G	Gentamicin	0.5 - 4	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - 8	0.5	0.5	0
	Kanamycin	1 - >64	2	4	1	1 - >64	2	4	2	1 - >64	2	4	6
	Streptomycin	2 - >128	8	16	9	2 - >128	8	16	8	2 - >128	32	128	55
J01M	Ciprofloxaxin	0.03 - 8	0.03	0.125	2	0.03 - 0.06	0.03	0.03	0	0.03 - 0.125	0.03	0.03	0
	Nalidixic acid	2 - >128	2	64	13	2 - 4	2	4	0	2 - 128	4	4	0
J01X	Colistin	2 - 4	2	2	0	2 - 2	2	2	0	2 - 2	2	2	0
	Carbadox	8 - 16	8	8	0	8 - 16	8	8	0	8 - 64	8	8	0
	Nitrofurantoin	32 - 128	32	32	1	32 - 32	32	32	0	32 - 64	32	32	0
Number of isolates					182				100				284

Table 33. Prevalence of Escherichia coli *in food samples collected from retail outlets, Denmark, 1999*

DANMAP 99

Origin	No. of samples	No. positive	% positive
Beef	147	77	52
Pork	147	80	54
Broiler meat	147	79	54
Fish	210	40	19
Lamb	320	83	28
Deer	218	39	18

Table 34. Susceptibility and occurence of resistance (%) in Escherichia coli *from food at retail outlets, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*E. coli*											
		Broiler meat				Beef				Pork			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - >32	1	>32	14	0.5 - >32	1	32	10	0.5 - >32	1	>32	14
J01B	Chloramphenicol	1 - >64	4	8	3	1 - >64	4	8	1	1 - 32	8	8	4
	Florfenicol	1 - 8	4	8	0	0.5 - 16	4	8	0	0.5 - 8	4	8	0
J01C	Ampicillin	0.5 - >32	2	>32	11	0.5 - >32	2	4	4	0.5 - >32	2	4	8
J01E	Sulfonamid	8 - >512	16	>512	20	8 - >512	16	>512	14	8 - >512	32	>512	15
	Trimethoprim	0.5 - >32	0.5	0.5	4	0.5 - >32	0.5	1	8	0.5 - >32	0.5	0.5	5
J01G	Gentamicin	0.5 - 2	0.5	1	0	0.5 - 1	0.5	0.5	0	0.5 - 2	0.5	1	0
	Kanamycin	1 - >64	2	4	1	1 - >64	2	4	3	1 - >64	4	4	3
	Streptomycin	2 - >128	8	16	9	2 - >128	8	32	10	2 - >128	8	64	19
J01M	Ciprofloxacin	0.03 - >8	0.03	0.25	3	0.03 - 0.03	0.03	0.03	0	0.03 - 4	0.03	0.03	1
	Nalidixic acid	2 - >128	2	128	16	2 - 8	2	4	0	2 - 4	2	4	0
J01X	Carbadox	8 - 16	8	8	0	8 - 16	8	8	0	8 - 8	8	8	0
	Colistin	2 - 16	2	2	1	2 - >64	2	2	1	2 - 2	2	2	0
	Nitrofurantoin	32 - 64	32	64	0	32 - 128	32	32	3	32 - 32	32	32	0
Number of isolates					79				77				80

DANMAP 99

ATC-group	Compound	*E. coli*															
		Lamb meat				Vegetables				Fish				Deer meat			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - >32	1	2	8	0.5 - >32	1	2	6	0.5 - >32	1	2	3	0.5 - 32	1	2	5
J01B	Chloramphenicol	1 - 8	4	8	0	1 - >64	8	8	3	1 - 8	8	8	0	1 - >64	4	8	3
	Florfenicol	0.5 - 8	4	8	0	0.5 - 8	4	8	0	0.5 - 8	4	8	0	0.5 - 8	4	8	0
J01C	Ampicillin	0.5 - >32	4	4	4	0.5 - >32	2	4	3	0.5 - >32	2	2	3	0.5 - 8	2	4	0
J01E	Sulfonamid	8 - >512	16	128	8	8 - >512	32	128	9	8 - >512	32	>512	18	8 - >512	32	64	5
	Trimethoprim	0.5 - >32	0.5	0.5	1	0.5 - >32	0.5	0.5	4	0.5 - >32	0.5	0.5	3	0.5 - 1	0.5	0.5	0
J01G	Gentamicin	0.5 - 2	0.5	1	0	0.5 - 2	0.5	1	0	0.5 - 1	0.5	1	0	0.5 - 4	0.5	0.5	0
	Kanamycin	1 - 16	2	4	0	1 - >64	4	8	1	1 -8	4	4	0	1 - 64	4	4	3
	Streptomycin	2 - >128	8	8	4	2 - >128	8	16	5	2 - 128	8	16	5	2 - 128	8	16	3
J01M	Ciprofloxacin	0.03 - 0.25	0.03	0.03	0	0.03 - 0.03	0.03	0.03	0	0.03 - 0.03	0.03	0.03	0	0.03 - 4	0.03	0.03	3
	Nalidixic acid	2 - >128	2	4	1	2 - 8	4	4	0	2 -8	2	4	0	2 - >128	4	4	3
J01X	Carbadox	8 - 16	8	8	0	8 - 16	8	8	0	8 - 8	8	8	0	8 - 32	8	8	0
	Colistin	2 - 2	2	2	0	2 - 4	2	2	0	2 - 8	2	2	0	2 - >64	2	2	5
	Nitrofurantoin	32 - 32	32	32	0	32 - 64	32	32	0	32 - 32	32	32	0	32 - 128	32	32	3
Number of isolates					83				80				40				39

