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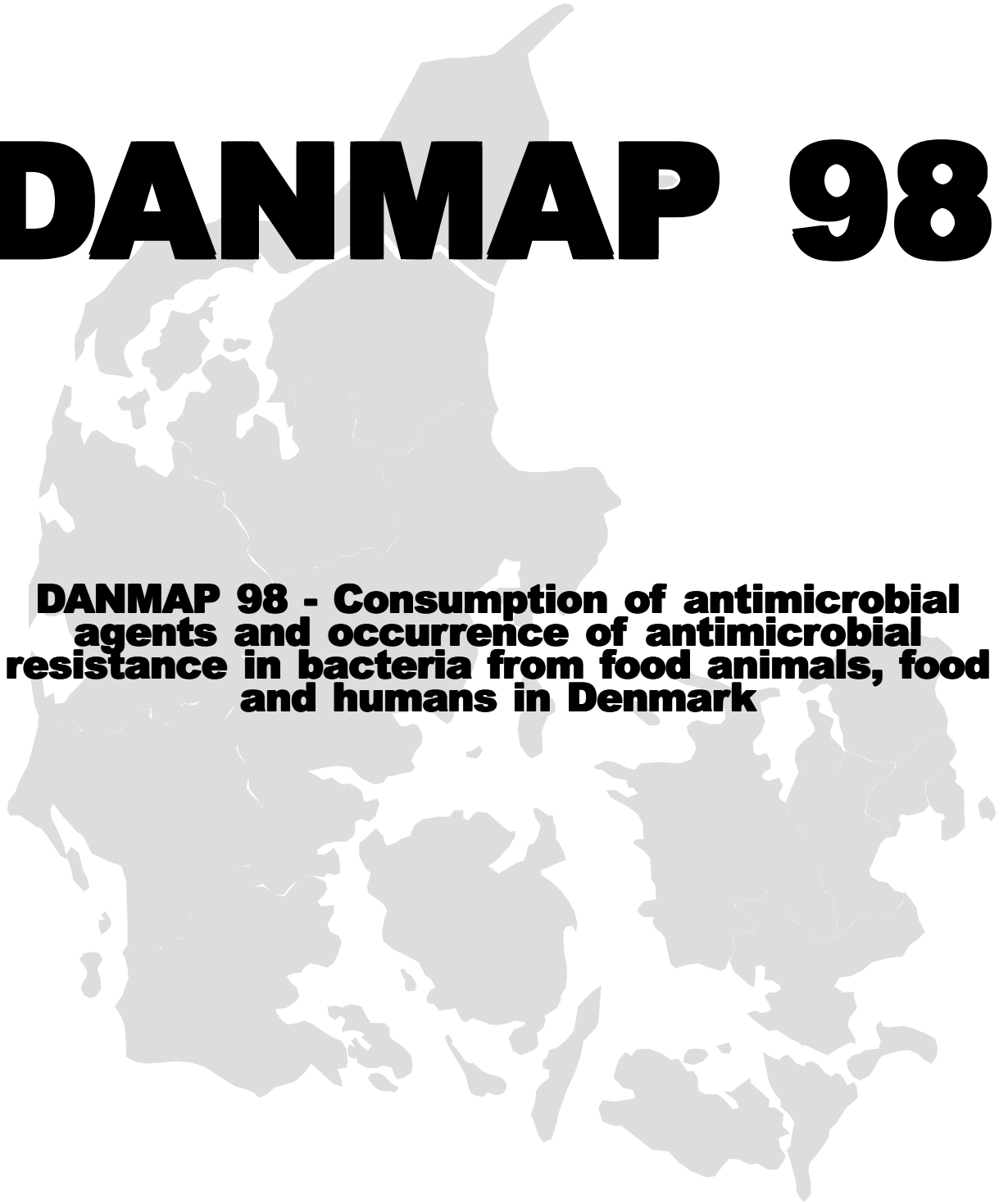

DANMAP 98 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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Contents

This publication is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of antimicrobial resistance monitoring in food animals, foods and humans in 1998 and is produced in collaboration between the Danish Veterinary Laboratory, The Veterinary and Food Administration, Danish Medicines Agency and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Food, Agriculture and Fisheries, and the Ministry of Health.

Sammendrag

DANMAP – det danske program for overvågning af antibiotikaresistens hos bakterier fra produktionsdyr, fødevarer og mennesker – blev etableret i 1995 og DANMAP 98 er den tredje rapport. Ud over resistensdata indeholder DANMAP en opgørelse af antibiotikaforbruget til dyr og mennesker. DANMAP er tilrettelagt for at give det bedst mulige grundlag for at sammenligne forekomst af antibiotikaresistens hos dyr og mennesker samt for at beskrive sammenhængen mellem antibiotikaforbrug og resistens.

Foruden overvågning omfatter DANMAP en betydelig forskningsaktivitet. Resultaterne fra denne forskning er ikke indeholdt i rapporten, men i Appendiks 2 findes en liste over de DANMAP artikler, der er publiceret i internationale tidsskrifter.

Denne rapport beskriver resultater for kalenderåret 1998. Den beskriver først forbruget af antibiotika til produktionsdyr, både til behandling, som vækstfremmere og som coccidiostatika. Dernæst følger en detaljeret analyse af forbruget af antibiotika til mennesker, inklusive en sammenligning mellem forbruget i de enkelte amter, samt en vurdering af udviklingen i forbruget over tid.

Resistensresultaterne præsenteres i afsnit med opgørelser for henholdsvis zoonotiske bakterier, indikatorbakterier (*Escherichia coli* og *Enterococcus faecium/Enterococcus faecalis*) samt for bakterier der stammer fra prøvemateriale fra syge dyr og mennesker. I hvert af disse afsnit beskriver vi først resultater for bakterier fra dyr, dernæst fra fødevarer og endelig for mennesker. Resultaterne vises i separate tabeller og figurer for hvert af disse tre reservoirer. Når det er muligt viser vi nogle af dem i "jord-til-bord" tabeller for at sammenligne, for eksempel, resistens hos *Salmonella* Typhimurium fra produktionsdyr, levnedsmidler og mennesker. De antibiotika, der indgår i undersøgelsen er udvalgt så alle relevante stofgrupper er repræsenteret. Vi har ikke undersøgt for resistens i de tilfælde hvor en bakterieart er naturligt resistent overfor et stof.

DANMAP programmet nyder betydelig international opmærksomhed. Det er derfor besluttet at udgive rapporten på engelsk. Nedenstående sammendrag beskriver de væsentligste udviklingstendenser i 1998.

Forbrug af antibiotika

I begyndelsen af 1998 vedtog de danske husdyrbrancher at reducere brugen af vækstfremmere, som siden februar 1998 kun har været brugt til svin op til 35 kg. Det medførte at vækstfremmerforbruget faldt med over 50 procent. Forbruget af antibiotika til behandling steg med knapt 3 procent, sammenholdt med at produktionen af slagtesvin og slagtekyllinger steg med henholdsvis 8 og 5 procent.

Nedgang i brug af vækstfremmere har således IKKE medført en kompensatorisk forøgelse i forbruget af antibiotika til behandling. Derimod tyder udviklingen i 1998 på et faldende forbrug af antibiotika pr. produceret dyr, også i forbruget til behandling af sygdom.

Ændringen i antibiotikaforbruget afspejler i hovedsagen en forøget anvendelse af de smalspektrede penicilliner og af sulfa/TMP til injektion, medens forbruget af de bredspektrede antibiotika som tetracykliner og kinoloner har været faldende. Denne udvikling svarer nøje til de anbefalinger vedrørende hensigtsmæssigt antibiotikaforbrug, som Statens Veterinære Serumlaboratorium har udsendt, uden at man dog kan sige med sikkerhed hvorvidt det er disse anbefalinger, der er årsagen til ændringerne.