Table 35. Occurrence of resistance (%) among Escherichia coli *from animals and food and percent change as compared to 1998, Denmark*

DANMAP 99

ATC-group	Compound	Broilers		Broiler meat		Cattle		Beef		Pigs		Pork	
		1999 %	% Change 98-99	1999 %	% Change 98-99	1999 %	% Change 98-99	1999 %	% Change 98-99	1999 %	% Change 98-99	1999 %	% Change 98-99
J01A	Tetracycline	16	- 2	14	- 5	8	- 6	10	- 1	34	- 3	14	- 15
J01B	Chloramphenicol	1	+ 1	3	- 2	0	0	1	- 2	4	- 2	4	- 1
	Florfenicol	0	a)	0	a)	0	a)	0	a)	0	a)	0	a)
J01C	Ampicillin	13	- 3	11	0	8	+ 4	4	- 7	13	+ 3	8	- 8
J01E	Sulfonamide	23	- 15	20	a)	7	- 17	14	a)	40	- 1	15	a)
	Trimethoprim	8	- 5	4	- 6	3	0	8	+ 1	10	0	5	+ 2
J01G	Gentamicin	0	0	0	- 1	0	0	0	- 1	0	0	0	0
	Kanamycin	1	+ 1	1	- 2	2	- 2	3	+ 1	6	- 1	3	- 1
	Streptomycin	9	- 10	9	- 2	8	0	10	+ 2	55	- 6	19	- 4
J01M	Ciprofloxacin	2	+ 2	3	+ 3	0	0	0	0	0	0	1	0
	Nalidixic acid	13	- 13	16	- 2	0	- 4	0	- 1	0	- 1	0	- 3
J01X	Colistin	0	- 2	1	1	0	0	1	0	0	0	0	0
	Carbadox	0	0	0	- 3	0	0	0	0	0	0	0	0
	Nitrofurantoin	1	0	0	a)	0	0	3	a)	0	0	0	a)
Number of isolates		182		79		100		77		284		80	

a) Not susceptibility tested in 1998

Escherichia coli from food

Samples of pork, beef, broiler meat and fish were examined for both enterococci and *E. coli*. Additionally, samples of lamb and deer were included. The prevalence of *E. coli* in food samples is shown in Table 33. Eighty isolates of indicator *E. coli* from vegetables originating from a special screening of vegetables collected from retail outlets are included in Table 34, which presents the results of the susceptibility testing. The highest levels of resistance were found in isolates from broiler meat and pork. The levels of resistance in 1999 were similar or slightly lower than those seen in 1998 with the exception of a decrease in tetracycline resistance in pork from 29% in 1998 to 14% in 1999. Nalidixic acid resistance was recorded in broiler meat (16%), which is the same level as in 1998. In lamb meat resistance levels are substancially lower than in broiler and pork meat.

Escherichia coli from farm to table

A comparison of resistance patterns in food animals and food samples is given in Table 35. Again, the majority of these food samples are of Danish origin. The number of *E. coli* isolates from foods of imported origin are pork: 9 out of 80 isolates, broilers: 9 out of 79 isolates, beef: 3 out of 77 isolates. As for 1998, the figures are very similar for broilers/broiler meat and cattle/beef. The level of resistance in isolates from pigs is higher than for pork, especially with regard to tetracycline, sulfonamide and streptomycin. This tendency was also observed last year.

Resistance in bacteria from diagnostic submissions

Bacteria from food animals

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

Escherichia coli

The *E. coli* serotypes included were O2 and O78 from broilers, F5 from cattle and O149 from pigs. Generally *E. coli* isolated from broilers were less often resistant than *E. coli* from pigs and cattle (Table 36).

A decline in resistance to nalidixic acid was observed among *E. coli* isolated from poultry and pigs from 51% and 41% in 1998, respectively, to 9% and 22% in 1999, respectively. None of the *E. coli* isolates from poultry were resistant to ciprofloxacin and for the pig isolates a decrease from 12% to 0% was observed from 1998 to 1999. In cattle the levels of nalidixic acid and ciprofloxacin resistance remained unchanged. Fewer *E. coli* isolates from cattle and poultry were resistant to sulfonamide in 1999 compared to 1998.

In addition, the percentage of poultry and pig isolates resistant to ampicillin declined from 1998 to 1999 and for the poultry isolates a reduction was observed in streptomycin resistance. The percentage of poultry isolates susceptible to all antimicrobials in the test panel increased significantly from 1998 to 1999 for the pig and cattle isolates the level remained unchanged.

Staphylococci

CNS and *S. aureus* were isolated from bovine mastitis. In general the isolates were susceptible to most antimicrobials in the test panel (Table 37).

When comparing results from 1998 and 1999 an increase in avilamycin resistance was observed for both CNS and *S. aureus*. This was a surprise due to a voluntary withdrawal of the growth promoters in early 1998 and because growth promoters were not used in dairy cattle. The percentage of *S. aureus* isolates resistant to sulfonamide was reduced from 1997 to 1998 and a further decrease was observed from 1998 to 1999.

Resistance among *S. hyicus* from pigs is generally more widespread than in isolates from cattle (Table 37). Erythromycin resistance decreased from 1997 to 1998 and a tendency towards a further reduction was observed from 1998 to 1999, although the reduction was not significant. The decline in erythromycin resistance observed from 1997 to 1998 was most likely caused by the withdrawal of tylosin as growth promoter. But macrolide are still used for therapy and the consumption increased from 1998 to 1999, therefore the selective pressure is still present. Resistance to sulfonamide, trimethoprim and streptomycin decreased from 1998 to 1999.

Table 36. Susceptibility and occurrence of resistance (%) among Escherichia coli *isolated from diagnostic submissions from animals, Denmark, 1999* DANMAP 99

ATC-group	Compound	*E. coli* from diagnostic submissions											
		Poultry				Cattle				Pigs			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	1 - >32	2	>32	49	0.5 - >32	>32	>32	69	0.5 - >32	32	>32	54
J01B	Chloramphenicol	2 - 8	4	8	0	4 - >64	8	64	12	2 - >64	4	>64	29
	Florfenicol	2 - 8	4	4	0	4 - >32	8	8	1	2 - 16	4	8	0
J01C	Ampicillin	1 - >32	2	4	9	1 - >32	>32	>32	86	0.5 ->32	2	>32	24
J01E	Sulfonamide	8 - >512	>512	>512	55	8 - >512	>512	>512	79	8 - >512	>512	>512	82
	Trimethoprim	0.5 - >32	0.5	>32	11	0.5 - >32	>32	>32	56	0.5 - >32	0.5	>32	30
J01G	Gentamicin	0.5 - 1	0.5	0.5	0	0.5 - 32	0.5	16	12	0.5 - >32	0.5	0.5	1
	Kanamycin	1 - 4	2	4	0	1 - >64	4	>64	29	1 - >64	2	>64	20
	Streptomycin	4 - >128	8	8	8	4 - >128	128	>128	82	2 - >128	32	>128	73
J01M	Ciprofloxaxin	0.03 - 0.25	0.03	0.03	0	0.03 - 8	0.03	0.25	6	0.03 - 1	0.03	0.25	0
	Nalidixic acid	2 - >128	2	4	9	2 - >128	4	>128	18	2 ->128	2	>128	22
J01X	Colistin	2 - 2	2	2	0	2 - >64	2	2	1	2 - >64	2	2	1
	Carbadox	8 - 16	8	8	0	8 - 8	8	8	0	8 - 32	8	16	0
	Nitrofurantoin	32 - 32	32	32	0	32 - 128	32	32	2	32 -128	32	64	5
Number of isolates					53				84				94

Bacteria from food

Staphylococcus aureus

Fiftytwo isolates from various food categories were tested for antimicrobial susceptibility, and the results are shown in Table 38. Approximately half of the isolates were from pork. Approximately one third of the isolates were resistant to penicillin and sulfonamide. Due to the low resistance levels observed during the last 4 years *S. aureus* from food will no longer be included in the monitoring programme.

Listeria monocytogenes

In 1999, 60 isolates of *L. monocytogenes* were tested for antimicrobial susceptibility. The isolates were mostly from sliced meat products and soft cheeses. The results are shown in Table 39. Antimicrobial resistance in *Listeria* from food has been monitored during the last 4 years. Since resistance levels are low this activity will not be continued.

Table 37. Susceptibility and occurrence of resistance (%) among staphylococci from diagnostic submissions from animals, Denmark, 1999

DANMAP 99

ATC-group	Compound	Staphylococci											
		Cattle								Pigs			
		CNS				*S. aureus*				*S. hyicus*			
		Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% resistant	Range	MIC 50	MIC 90	% 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	24
J01B	Chloramphenicol	2 - 8	8	8	0	4 - 16	8	8	0	4 - 16	8	8	0
J01C	Oxacillin	0.5 - >4	0.5	0.5	1	0.5 - 2	0.5	0.5	0	0.5 - 0.5	0.5	0.5	0
	Penicillin	0.06 - 2	0.06	0.5	16	0.06 - 8	0.06	1	17	0.06 - >16	1	16	75
J01E	Sulfonamide	16 - >512	64	256	8	16 - >512	32	64	3	16 - >512	32	128	5
	Trimethoprim	1 - 32	4	8	8	1 - 8	1	2	0	2 - >32	8	>32	22
J01F	Erythromycin	0.25 - >16	0.5	0.5	1	0.25 - >16	0.25	0.5	1	0.25 - >16	0.5	>16	15
J01G	Gentamicin	1 - 8	1	1	0	1 - 2	1	1	0	1 - 1	1	1	0
	Kanamycin	4 - 128	4	4	1	4 - 8	4	4	0	4 - >128	4	4	3
	Streptomycin	4 - >64	4	8	8	4 - >64	4	16	4	4 - >64	8	>64	36
J01M	Ciprofloxacin	0.25 - 0.5	0.25	0.25	0	0.25 - 2	0.25	0.5	0	0.25 - 8	0.25	0.5	3
J01X	Vancomycin	1 - 2	1	2	0	1 - 2	1	1	0	1 - 2	1	1	0
	Synercid a)	0.5 - 1	0.5	0.5	0	0.5 - 1	0.5	0.5	0	0.5 - 4	0.5	1	2
	Avilamycin	2 - 32	4	16	13	2 - 16	4	8	7	4 - 16	8	8	2
	Bacitracin	8 - 256	32	128	32	8 - 128	8	32	1	16 - 128	32	128	10
Number of isolates					88				184				59

a) Synercid consists of dalfopristin and quinopristin

Table 38. Susceptibility and occurrence of resistance (%) among Staphylococcus aureus *from food from retail outlets, Denmark, 1999*