Detaljerede analyser af sammenhængen mellem forbrug af antibiotika og resistensforekomst vanskeliggøres af, at der mangler oplysning om antibiotikaforbruget til de enkelte dyrearter.

I modsætning til udviklingen indenfor husdyrproduktionen skete der i 1998 en stigning i forbruget af antibiotika til behandling af mennesker. Stigningen (regnet som definerede døgndoser, DDD) fandt især sted i primærsektoren og forbruget af penicilliner var årsag til en væsentlig del af stigningen. Imidlertid er der også sket en stigning i forbruget af makrolider.

Resistens hos zoonotiske bakterier

I 1998 har vi set en stigning i forekomsten af resistens overfor kinolonet nalidixan hos *Salmonella* Enteritidis og *Salmonella* Typhimurium fra fjerkræ. Der blev ligeledes set en stigning i nalidixan resistens hos *S.* Typhimurium fra mennesker. Sidstnævnte stigning var et resultat af et levnedsmiddelbårent

udbrud af nalidixan resistent *S.* Typhimurium DT104 med oprindelse i svinekød.

Afbildning af MIC-fordelingen (Minimum Inhibitory Concentration) for såvel nalidixan som ciprofloxacin hos *S.* Typhimurium fra svin viser imidlertid også en bekymrende forskydning i retning af mindre følsomhed. Denne udvikling i retning af øget resistensforekomst har fundet sted på trods af et beskedent fald i forbruget af kinoloner.

En særlig undersøgelse af antibiotikaresistens hos Salmonella fra importeret fjerkræ viste at disse var hyppigere resistente end tilfældet var for Salmonella fra dansk fjerkræ.

Campylobacter jejuni fra slagtekyllinger og kvæg var følsomme overfor de fleste af de antibiotika de blev undersøgt for og der er ikke sket større ændringer i forhold til 1997. Det er interessant, at resistens overfor kinoloner var lige hyppig blandt *C. jejuni* fra fjerkrækød og fra mennesker, men noget højere end den forekomst der blev fundet hos isolater fra levende kyllinger. Årsagerne til den højere forekomst i kød end i de levende dyr kendes ikke med sikkerhed, men en del af forklaringen kan være at der i prøverne af kyllingekød indgik importerede produkter. Altså den samme tendens til højere forekomst i importerede produkter end i danske som vi også så for salmonella.

Det fremgår at *Campylobacter coli* generelt havde en højere resistensforekomst end *C. jejuni*, særligt overfor kinoloner, hvor 13 og 17 procent af isolaterne fra henholdsvis slagtekyllinger og fjerkræ var resistente overfor ciprofloxacin. *C. coli* er ansvarlig for under 10 procent af campylobacterinfektionerne hos mennesker og *C. jejuni* for ca. 90 procent. I lyset af udbredelsen af kinolonresistens hos *C. coli* og hos *C. jejuni* fra mennesker betyder det at der ikke altid kan forventes effekt af kinolonbehandling ved de få tilfælde af behandlingskrævende campylobacter infektion hos mennesker.

Resistens hos indikatorbakterier

Der blev påvist stigende resistens overfor kinoloner hos *Escherichia coli* isoleret fra den raske population af slagtekyllinger, og i mindre grad hos *E. coli* fra raske slagtesvin og kvæg. For enterokokkernes vedkommende så vi i 1998 et fald i resistens overfor vækstfremmerne avoparcin og avilamycin, en tendens der falder sammen med ændringer i forbruget af de pågældende stoffer. For både *Enterococcus faecium* og *Enterococcus faecalis* så vi store forskelle i resistensforekomst blandt isolater fra, for eksempel, svin og svinekød. En forklaring på dette kan være at bakteriefloraen i levnedsmiddelprøver i detailleddet i vidt omfang afspejler krydskontaminering i forbindelse med forarbejdning og distribution, i stedet for næsten udelukkende forurening, der har fundet sted under slagtningen, eller der kan være sket en selektion af kloner, der bedre kan overleve i det ekstraintestinale miljø.

Resistens hos bakterier fra diagnostiske indsendelser

E. coli fra diagnostiske indsendelser fra dyr var langt hyppigere resistente end *E. coli* fra raske dyr. En mulig forklaring herpå kan være at indsendelse af prøver i forbindelse med sygdom først sker når behandling har været forsøgt og der derfor er sket en selektion af resistente stammer. Eller det kan være at de bakteriestammer, der er årsag til sygdom hyppigere er resistente end stammer der ikke medfører sygdom.

E. coli fra sygdom hos kvæg og svin er ofte resistente overfor chloramphenicol, selv om dette stof ikke har været brugt til behandling af produktionsdyr i 20 år. Isolaterne fra kvæg er endvidere også hyppigt resistente overfor gentamicin, som hos produktionsdyrene kun er godkendt til behandling af svin. Disse eksempler viser, at der ikke altid eksisterer en simpel sammenhæng mellem forbruget af et stof og udbredelsen af resistens mod det samme stof.

Vi så stigende resistens mod ampicillin hos *E. coli* fra mennesker i 1998. Forbruget af penicilliner steg ligeledes men det er usikkert, hvorvidt dette udgør hele forklaringen eller hvorvidt andre omstændigheder, f.eks. ændringer i serotypefordelingen, også har spillet en rolle. For *Staphylococcus aureus* isoleret fra blod har resistensforholdene været stabile i en årrække, medens der har været stigende resistens overfor makrolider hos andre *S. aureus*. Den stigende forekomst af resistens hos *Streptococcus pneumoniae* overfor penicilliner og makrolider er årsag til nogen bekymring.

Kommentarer

Forbruget af antibiotika til dyr er på vej ned. Dette gælder i særdeleshed for forbruget af vækstfremmere men også for forbruget af antibiotika til behandling. Det danske forbud mod vækstfremmeren avoparcin og det frivillige stop for brugen af avilamycin har resulteret i fald i resistens overfor disse stoffer.

Selv om resistenssituationen i Danmark stadig kan betragtes som favorabel er der nogle tendenser, der giver anledning til bekymring.

En sådan tendens er den stigende hyppighed af resistens overfor kinoloner blandt bakterier fra produktionsdyr. Vi har observeret øget resistens overfor nalidixan hos *S.* Enteritidis og *S.* Typhimurium fra fjerkræ, hos *Campylobacter coli* hos svin og hos alle *E. coli*, uanset om bakterierne kom fra diagnostiske indsendelser eller fra raske dyr. Denne udvikling fandt sted på trods af et lille fald i forbruget af kinoloner.

Denne udvikling må ses som et varsel om mulige kommende behandlingsproblemer ikke bare hos produktionsdyr men også – for så vidt angår kinolonresistente zoonotiske bakterier – hos mennesker, hvor kinoloner anvendes som standardterapi. Udviklingen understreger behovet for en restriktiv anvendelse af kinoloner.

På nuværende tidspunkt er gentamicinresistens sjældent forekommende hos bakterier fra husdyr. Imidlertid giver stoffet apramycin som i 1998 blev markedsført til anvendelse hos kvæg og svin anledning til krydsresistens overfor gentamicin. Skulle apramycin finde udbredt anvendelse fremover kan et resultat derfor blive øget udbredelse af gentamicinresistens. Bliver det tilfældet vil det være en forværring af de sundhedsmæssige bekymringer vedrørende kinolonresistens, fordi en stigende andel af de kinolonresistente bakterier, f.eks. salmonella, tillige kan være resistente overfor gentamicin.

En anden udvikling der giver anledning til bekymring er den stigende udbredelse af resistens overfor penicillin og makrolider hos pneumokokker hos mennesker. Denne udvikling synes at hænge sammen med et forøget forbrug af makrolider til behandling af mennesker i de seneste år.

Endelig har vi vist, at resistensforekomsten for nogle bakterier i importerede produkter har indflydelse på effekten af forbedringer, der finder sted efter dansk initiativ.