DANMAP 99

ATC-group	Compound	*S. aureus*			
		Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - 1	0.5	0.5	0
J01B	Chloramphenicol	4 - 8	8	8	0
J01C	Oxacillin	0.5 - 0.5	0.5	0.5	0
	Penicillin	0.06 - >16	0.06	8	29
J01E	Sulfonamide	16 - >512	128	>512	35
	Trimethoprim	1 - 4	2	4	0
J01F	Erythromycin	0.25 - >16	0.5	0.5	8
J01G	Gentamycin	1 - 1	1	1	0
	Kanamycin	4 - 8	4	4	0
	Streptomycin	4 - >64	8	16	2
J01M	Ciprofloxacin	0.25 - 1	0.25	0.5	0
J01X	Vancomycin	1 - 2	1	1	0
	Synercid a)	0.5 - 1	0.5	1	0
	Avilamycin	2 - 16	4	8	4
	Bacitracin	8 - 64	8	32	0
Number of isolates					52

a) Synercid consists of dalfopristin and quinopristin

Table 39. Susceptibility and occurrence of resistance (%) among Listeria monocytogenes *from food from retail outlets, Denmark, 1999*

DANMAP 99

ATC group	Compound	*L. monocytogenes*			
		Range	MIC 50	MIC 90	% resistant
J01A	Tetracycline	0.5 - 32	0.5	1	3
J01B	Chloramphenicol	1 - 16	4	8	0
J01C	Oxacillin	0.5 - >4	0.5	2	8
	Penicillin	0.06 - 0.5	0.06	0.25	0
J01E	Sulfonamide	16 - >512	16	128	10
	Trimethoprim	1 - 1	1	1	0
J01F	Erythromycin	0.25 - 0.25	0.25	0.25	0
J01G	Gentamycin	1 - 1	1	1	0
	Kanamycin	4 - 16	4	4	0
	Streptomycin	4 - >64	4	4	2
J01M	Ciprofloxacin	0.25 - 2	0.25	0.5	0
J01X	Vancomycin	1 - 8	1	1	0
	Synercid a)	0.5 - 2	0.5	1	0
	Avilamycin	1 - 2	1	1	0
Number of isolates					60

a) Synercid consists of dalfopristin and quinopristin

Bacteria from humans

As in 1998, this report includes data from the clinical microbiology laboratories serving the Copenhagen Municipality and Roskilde, Aarhus and North Jutland counties, representing approximately 35% of the Danish population. While the 3 laboratories in Roskilde, Aarhus and North Jutland counties represent close to 100% of the bed-days in these counties, the clinical microbiology laboratory serving the Copenhagen Municipality represents only one third of the total number of bed-days in this municipality. More information on demographics is presented in Table 2.

Escherichia coli

The results for the period 1995-1999 are presented in Table 40 and Figure 14. 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 while comparing resistance levels among the four counties, the general increase observed in 1998 went on in 1999, bringing ampicillin resistance in *E. coli* blood isolates to approximately 40%. This trend towards increase of resistance could not be related to the consumption of any class of antimicrobials, including penicillins with extended spectrum (J01CA) that comprises ampicillin and amoxicillin, in primary health care or in hospitals (Tables 7 and 8). Consumption of antimicrobials was expressed as the number of DDD per person-days which is a broad measurement unit. The use of other units of measurement such as the number of antimicrobial prescriptions per person-days or the percentage of the population exposed to antimicrobials should be explored. Case-control studies on antimicrobial exposure in bacteriemic patients should be performed before ruling out the participation of antimicrobial use in this general increase in ampicillin-resistant *E. coli*. Finally, gentamicin and cefuroxime resistance in *E. coli* blood isolates remained low in 1999, including in Aarhus county which has reported much higher levels of cefuroxime resistance in the past.

The right hand side of Figure 14 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 approximately 35%, 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 fact that sulfonamide use is very low in Danish hospitals (Table 8). As in blood isolates, ampicillin resistance in *E. coli* urine isolates was approximately 40% and showed an increasing trend during the past years. Finally, ciprofloxacin resistance in *E. coli* urine isolates remained very low in 1999.

Streptococcus pneumoniae

As national reference centre, the *Streptococcus* Laboratory at the Statens Serum Institut performs typing and susceptibility testing on *S. pneumoniae* isolates referred by the Danish local clinical microbiology laboratories. In 1999, susceptibility testing was performed on 880 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 has been rare until 1995 when it started to increase to reach approximately 4% in *S. pneumoniae* blood and

Table 40. Occurrence of resistance (%) among Escherichia coli *isolates from human diagnostic samples in 4 counties, Denmark, 1999*

DANMAP 99

ATC group	Compound	Blood isolates				Urine isolates						
		Hospitals in county no.: a)				Hospitals in county no.:				Primary health care in county no.:		
		1	5	14	16	1	5	14	16	1	14	16
J01C	Ampicillin	42	38	45	42	36	38	44	37	37	42	41
J01D	Cefuroxime	1	1	3	2		1					
J01E	Sulfamethizol					35	36	33	34	38	34	40
J01G	Gentamicin	1	1	2	1							
J01M	Ciprofloxacin		2			2		2	1	2	2	1
	Nalidixic acid								4			4
Number of isolates		337	162	585	378	5,228	2,009	4,998	3,172	4,107	3,191	2,501

a) County no.: 1, Copenhagen Municipality; 5, Roskilde County; 14, Aarhus County; 16, North Jutland

spinal fluid isolates in 1999 (Figure 15). In these isolates, erythromycin resistance paralleled penicillin resistance and reached 3.4% in 1999. Indeed, a majority of the penicillin-resistant isolates were also resistant to erythromycin. Although there has been an increase in the use of penicillins and macrolides in primary health care between 1996 and 1998, this trend stopped in 1999 when the use of penicillins overall (J01C) decreased by 4.6% and the use of macrolides (J01FA) decreased by 4.1%, as compared to 1998 (Table 7).

A closer look at macrolide use in primary health care shows that, while erythromycin and roxithromycin use decreased slowly between 1994 and 1999, azithromycin use increased gradually. Azithromycin is now the second most used macrolide in the Danish community (Figure 4). Although more research is needed, it is striking to observe that after years of intensive use of penicillins and macrolides that did not result in penicillin or erythromycin resistance, the emergence of penicillin- and erythromycin-resistant *S. pneumoniae* isolates followed the introduction of azithromycin and then paralleled its use in the Danish primary health care sector.

Coagulase-negative staphylococci

Figure 16 shows the level of resistance to selected antimicrobials among coagulase-negative staphylococci blood isolates from the four participating counties. Penicillin resistance in coagulase-negative staphylococci blood isolates was about 80%. Depending on the county, methicillin resistance varied from approximately 15% 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. In the clinical microbiology laboratories serving the Copenhagen Municipality and Roskilde county, all coagulase-negative staphylococci isolated from blood cultures are tested, while in the two other laboratories only isolates of clinical significance are tested. 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 25%.

Staphylococcus aureus

The *Staphylococcus* Laboratory at the Statens Serum Institut has a very long tradition for phage typing of *Staphylococcus aureus*. Isolates are also systematically tested for antimicrobial susceptibility. Figure 17 shows the trends in resistance to selected antimicrobials for *S. aureus* blood isolates since 1960. More than 25,000 isolates have been registered since this date. The resistance figures for 1998 are based on 1,322 non duplicate *S. aureus* blood isolates.

Penicillin resistance in *S. aureus* isolates increased from approximately 70% in 1960 to approximately 85% in the 1990s. In the beginning of this period, penicillin resistance was much more prevalent in hospital- than in community-acquired *S. aureus* isolates. Today, the susceptibility pattern is almost identical for the two sources. Studies on *S. aureus* strains originating from the normal Danish population find that approximately 75% of the isolates are resistant to penicillin.

Figure 17 shows that Denmark experienced an epidemic of methicillin-resistant *S. aureus* (MRSA) from the end of the 1960s until the mid-1970s. At the peak of the epidemic, more than 30% of *S. aureus* blood isolates were MRSA. The exact reason for the disappearance of MRSA strains from Denmark is unknown. However, it paralleled a marked reduction in the consumption of broad-spectrum antimicrobials such as tetracycline and streptomycin to which the MRSA strains were also resistant. Additionally, it coincided with the development of clinical microbiology as a medical discipline, with an increasing awareness of the importance of hospital hygiene and with an intensive campaign to teach Danish physicians the principles of prudent use of antimicrobials. Since the beginning of the 1980s, MRSA represent 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. Resistance to all other antimicrobials presented in Figure 17 remains below 5%.

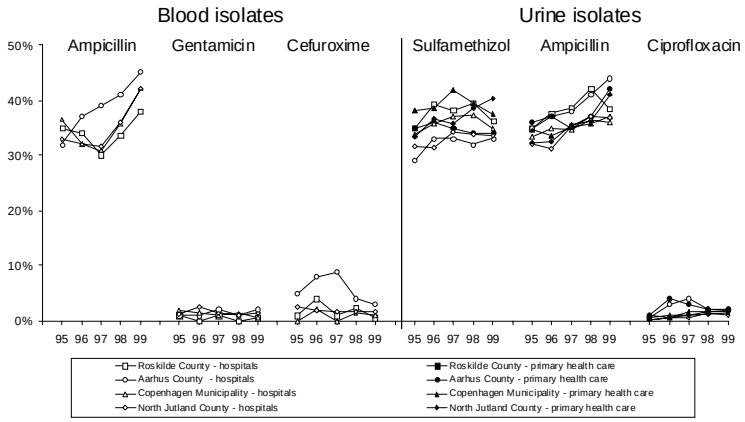


Figure 14. Resistance (%) to selected antimicrobials in Escherichia coli *blood and urine isolates from humans, Denmark, 1995-1999*

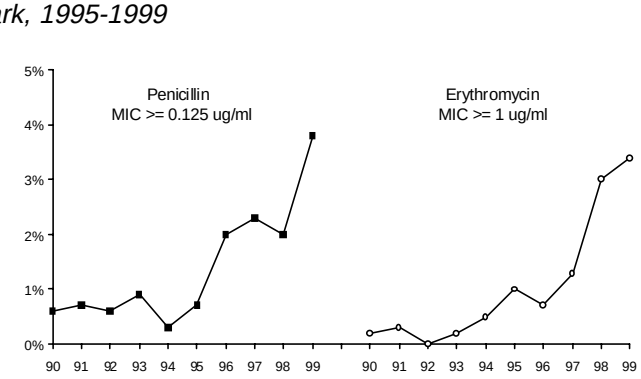


Figure 15. Resistance (%) to selected antimicrobials in Streptococcus pneumoniae *blood and spinal fluid isolates from humans, Denmark, 1990-1999*