Summary

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme was set up in 1995 and DANMAP 98 is the third report from the programme. DANMAP monitors antimicrobial resistance in bacteria from food animals, foods and humans and has been designed to provide a basis for comparison of the occurrence of resistance in these reservoirs. DANMAP also reports data on the consumption of antimicrobials in animals and in humans, and on associations between the use of antimicrobials and trends in antimicrobial resistance.

The DANMAP programme includes – in addition to resistance monitoring – considerable research activity. The results of this research are not reported here, however Appendix 2 of this report shows a list of DANMAP publications that have appeared in the international scientific literature.

This report presents data for 1998. Consumption data include the use of antimicrobials in food animals, including the use of antimicrobials for therapy, for growth promotion and as coccidiostats. For antibacterials used in human medicine, there is a detailed analysis of the consumption, including a comparison of the consumption in the Danish counties and an analysis of the trend over time.

The resistance data are presented in sections describing the results for zoonotic bacteria, indicator bacteria (*Escherichia coli* and *Enterococcus faecium/ Enterococcus faecalis*), and bacteria isolated from diagnostic submissions. These sections describe the results for isolates from food animals, from foods and finally from humans.

The data are presented in separate tables for each of the reservoirs but some results are compared in “pen to plate” tables, comparing, for example, resistance in *Salmonella* Typhimurium from food animals, foods and humans.

The panels of antimicrobials have been selected in order to represent all the relevant classes. We have not reported resistance to antimicrobials to which the bacterial species lack a target site (are intrinsically resistant).

Consumption of antimicrobial agents

In early 1998 the Danish food animal industries reduced the use of antimicrobial growth promoters which are now used only in pigs up to 35 kg live weight. This has resulted in a decreased consumption of growth promoters by more than 50 percent. While the production of broilers and slaughter pigs in 1998 increased by five and eight percent, respectively, the consumption of antimicrobials for therapy only showed an overall increase of 2.9 percent. The increase mainly reflects an increased use of narrow spectrum penicillins and sulfonamide/trimethoprim for injection, while the consumption of wide spectrum agents such as fluoroquinolones and tetracycline has decreased.

The decrease in the use of antimicrobial growth promoters has NOT resulted in a compensatory increase in the use of antimicrobials for therapy. In contrast, there has been a substantial decline in the consumption of antimicrobials per food animal produced, including a decline in the consumption of therapeutics.

Detailed examination of the associations between resistance frequencies and consumption of antimicrobials is difficult because we have very little information on the consumption of antimicrobials in the individual animal species.

In contrast to the situation in animals there has been an increase in the consumption of antibacterials (calculated as defined daily doses, DDD) in human medicine. The increase was seen mainly in primary health care and penicillins accounted for much of it. However, there has also been a considerable increase in the consumption of macrolides.

Antimicrobial resistance in zoonotic bacteria

In 1998 an increase in nalidixic acid resistance was seen in *Salmonella* Enteritidis and *Salmonella* Typhimurium from poultry. A similar increase was seen in *S.* Typhimurium from humans. However, this was the result of a food borne outbreak caused by nalidixic acid resistant *S.* Typhimurium DT104 in pork and was not associated with poultry. Graphical presentation of the data reveals that there is a worrying general shift towards higher MIC's, respectively smaller inhibition zones for nalidixic acid and ciprofloxacin in isolates from animals, food and humans. This shift has occurred in spite of a small decline in the use of fluoroquinolones in food animals.

A special study on resistance in Salmonella isolated from imported poultry revealed that the frequency of resistance was generally higher than the one seen in isolates from domestic (Danish) products.

Campylobacter jejuni isolates from broilers and cattle were generally susceptible to most of the antimicrobials included in the test panel and no major changes have occurred compared with 1997. It is noteworthy that the frequency of resistance to quinolones was similar in isolates from broiler meat and from humans but maked higher than the frequency among isolates from Danish broilers.

One contributing factor may be the inclusion of results from isolates from imported products, but further studies are needed to clarify this issue. The predominating species in pigs, *Campylobacter coli*, was generally more resistant to antimicrobials, in particular to quinolones, than *C. jejuni*.

Resistance in *Campylobacter*, particularly resistance to fluoroquinolones and erythromycin, is a potential public health problem. Fluoroquinolones are often the "drug of choice" for empirical treatment of non-specific diarrhoea in humans and erythromycin is often administered to patients with campylobacteriosis.

Antimicrobial resistance in indicator bacteria

An increase in resistance to quinolones was observed in *Escherichia coli* isolates from the normal broiler population and, to a lesser extent, in isolates from slaughter pigs and cattle. For the enterococci, we have observed a decreasing trend in the frequency of resistance to the growth promoters avoparcin and avilamycin, a trend which appears to be associated with the lower consumption of these compounds. For both *Enterococcus faecium* and *Enterococcus faecalis,* we observed discrepancies in the frequencies of resistance in, for example, pigs and pork. A likely explanation is that the microflora of foods in retail outlets is the cumulated result of contamination occurring at all stages of processing and distribution rather than just

contamination occurring during slaughter. Furthermore, there could be a selection in food of clones that are better adapted for survival outside the gastrointestinal tract.

Antimicrobial resistance in pathogenic bacteria

Comparison of results for the *E. coli* serotypes from diagnostic submissions with results for isolates from the normal animal populations revealed that the former were more often resistant to antimicrobials. Isolates from cattle and pigs have a high frequency of resistance to chloramphenicol, although this compound has not been used in food animals for 20 years. In addition, isolates from cattle were often resistant to gentamicin, which only is used in pigs. These examples show that there may not always be a simple association between the consumption of a compound and the occurrence of resistance to that compound, for example because of co-selection.

In *E. coli* from human patients, there was an increasing frequency of resistance to ampicillin from 1997 to 1998. The consumption of penicillins also increased, but it is uncertain whether the increased consumption is the only reason for the observed increase in resistance or whether other factors, for example changes in the predominating serotypes, also played a role. For *Staphylococcus aureus* blood isolates the resistance situation has been stable for a number of years; however, when looking at all clinical isolates of *S. aureus* there has been an increase in resistance to macrolides. For *Streptococcus pneumoniae,* the increasing frequency of resistance to penicillin and to macrolides is a cause for concern.

Comments

Consumption of antimicrobials in food animals is declining. This applies in particular to the consumption of growth promoters but also to the consumption of therapeutics. A number of regulatory and voluntary initiatives seem to have resulted in this positive trend. The Danish ban on the growth promoter avoparcin and the voluntary stop for the use of avilamycin has resulted in a decline in the frequency of resistance to these compounds.

The overall situation in Denmark is still favourable, but there are some trends that are a cause of concern.

One is the increasing frequency of quinolone resistance in bacteria from food animals, and the general decreasing trend in the susceptibility of isolates from animals, foods and humans. While the increase was not uniform, we did find increased resistance to quinolones (nalidixic acid) among *Salmonella* Enteritidis and *Salmonella* Typhimurium from poultry, among *Campylobacter coli* from pigs and among all *Escherichia coli*, irrespective of whether they came from the healthy or the diseased populations. This increase occurred in spite of a small decline in the consumption of quinolones in animals.

This trend may be a warning of potential future treatment failures in food animals but also - in the case of infections with quinolone resistant zoonotic bacteria - in humans, where quinolones often are used in first line therapy.

So far, resistance to gentamicin is generally infrequent in bacteria from food animals. However, apramycin, which was recently registered in Denmark, causes cross-resistance to gentamicin. We have so far observed very little resistance to apramycin, but should its use become widespread it may result in increasing resistance to gentamicin. Such resistance would exacerbate the concerns about quinolone resistance, seen from the public health point of view.

Another trend that causes concern is the increasing resistance to macrolides among pneumococci from human patients. This trend seems to be associated with increased consumption of macrolides over the past few years.

Finally, it has been documented that the resistance pattern for some bacteria in imported products can modify the effect of improvements based upon national initiatives.