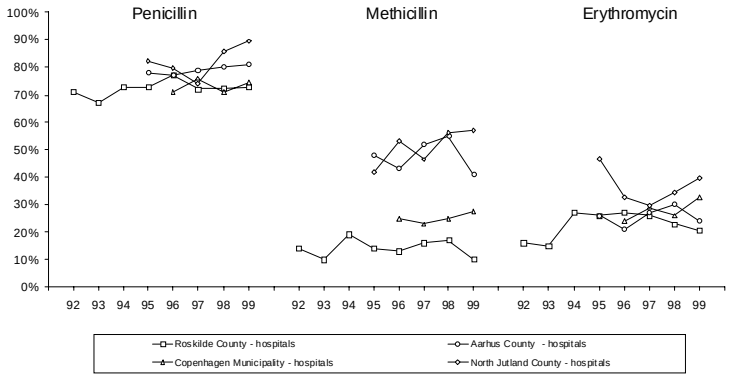


Figure 16. Resistance (%) to selected antimicrobials in coagulase-negative staphylococci blood isolates from humans, Denmark, 1992-1999

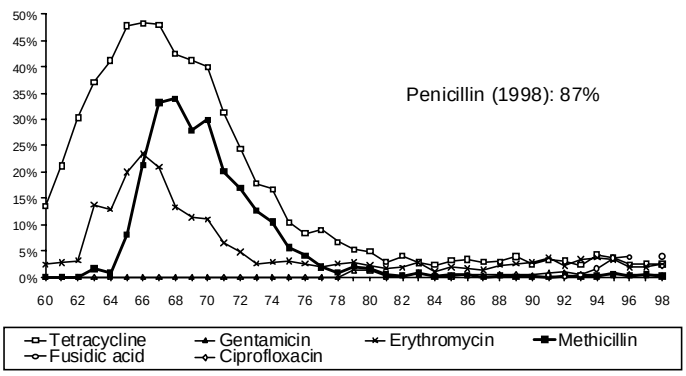


Figure 17. Resistance (%) to selected antimicrobials in Staphylococcus aureus *blood isolates from humans, Denmark, 1960-1998*

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.

The Danish Veterinary and Food Administration would like to acknowledge the assistance of the staff at 22 participating Municipal Food and Environmental Laboratories. The help of the following persons is especially appreciated: Finn Madsen, Lis Nielsen, Marie Thisgård, H.C. Rasmussen, Carl Lund, Solveig W. Andersen, Carsten Grønbæk, Morten Lisby, V. Quistgaard Nielsen, Brit Qviste Larsen, Morten Østergård, Marianne Møller, U.S. Mikkelsen, Ulla Møller, Marius Olesen, Anne Rahbek, Flemming Boisen, Per Rasmussen, Turid Smith, Maja Kraglund Holfort, Pia Rinas, and Christian Aaen.

Statens Serum Institut would like to thank the Danish Medicines Agency for providing data on consumption of antimicrobials in humans.

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 registered 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 consumption of therapeutics presented in this report comes from 2 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 consumption. 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 were rounded, so that quantities between 1 and 25 are shown as ”< 25”; quantities between 25 and 1000 were rounded to the nearest 50, and quantities over 1000 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 from 1997 onwards.

In Denmark, all antimicrobials for use in humans are prescription-only medicines. All antimicrobials are sold by pharmacies in defined packages. Each package is uniquely identified by a code which can be related to the size of the package (by content and in Defined Daily Doses or DDD), to the code of the antimicrobial in the Anatomical Therapeutic Chemical (ATC) classification system and to the name of the producer. In addition, the following information is collected for each transaction: social security number (CPR-number) of the patient, code identifying the prescribing physician, date and place (pharmacy, hospital pharmacy, institution) of the transaction, and information regarding 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, or group J01 of the ATC classification system, in primary health care and in hospitals. Consumption in the primary health care sector is reported at the national as well as the regional (county) level. Denmark is divided in 16 counties (Greenland and the Faroe Islands excluded)

that have very different demographic and social characteristics. In this report, counties are sometimes identified by a number as shown in Table 2. University hospitals are located in counties 1, 3, 9, 14 and 16, whereas counties 6, 7, 8, 10, 11, 13, 15 and 16 represent rural areas with a low population density.

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) using ATC codes at the level 2, 3 and 4. Although the WHO recommends expressing hospital consumption as a number of DDD per 100 bed-days, this proved impossible for the present report since information on hospital bed-days for 1999 was not available at the time of writing. Hospital consumption is therefore also expressed as a number of DDD/1,000 inhabitant-days. Data from 1997 show that, with the exception of the Copenhagen and Frederiksberg municipalities combined, the number of hospital bed-days in each county was correlated to the number of inhabitant-days (Figure 1), thus allowing comparisons of hospital use among counties as shown in Figure 7.

Collection of bacterial isolates

Isolates from animals

Bacterial isolates included in the monitoring programme are collected from animals at slaughter (*Escherichia 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 food

All food samples were collected at retail outlets by the Municipal Food and Environmental Laboratories (MFEL) during the course of routine inspection carried out by the laboratories, or on request specifically for the DANMAP surveillance programme. The collection of food samples for analyses of indicator bacteria (enterococci and *E. coli*) was planned and coordinated by the Danish Veterinary and Food Administration (DVFA). The samples collected consisted of both Danish and imported foods.

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 1999. For *Campylobacter* spp., the recruitment of isolates in 1999 was very different from the one performed in 1998 where only non-imported cases were reported and several isolates from outbreaks were included in the database. Comparison of resistance levels in *Campylobacter jejuni* between 1998 and 1999 is therefore not possible and is not reported.