Demographic data

Table 1 shows the production of food animals. For broilers and pigs the number of animals slaughtered in 1998 increased by 5 and 8 percent, respectively, while for cattle there was a decline of 7 percent. The proportion of imported meat has been estimated at 30-40 percent for beef and 25-35 percent for poultry. Only a very small proportion of total pork meat is imported. Table 2 shows detailed information on the distribution of the human population.

Table 1 Food animal production (1,000's) and the production of meat in Denmark, 1990-1998 (million kg)

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Year	Poultry		Cattle		Pigs	
	1,000 heads	mio. kg	1,000 heads	mio. kg	1,000 heads	mio. kg
1990	106,675	133.3	789	219	16,427	1,260
1992	122,125	160	862	236	18,559	1,442
1994	137,903	185	813	210	20,760	1,604
1997	130,618	185	791	195	21,180	1,639
1998	138,000	a	735	a	23,032	a

a) Data on the production of meat in 1998 was not yet available

Table 2 Distribution of human population by county in Denmark, 1996-1998

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County no.	County name (1)	Inhabitants January 1998	Population density inhab./km^2	1,000 bed days (2) 1997	No. hospitals 1997	inhab./GP 1996
1	Copenhagen Municipality	487,696	5,529	1,049	5	1,415
2	Frederiksberg Municipality	89,507	10,206	114	1	1,741
3	Copenhagen	610,261	1,160	659	4	1,590
4	Frederiksborg	359,839	267	333	3	1,615
5	Roskilde	228,202	256	223	2	1,646
6	Vestsjælland	292,146	98	290	6	1,567
7	Storstrøm	258,295	76	281	5	1,551
8	Bornholm	44,786	76	45	1	1,329
9	Funen	471,873	135	548	10	1,553
10	Sønderjylland	253,836	65	231	4	1,601
11	Ribe	223,818	72	222	5	1,582
12	Vejle	344,507	115	373	6	1,618
13	Ringkøbing	271,978	56	261	5	1,617
14	Aarhus	631,586	139	683	10	1,579
15	Viborg	233,143	57	250	5	1,580
16	Nordjylland	493,114	80	509	7	1,534
	Total, Denmark (3)	5,294,860	123	6,072	79	1,566

1: The municipalities of Copenhagen and Frederiksberg both have status of county

2: Excl. psychiatry and private hospitals. Preliminary data from The National Board of Health

3: The sum of inhabitants does not correspond to the total. The total includes people residing abroad and people with no permanent address

Consumption of antimicrobials

The Danish Medicines Agency collects data on the consumption of antimicrobials for therapy in humans and in animals. Data on the consumption of antimicrobial growth promoters and coccidiostats are collected by the Danish Plant Directorate. Further details on the data collection may be found in Appendix 1.

Consumption in food animals

At present, information is available on the total consumption of antimicrobials for therapy, for growth promotion and as coccidiostats. For therapeutics, the preparations obviously intended for use in pet animals have been excluded. However, other than that we have no information on the quantities used in the individual animal species. Coccidiostats are used almost exclusively in broilers.

Therapeutics

Table 3 shows the consumption of therapeutics in 1997 and 1998 by group of compound and route of administration. The therapeutics are prescription-only medicines and are not used for growth promotion. They may be used for feed medication, however, only under prescription by a veterinarian. The macrolides tylosin and spiramycin represent an exception as they are approved for both growth promotion and for therapy. However, our data collection system enables us to discriminate between the consumption of identical antimicrobials for these two different purposes.

The total consumption in 1998 increased by 2.9 percent as compared with 1997, somewhat less than the 16 percent increase from 1996 to 1997. The increase is likely to be a result of increased animal production from 1997 to 1998, and the total consumption is still lower than during the mid-1990's (Table 4).

In 1997, the Danish Veterinary Laboratory issued a set of guidelines on prudent use of antimicrobials in veterinary practice *(Pedersen et al., Veterinary Record, in press*). The guidelines recommend using narrow spectrum antimicrobials whenever possible, and, while it is not known for certain to what extent they are followed by veterinary practitioners, the results do show a 9 percent increase in the use of narrow spectrum penicillins in particulary injectables, and a decline in the consumption of, among others, tetracycline and quinolones. These changes are highly favourable from a resistance point of view. These results show that a reduction in the use of antimicrobial growth promoters (see below) is not accompanied by an increased use of antibiotics for treatment.

In September 1998, following a foodborne outbreak of salmonellosis caused by nalidixic acid resistant *S.* Typhimurium DT104, the Danish authorities issued a recommendation to veterinarians to show restraint in their use of fluoroquinolones. This may have contributed to the observed decrease. In February 1999, Bayer, the manufacturer of the most widely

Table 3 Consumption of antimicrobials (kg active compound) in food animals by ATC group and route of administration, Denmark, 1997-1998

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ATC-group	Compound	Oral		Injection		Intramammary		Intrauterine		Total	
		1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
J01A	Tetracyclines	11,300	9,600	2,300	2,400	50	< 25	< 25	< 25	13,700	12,100
J01C	Penicillins										
	Extended Spectrum Penicillins	3,900	4,000	2,100	2,500	100	150	0	0	6,200	6,600
	Narrow Spectrum	0	0	12,900	14,100	100	150	50	50	13,100	14,300
J01D	Cephalosporins	0	0	50	50	< 25	< 25	0	0	50	50
J01E	Sulfonamides	0	0	400	0	0	0	1,000	1,000	1,400	1,000
	Sulfonamides + TMP	4,000	4,400	2,900	3,300	0	0	0	0	6,900	7,700
J01F	Macrolides	1,800	2,100	2,000	1,800	0	0	0	0	3,700	4,000
J01G	Aminoglycosides	3,900	4,600	2,900	2,900	150	150	150	100	7,000	7,800
J01M	Quinolones	250	250	200	150	0	0	0	0	450	400
J01X	Others	2,900	3,100	200	250	< 25	< 25	0	0	3,100	3,400
J01	Total	28,000	28,100	25,900	27,500	450	450	1,200	1,200	55,700	57,300

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 1996-1998: Danish Medicines Agency

DANMAP 98

ATC-group	Compound	1986	1988	1990	1992	1994	1996	1997	1998
J01A	Tetracyclines	3,800	3,600	9,300	22,000	36,500	12,900	13,700	12,100
J01C	Penicillins	3,700	3,800	5,000	6,700	9,400	7,200	13,100	14,300
J01C/J01D	Semisyn. pen. etc.	850	1,000	1,200	2,500	4,400	5,800	6,200	6,700
J01E	Sulfa/TMP	2,500	2,200	3,800	7,900	9,500	4,800	6,900	7,700
J01E	Sulfonamides	22,300	24,200	8,700	5,900	5,600	2,100	1,400	1,000
J01F	Macrolides *a)*	10,100	9,300	10,900	12,900	11,400	7,600	6,700	7,100
J01G	Aminoglycosides	7,800	7,400	7,700	8,500	8,600	7,100	7,000	7,800
	Others	13,800	6,900	6,700	6,800	4,400	600	650	650
J01	Total	64,800	58,400	53,400	73,200	89,900	48,000	55,700	57,300

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

used fluoroquinolone, enrofloxacin, issued a statement that, in response to concerns about imprudent use the company would stop producing an enrofloxacin formulation mainly used for feed medication.

In 1998, apramycin was marketed for the first time in Denmark. DANMAP will provide baseline data for the developments in the occurrence of resistance to apramycin.

Growth promoters

In January 1998, the Danish minister for Food, Agriculture and Fisheries banned the use of virginiamycin. At the same time, the Danish food animal industry adopted a voluntary stop on the use of all antimicrobials for growth promotion except in pigs up to 35 kg live weight; however, the latter use will be phased out during 1999. Changes in the consumption of growth promoters reflect this ban (Table 5). The total consumption is down by more than 50 percentas compared to 1997. However the consumption of olaquindox, which is used in weaner pigs only, has increased by more than 60 percent.

Among broiler producers there was concern that the stop of growth promoters use would lead to an increase in the incidence of disease problems and a reduced productivity. According to the industry, this has not been the case (see Box page 12). There have been few disease problems, no increased use of therapeutics and the Danish broiler industry is still able to compete in the international market place.

Coccidiostats

The use of anticoccidials was not affected by the stop for use of growth promoters in broilers. The consumption of coccidiostats in poultry is presented in Table 6. The total consumption has remained fairly constant over the past 3 years although there have been changes in the preferred individual agents.

Consumption in humans

Tables 7 and 8 present the consumption of antibacterials for systemic therapy in primary health care and in hospitals, respectively. The total consumption is found by simple addition of figures in the tables.

Table 5 Consumption of antimicrobial growth promoters (kg active compound), Denmark 1990-1998

DANMAP 98

Antibiotic group	Growth promoter	1990	1992	1994	1996	1997	1998
Bacitracin	Bacitracin	3,983	5,657	13,689	8,399	8,544	3,945
Flavofosfolipol	Flavomycin	494	1,299	77	18	93	6
Glycopeptide	Avoparcin	13,718	17,210	24,117	0	0	0
Ionophore	Monensin	2,381	3,700	4,755	4,741	3,008	935
	Salinomycin	12	-	213	759	460	113
Macrolides	Spiramycin	-	-	95	15	3	0.3
	Tylosin	42,632	26,980	37,111	68,350	62,009	13,148
Oligosaccharides	Avilamycin	10	853	433	2,740	670	7
Quinoxalines	Carbadox	850	7,221	10,012	1,985	4,153	1,803
	Olaquindox	11,391	21,193	22,483	13,486	17,595	28,445
Streptogramins	Virginiamycin	3,837	15,537	2,801	5,055	10,644	892
Total		79,308	99,650	115,786	105,548	107,179	49,294

Consumption of antibiotics following discontinued use of growth promoters in broilers

In the beginning of 1998 the Danish food animal industries adopted a voluntary ban on the use of antimicrobial growth promoters. A number of scientific studies have been initiated to determine the effects of this stop on productivity, morbidity and on the consumption of therapeutics in pigs and broilers. The results of these studies are not yet available; however, the editors have asked Danpo A/S to provide information on the effects of the ban in their part of the production. Danpo A/S is a subsidiary of Scandinavian Poultry and slaughters and processes about 40 percent of the Danish broiler production, mostly for export.

According to statistics compiled by the Danish Poultry Council and based on almost all commercial flocks produced in 1998, the mean feed consumption at 42 days of age did increase from 1.78 kg feed to 1.82 kg per kg live bird after the ban and it has remained higher than 1.81 kg. At the same time, the slaughter weight at 42 days decreased by about 30 grams to 1,930 grams; however, it has since increased and is now higher than 2,000 grams.

There has been no increase in the overall morbidity and no significant increase in the consumption of antimicrobials for therapy in commercial Danish broiler flocks since the use of growth promoters was discontinued. However, as expected, the industry has observed an increase in the number of flocks suffering from the *Clostridium perfringens* related diseases necrotic enteritis and chronic hepatitis.

In 1998 the Danpo production amounted to 48.2 million birds from about 1,700 flocks. During this year necrotic enteritis was diagnosed in 25 flocks compared with 1-2 flocks only when growth promoters were used. A total of about 24 kg amoxicillin (active compound) was used to treat the outbreaks. Alternatively, without the voluntary stop, the Danpo flocks would have used approximately 1,500 kg active compound of antimicrobials for growth promotion, assuming a mean inclusion rate in the feed of 10 ppm.

In other words, for the segment of the Danish broiler production that comes under the Danpo umbrella, the discontinued use of growth promoters has reduced very significantly the total consumption of antimicrobials with only a minor increase in morbidity in the broiler flocks and with no loss of productivity.

Further details may be obtained by contacting Niels Tornøe, DVM, Ph.D., Danpo A/S, Aagade 2, Farre, DK-7323 Give, Denmark.

The overall consumption of antibacterials in primary health care and hospitals increased by 4.4 percent from 1997 to 1998 and reached 4,794 DDD per 1,000 inhabitants. This corresponds to a total consumption in primary health care and in hospitals of 13.1 DDD/ 1,000 inhabitants x day in 1998. In 1998, 9.6 percent of the total consumption was used in hospitals. The most commonly used antibacterials were the ones that are favourable from a resistance point of view: penicillins (J01C) accounted for 64 percent of the consumption, whereas cephalosporins (J01D) and fluoroquinolones (J01M) only accounted for 1 and 2 percent, respectively.

Primary health care sector

The consumption of antibacterials in primary health care increased from 11.3 DDD/1,000 inhabitants x day in 1996-1997 to 11.9 in 1998. The number of persons prescribed antibacterials at least once during the year increased by approximately 68,000 from 1997 to 1998. Thirty-four percent of these were children under 15 years.

The greatest increase in consumption was observed in the penicillin group (J01C) and amounted to 113 DDD/1,000 inhabitants (relative increase: 4 percent). However, with 12 percent, the macrolide group had

Table 6 Consumption of coccidiostats in poultry (kg active compound), Denmark, 1990-1998 DANMAP 98

Coccidiostats	1990	1992	1994	1996	1997	1998
Amprolium/Ethopabat	3,562	2,716	2,342	1,339	488	275
Dimetridazol	-	-	-	38	542	-
DOT	-	-	300	-	352	-
Monensin	-	108	1,016	3,405	3,690	3,709
Robenidin	33	295	858	293	672	367
Ronidazol	36	-	-	-	-	-
Metichlorpindol/Methylbenzoat	89	1,503	3,360	4,857	3,928	930
Lasalocid	75	-	5	773	1,359	1,677
Halofuginon	-	-	19	8	7	-
Narasin	1,588	5,157	6,370	3,905	1,777	3,177
Salinomycin	7,783	10,298	6,018	4,531	4,197	7,884
Nicarbazin	-	-	-	115	4	36
Nifursol	-	395	-	146	-	234
Diclazuril	-	-	18	34	21	3
Nitrovin	403	-	-	-	-	-
Total	13,569	20,472	20,306	19,444	17,037	18,292

the largest relative increase, corresponding to 89 DDD/1,000 inhabitants x year.

The consumption of macrolides has increased since 1994 when it accounted for 16 percent of the total consumption, as compared to 19 percent in 1998. There was a general high consumption in 1998 and a major increase during the last two months of the year. In December 1998, 90,000 prescriptions were redeemed as compared to an average level of around 40,000 in previous years. This increase was due to an epidemic of Mycoplasma upper respiratory tract infection during the fourth quarter of 1998.

The consumption during 1998 (2.2 DDD/1,000 inhabitants x day) was similar to the amount used in Finland in the late eighties **(***Seppälä et al., N Engl J Med, 1997, 337, 441-446; Seppälä et al., N Engl J Med, 1992, 326, 292-297*). In Finland this led to a sudden rise in the frequency of macrolide resistant group A streptococci with a couple of years delay. To control this problem, a big and successful effort was made to reduce the consumption of macrolides. At present the levels of macrolide resistance in group A streptococci and of macrolide and penicillin resistance in *Streptococcus pneumoniae* in Denmark are low. However, a reduction in the consumption of macrolides, preferably to a level around 1.0-1.5 DDD/1,000 inhabitants x day is recommended in order to prevent an increase in resistance.

Consumption in individual counties

The consumption by class of antibacterials for each county is shown in Figure 1. Please note that the scale differs for each of the therapeutic groups. Figure 2 shows the change over time in the consumption of antibacterials for systemic use in the individual counties.There were major regional differences in the consumption of antibacterials during 1998. The lowest consumption, 9.6 DDD/1,000 inhabitants x day, was observed in Aarhus and Bornholm counties. Inversely, Frederiksberg Municipality and Copenhagen and Ribe counties used as much as 13.4 and 13.2 DDD/ 1000 inhabitants per day, respectively, which is 29 percent higher than in Aarhus and Bornholm counties. We can offer no explanation for these differences. The increase in the consumption also differed greatly between counties, ranging from just over 1 percent in Ribe county to over 7 percent in Vestsjælland, Ringkøbing and Roskilde counties.