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, alle *S. pneumoniae* blood and spinal fluid isolates nationwide are sent to the *Streptococcus* reference laboratory 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 four participating laboratories serving the Copenhagen Municipality, Roskilde county, Aarhus county and the county of North Jutland, 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 PBS 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 PBS of pooled droppings, 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 5% hydrogen for 1-5 days at 42°C. Selective enrichment was done by inoculation of Preston broth at a ratio of 1:10, followed by incubation in microaerophilic atmosphere for 24 h at 42°C. Ten 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 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 food samples

The primary isolation of indicator organisms from food samples was performed by the MFEL. Subsequently, isolates were shipped to the DVFA in standard transport media. Verification of identity and determination of antimicrobial resistance was performed by the DVFA. Only one strain of E. coli and/or Enterococcus from each food sample was tested for antimicrobial susceptibility.

The isolation method for *E. coli* employed 5 grams of food incubated at 44°C for 18-24 hours in 45 ml of MacConkey- or laurylsulfate-broth. The broth culture was streak-inoculated onto violet red bile agar and incubated for 48 hours at 44°C. Presumptive *E. coli* were subcultured onto blood agar, transferred to standard transport medium and shipped to DVFA. The isolates were identified *as E. coli* by standard morphological examinations and biochemical tests, including an api 20E test (bioMérieux, France).

Analysis for enterococci was carried out by adding 5 g of the sample to 45 ml of azide dextrose broth which was incubated at 44°C for 18-24 hours, and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours the plates were examined for growth, and typical red colonies were subcultured on blood agar, then transferred to transport medium and shipped to the DVFA. The isolates were identified *as E. faecium* or *E. faecalis* by standard morphological examinations and biochemical tests, including an API 20STREP test (bioMérieux, France).

A few of the *Enterococcus* and *E. coli* strains were isolated in accordance with the Nordic Committee on Food Analysis (NMKL) No. 68, 2nd ed., 1992 (*Enterococcus*) and NMKL No. 125, 2nd ed., 1995 (*E. coli*).

Salmonella, Campylobacter, L. monocytogenes, and *S. aureus* were isolated as specified by DVFA in "Cirkulære om mikrobiologiske undersøgelser af levnedsmidler af 3. december 1997" (Circular on microbiological Examination of Foodstuffs of December 3rd, 1997, ISBN: 87-601-3122-5, only available in Danish). The following methods for isolation are in accordance with this circular. Salmonella isolates were isolated according to NMKL no. 71, 4th ed., 1991 Sero- and phagetyping was performed at DVL.Thermotolerant *Campylobacter* were isolated and identified according to NMKL No. 119, second ed. 1990, *S. aureus* according to NMKL no., 66, second ed., 1992, and *L. monocytogenes* in accordance with NMKL no. 136 1990. All strains submitted by the MFEL were identified to species level at the DVFA, using standard morphological examinations and biochemical tests.

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

All other susceptibility testing was done with Sensititre (Trek Diagnostic Systems Ltd.), a commercially available MIC technique using dehydrated antimicrobials in microtitre wells. The wells were inoculated according to NCCLS guidelines and incubated aerobically at 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, a set of wells were inoculated and incubated with the test strains. With plate dilution all 4 control strains were included on each plate. The MIC values for the control strains were evaluated daily and the tests re-done if the values were out of range.

Isolates from humans
Gastrointestinal pathogens. Susceptibility testing for *Salmonella* sp. isolates was performed using the tablet diffusion method (Neo-Sensitabs®, A/S Rosco, Taastrup, 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-supplemented agar (SSI Diagnostika) and the breakpoints defined in Table A2.

Staphylococcus aureus. The *Staphylococcus* reference laboratory 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* reference laboratory at the Statens Serum Institut is screens for penicillin-resistant *S. pneumoniae* using a 1mg oxacillin tablet (Neo-Sensitabs®, A/S Rosco) on 10% horse blood agar (SSI Diagnostika). Penicillin

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

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Antimicrobial agent	*E. coli, Salmonella*		Staphylococci, *Listeria*		Enterococci		*Campylobacter*	
	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range
Ampicillin	16	0.5-32					16	1-32
Avilamycin			8	1-32	8	1-32		
Bacitracin			64	8-256	64	8-256		
Carbadox	64	8-128					64	0.06-32
Chloramphenicol	16	1-64	16	1-64	16	2-64	16	1-64
Ciprofloxacin	2	0.03-8	2	0.25-8			1 a)	0.03-16
Colistin	8	2-64					32	0.5-64
Erythromycin			4	0.25-16	4	1-32	16	0.25-32
Flavomycin					8	0.5-32		
Florfenicol	16	0.5-32						
Gentamicin	8	0.5-32	8	1-32	512	128-2,048	8 a)	0.5-32
Kanamycin	32	1-64	32	4-128	1,024	128-2,048		
Nalidixic acid	16	2-128					32 a)	1-128
Neomycin							8	1-64
Nitrofurantoin	64	32 - 128			64	64 - 256		
Oxacillin + 2% NaCl			2	0.5-4				
Penicillin			0.12 b)	0.06-16	8	2-128		
Salinomycin					8	1-32		
Streptomycin	16	2-128	16	4-64	1,024	128-2,048	8 a)	1-64
Sulfonamethoxazole	256	8-512	256	16-512			256	8-512
Synercid c)			2	0.5-16	2	0.5-32		
Tetracycline	8	0.5-32	8	0.5-32	8	1-32	8 a)	0.5-32
Trimethoprim	8	0.5-32	8	1-32				
Vancomycin			16	1-32	16	1-32		
Virginiamycin			4		4	0.5-32		

a) New breakpoints compared to previous years
b) *Listeria* breakpoint for penicillin is 2 µg/ml (according to NCCLS)
c) Synercid consists of dalfopristin and quinopristin

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.** The clinical microbiology laboratories serving the Roskilde and Nordjylland counties use 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.

During 1999, the clinical microbiology laboratory serving the Copenhagen Municipality changed susceptibility testing method. Until approximately June 1999, this laboratory used the tablet diffusion as described above. From this date onwards, this laboratory started using the disk diffusion method (Oxoid, Basingstoke, UK) on 5% horse blood Iso-Sensitest (ISA) medium (Oxoid) and the breakpoints defined for this medium by the Swedish Reference Group for Antibiotics (Available from: URL: http://www.ltkronoberg.se/ext/raf/ZONTAB/Zontab.htm).

The clinical microbiology laboratory serving Aarhus county is using the disk pre-diffusion method with in-house disks on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this method (Schumacher H, et al. APMIS 1998;106: 979-86; Schumacher H, et al. J Antimicrob Chemother 2000; in press).

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.

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Antimicrobial agent	Species	
	Salmonella enterica	*Campylobacter*
Ampicillin	28 mm	-
Apramycin	20 mm	24 mm
Ceftiofur	20 mm	-
Chloramphenicol	24 mm	33 mm
Colistin	17 mm	18 mm
Ciprofloxacin	- a)	27 mm
Erythromycin	-	27 mm
Gentamicin	22 mm	30 mm
Kanamycin	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) From 1999 onwards, resistance to quinolones is based on susceptibility results for nalidixic acid.

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

Performance test

In order to ascertain the comparability of susceptibility results presented in this report, we carried out a performance test in four laboratories including the Department of Gastrointestinal Infections and the Clinical Microbiology Laboratory at the Statens Serum institut, the Danish Veterinary Laboratory, and the laboratory at the Danish Veterinary and Food Administration. It was performed by testing two collections of bacterial strains, one composed of *Enterobacteriaceae* (10 strains of *E. coli*, 2 *Salmonella* sp., 2 *Klebsiella oxytoca* and 3 *Enterobacter cloacae*) and the other composed of enterococci (5 *E. faecium*, 3 *E. faecalis* and 2 *E. casseliflavus*). This means that the number of susceptibility tests for evaluating performance within DANMAP more than doubled as compared to 1998.

The results of the performance test are presented in Table A3 which shows the gross results as the number of strains reported either susceptible or resistant in relation to the total number of tests performed for each antimicrobial. Overall, there was agreement for 98.2% of the tests performed.

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 or mm inhibition zones) 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. 6.12.

Data on food isolates

Results from the analysis of food samples were reported from the MFEL to the DVFA via the Food Microbiology Database (LMD) or mailed as written data sheets. The results of all the susceptibility tests were stored as MIC-values in a Microsoft Access database. For each bacterial isolate information is available on the type of food sample, bacterial species, date of examination of the sample, the MFEL that collected and processed the sample, and a MFEL identification number, which makes it possible to obtain further information about the isolate from the laboratory. Furthermore, information about the country of origin was recorded whenever possible.

The results of the susceptibility testing of the isolates are shown for each species as the percentage of strains resistant to a specific antimicrobial.

Data on human isolates

Data on susceptibility testing of gastrointestinal pathogens were stored as zone diameters (mm) in a Microsoft Excel database at the Department of Gastrointestinal Infections at the Statens Serum Institut. These data were analyzed using Microsoft Excel and Epi Info v. 6.04c.

Data on susceptibility testing of *Staphylococcus aureus* isolates associated with bacteremia were stored in a Microsoft Access database. Only one *S. aureus* isolate per bacteriemic episode and per patient is registered in this database.

Data on susceptibility testing of *Streptococcus pneumoniae* isolates were stored as MICs in a Microsoft Access database. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed using this software.

The four other clinical microbiology laboratories provided compiled data on resistance levels in *Escherichia coli* blood and urine isolates and in coagulase-negative staphylococcus blood isolates. In three of these laboratories, data were extracted from the laboratory information system, i.e. ADBakt (Autonik AB, Skoldinge, Sweden) for Copenhagen Municipality (Hvidovre Hospital) and the county of North Jutland (Aalborg Hospital), and MADS (Clinical Microbiology Laboratory, Aarhus Kommunehospital, Aarhus, Denmark) for Aarhus county. 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 were asked to

remove duplicate isolates from the same patient within a window of 30 days, only the laboratories serving the Copenhagen Municipality and the county of North Jutland were able to comply with this rule. In the two other laboratories, this task would have required too much additional work to be fulfilled.

Table A3. Results of performance testing (correct results/no. of tests) among laboratories participating in DANMAP, 1999

DANMAP 99

Antimicrobial agent	*Enterobacteriaceae*		*Enterococci*		Total
	S a)	R	S	R	
Penicillin	-	-	25/25	23/25	48/50
Ampicillin	42/42	66/66	30/30	20/20	158/158
Cefuroxime	52/52	20/20	-	-	72/72
Erythromycin	-	-	32/32	10/10	42/42
Tetracycline	48/48	54/54	25/25	24/25	151/152
Gentamicin	72/72	59/60	45/45	5/5 b)	181/182
Nalidixic acid	96/96	6/6	-	-	102/102
Ciprofloxacin	96/96	4/6	-	-	100/102
Sulfonamide	53/60	42/42	-	-	95/102
Trimethoprim	72/72	30/30	-	-	102/102
Vancomycin	-	-	41/45	5/5	46/50
Teicoplanin	-	-	27/27	3/3	30/30
Nitrofurantoin	76/78	20/24	-	-	96/102
Total	607/616	301/308	225/229	90/93	1,223/1,246

a) S, susceptible; R, resistant

b) For enterococci, R means high-level gentamicin resistance (MIC > 1,024 mg/l).

Appendix 2

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