Consumption of antibacterials in hospitals

Figure 3 shows the consumption of antibacterials in hospitals in the different counties. There is some delay in getting the final data on the number of bed days. Instead we have used DDD per 1,000 inhabitants as denominator. There have been changes in the health care structure in the Copenhagen area (counties No 1 and 2) in 1997 and 1998. Data from these counties are not readily comparable (indicated by grey shade in figures no. 3 and 4).

In general, the consumption is similar in the counties, however a number of differences deserves a comment. There was an almost three times higher consumption in Copenhagen Municipality (county 1) than the national average. This is explained by the fact that the university hospital in Copenhagen recruits patients from all the other counties and also from Greenland and the Faeroe islands, so the denominator is underestimated. The patient population is also different and represent the more complicated cases.

Table 7 Use of antibacterials for systemic use in human primary health care (DDD per 1,000 inhabitants), Denmark, 1994-1998

DANMAP 98

ATC-group	Therapeutic group	1994	1995	1996	1997	1998	Change (97-98) %
J01A	Tetracyclines	628	578	391	357	359	1
J01B	Chloramphenicol	0	0	0	0	0	-25
J01C	Penicillins	2,802	2,835	2,640	2,669	2,782	4
J01D	Cephalosporins	6	6	6	6	6	2
J01E	Sulfonamides	312	302	292	290	282	-3
J01F	Macrolides and lincosamides	756	771	691	716	805	12
J01G	Aminoglycosides	1	1	0	0	0	36
J01M	Quinolones	132	113	87	80	86	7
J01X	Other	15	15	15	16	16	-2
Total		4,652	4,620	4,122	4,134	4,334	5

The counties with a high consumption are those with a university hospital (counties 2, 3, 9, 14 and 16). There has been a negligible (0.2 percent) increase in the total consumption from 1997 to 1998. Figure 4 presents the consumption of antibacterials by therapeutic groups for each county. There are some major differences. The change from a high consumption of tetracycline in the hospital in the small county of Bornholm (8) is explained by a change in the first choice antibiotic for treatment of dermatological ailments from tetracycline to other agents. The decrease, while large in relative terms, represents only 633 DDD.

In primary health care, the consumption of cephalosporins and quinolones in hospitals was low and amounted to 0.12 and 0.05 DDD/1000 inhabitants x day, respectively.

Table 8 Use of antibacterials for systemic use in hospitals (DDD per 1,000 inhabitants), Denmark, 1997-1998

DANMAP 98

ATC-group	Therapeutic group	1997	1998	Change (97-98) %
J01A	Tetracyclines	4.24	4.05	-4
J01B	Chloramphenicol	0.01	0.02	100
J01C	Penicillins	273	280	3
J01D	Cephalosporins	40.3	42.7	6
J01E	Sulfonamides	25.1	26.5	6
J01F	Macrolides and lincosamides	40.7	41	1
J01G	Aminoglycosides	38.5	25.1	-35
J01M	Quinolones	16.6	17.5	5
J01X	Other	21.9	22.7	4
Total		459	460	0

DANMAP 1998

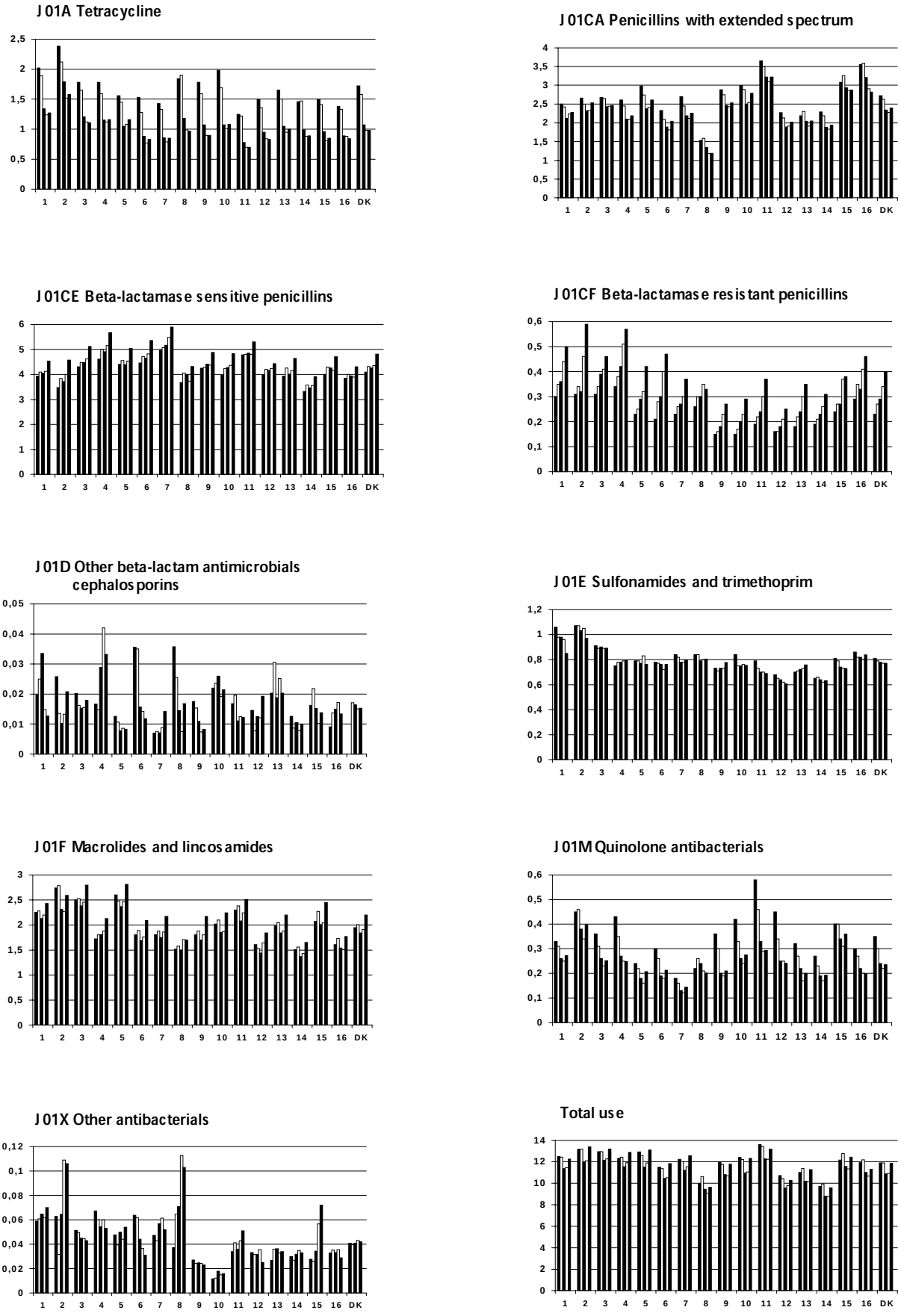


Figure 1 Annual use of different classes of antibacterials in primary health care (DDD/1,000 inhabitants x day), Denmark, 1994-1998. Numbers on horizontal axis represent counties. For county codes, please see next page.

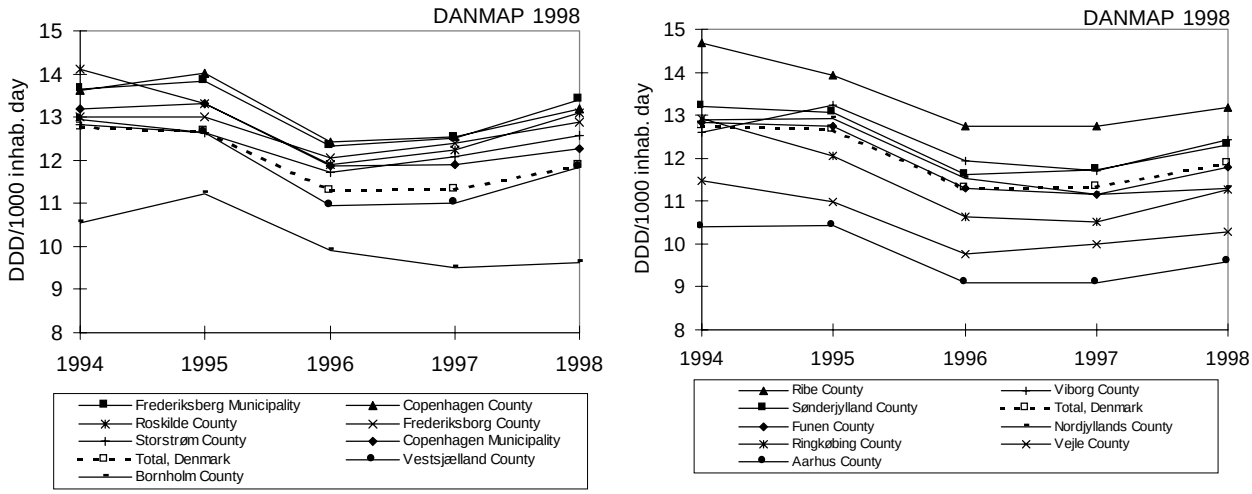


Figure 2 Trend in total use of antibacterials in primary health care in individual counties, Denmark, 1994-1998

Legends for Figures 1, 3 and 4

County codes:

1. Copenhagen Municipality
2. Frederiksberg Municipality
3. Copenhagen County
4. Frederiksborg County
5. Roskilde County
6. Vestsjælland County
7. Storstrøm County
8. Bornholm County
9. Funen County
10. Sønderjylland County
11. Ribe County
12. Vejle County
13. Ringkøbing County
14. Aarhus County
15. Viborg County
16. Nordjylland County

DK Denmark

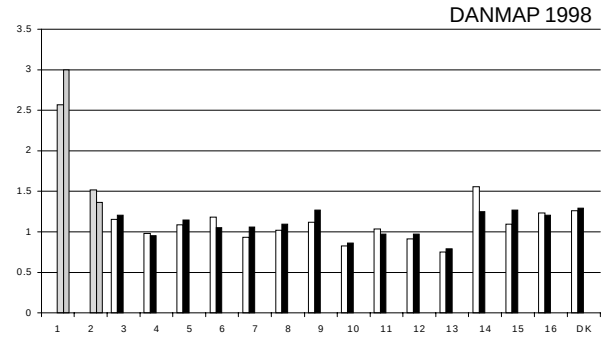


Figure 3 Total use of antibacterials in Danish hospitals in different counties (DDD/1,000 inhabitants x day), Denmark, 1997-1998

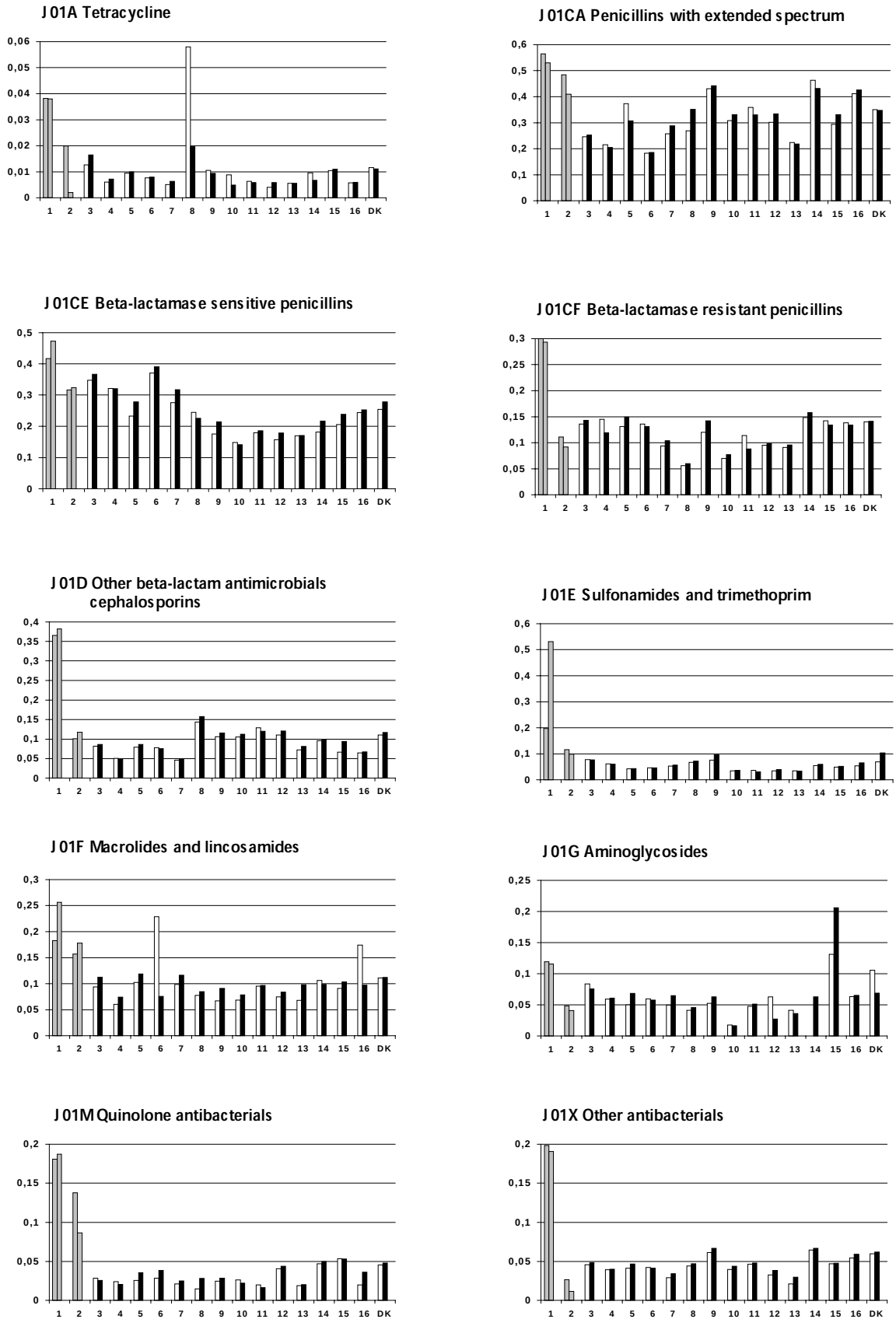


Figure 4 Annual use of different classes of antibacterials in hospitals (DDD/1,000 inhabitants x day), Denmark,1997-1998. Number on horizontal axis represent counties. For county codes, please see previous page.

Resistance in Zoonotic bacteria

Table 9 Distribution (%) of Salmonella *serotypes isolated from animals and food, Denmark, 1998*

DANMAP 1998

Serotypes	Poultry	Broiler meat 1)	Other poultry meat 1)	Cattle	Beef 1)	Pigs	Pork 1)
4.12:b:-	8	0	1	0	0	0	0
Derby	1	0	5	3	0	9	11
Dublin	0	0	0	53	40	0	4
Enteritidis	47	13	4	1	0	1	2
Hadar	5	15	13	1	0	0	4
Indiana	5	6	4	0	0	0	0
Infantis	11	2	1	1	0	6	7
Typhimurium	13	4	14	34	20	72	53
Others	10	60	55	7	40	13	19
Number of isolates	240	103	83	77	10	248	109

1) Includes samples of imported products

Salmonella

Salmonellosis is the most common foodborne zoonosis in Denmark. The majority of human *Salmonella* infections in Denmark are caused by *Salmonella* Enteritidis (67 percent of all cases). *S.* Enteritidis is the predominant serotype in egg layers and also occurs in broilers but is rare in other animal species (*Annual Report on Zoonoses in Denmark 1998*). The second most important serotype in Denmark is *Salmonella* Typhimurium (17 percent of all human cases). It is the predominant serotype in pigs but is also prevalent in other food animal species.

Table 10 Distribution (%) of Salmonella *Typhimurium phage types from animals, pork and humans, Denmark, 1998*

DANMAP 1998

Phage type	Poultry	Cattle	Pigs	Pork	Humans
1	3	-	-	-	-
10	-	-	1	3	1
12	6	50	52	47	43
15a	-	-	1	2	1
17	3	4	6	7	2
18	-	-	-	2	-
40	-	4	-	-	-
41	3	-	-	-	1
66	6	8	9	7	5
86	-	-	1	-	-
104	-	15	3	5	13
107	-	8	1	-	1
108	-	-	1	-	-
109	-	-	1	-	-
110	35	-	1	-	1
120	-	-	2	2	2
135	10	-	3	-	4
177	13	-	-	-	1
186	-	-	1	-	-
193	10	4	3	2	3
208	-	-	1	-	-
288	-	-	-	4	4
fi/ru	-	4	1	-	-
nt	-	-	3	7	-
rdnc	10	-	10	5	-
u302	-	4	1	-	2
Others/ unknown	3	-	-	9	18
Number of isolates	32	26	178	58	474

Four isolates from broiler meat, 11 isolates from other poultry meat and 2 isolates from beef are not included in this table. The human isolates represent a random subsample of those susceptibility tested.

Salmonella from food animals

The tendency of *Salmonella* to become resistant to antibiotics depends to some extent on the serotype (*S.* Enteritidis and *S.* Dublin in contrast to *S.* Typhimurium) or even phage type (e.g. *S.* Typhimurium DT104). Therefore, the distribution of serotypes and phage types must be considered when the occurrence of resistance is compared between isolates from different animal species. Table 9 and 10 show the distribution of serotypes and *S.* Typhimurium phage types of isolates included in this report.

The occurrence of resistance among all *Salmonella* serotypes isolated from animals in 1998 is presented in Table 11 and the results for *S.* Enteritidis from layers and broilers and *S.* Typhimurium from broilers and layers, cattle and pigs are presented in Table 12.

We examined the proportions of *Salmonella* isolates from animals resistant to antimicrobials important in human medicine (ampicillin, gentamicin, streptomycin, tetracycline, and nalidixic acid as representative of the quinolones. Figures 5 and 6 show that the proportions of isolates resistant to two or more of these compounds is less than 15 percent.

Table 11 Susceptibility and occurrence of resistance among Salmonella *and* Yersinia *from food animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Salmonella enterica												Yersinia enterocolitica			
		Poultry				Cattle				Pigs				Pigs			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5-32	1	4	4	0.5-32	1	32	17	0.5-32	2	32	16	0.5-8	1	2	0
J01B	Chloramphenicol	2-16	8	8	0	2-64	4	8	9	2-64	4	8	5	1-8	4	4	0
J01C	Ampicillin	0.5-32	2	4	7	0.5-32	1	4	8	0.5-32	1	2	6	16-32	32	32	90
J01E	Sulfonamid	16-512	64	128	8	16-512	32	512	21	8-512	64	512	25	8-32	8	32	0
	Trimethoprim	0.5-32	0.5	1	5	0.5-1	0.5	0.5	0	0.5-32	0.5	32	11	1-4	2	4	0
J01G	Apramycin	1-8	2	4	0	1-8	2	4	0	1-8	2	4	0	1-8	4	8	0
	Gentamicin	0.5-2	0.5	1	0	0.5-2	0.5	1	0	0.5-8	0.5	1	0	0.5-2	0.5	1	0
	Kanamycin	1-8	2	4	0	1-64	2	4	1	1-64	2	4	6	1-8	2	4	0
	Streptomycin	2-128	8	16	9	2-128	16	64	25	4-128	8	64	15	2-128	8	16	8
J01M	Ciprofloxacin	0.125-1	0.125	0.125	0	0.125-1	0.125	0.125	0	0.125-0.25	0.125	0.125	0	0.125-0.25	0.125	0.125	0
	Nalidixic acid	2-128	4	8	7	2-128	4	8	5	2-128	4	8	2	2-4	2	2	0
J01X	Colistin	1-8	1	2	0	1-64	2	4	4	1-64	1	2	1	1-4	1	1	0
	Carbadox	8-64	8	16	0	8-64	16	32	0	8-64	8	16	0	8-16	8	8	0
	Olaquindox	2-64	16	32	0	8-64	32	32	0	8-128	16	32	0	4-64	16	16	0
	Nitrofurantoin	32-128	32	64	0	32-64	32	64	0	32-128	32	32	1	32-32	32	32	0
Number of isolates					240				77				248				49

Table 12 Susceptibility and occurrence of resistance among Salmonella *Enteritidis and* Salmonella *Typhimurium from food animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	S. Enteritidis				S. Typhimurium											
		Poultry				Poultry				Cattle				Pigs			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5-32	1	4	2	1-32	2	4	6	0.5-32	2	32	23	1-32	2	32	16
J01B	Chloramphen	2-8	4	8	0	4-8	8	8	0	2-64	4	64	19	2-64	4	8	6
J01C	Ampicillin	1-32	2	2	4	1-32	2	16	9	0.5-32	2	32	19	0.5-32	1	4	7
J01E	Sulfonamid	32-512	64	64	3	16-512	32	512	19	32-512	64	512	38	8-512	64	512	29
	Trimethoprim	0.5-32	0.5	1	3	0.5-32	0.5	2	9	0.5-1	0.5	0.5	0	0.5-32	0.5	32	11
J01G	Apramycin	1-8	2	4	0	1-8	2	4	0	2-8	2	4	0	1-8	2	4	0
	Gentamicin	0.5-1	0.5	0.5	0	0.5-2	0.5	1	0	0.5-2	0.5	1	0	0.5-8	0.5	1	0
	Kanamycin	1-8	2	4	0	1-4	2	4	0	1-64	2	4	4	1-64	2	4	6
	Streptomycin	2-64	4	8	2	2-128	8	32	22	8-128	8	128	27	4-128	8	128	17
J01M	Ciprofloxacin	0.125-1	0.125	0.125	0	0.125-0.5	0.125	0.125	0	0.125-0.25	0.125	0.125	0	0.125-0.25	0.125	0.125	0
	Nalidixic acid	2-128	4	8	7	2-128	4	8	6	2-128	4	8	8	2-128	4	8	2
J01X	Colistin	1-8	1	2	0	1-2	1	1	0	1-4	1	2	0	1-4	1	2	0
	Carbadox	8-32	16	16	0	8-32	16	16	0	8-32	8	16	0	8-64	8	16	0
	Olaquindox	16-64	16	32	0	16-32	16	32	0	8-32	16	16	0	8-128	16	32	1
	Nitrofurantoin	32-64	32	64	0	32-64	32	64	0	32-64	32	64	0	32-128	32	64	1
Number of isolates					113				32				26				178

Salmonella from foods

A total of 341 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 wholesalers and in retail outlets. The *Salmonella* isolates originated from beef (3 percent), pork (32 percent), broiler meat (30 percent), other poultry meat (24 percent) and other foodstuffs (11 percent).

The serotype distribution of the *Salmonella* isolated from foods is given in Table 9. *S.* Typhimurium is the predominant serotype in pork, with 53 out of a total of 109 isolates. The phage type distribution of *S.* Typhimurium is shown in Table 10, and it is apparent, that approximately half of the *S.* Typhimurium isolates from pork belong to DT12. A total of 6 penta-resistant *S.* Typhimurium DT 104 (three from pork, one from beef, one from broiler meat and one from other poultry products) were tested for antimicrobial susceptibility.

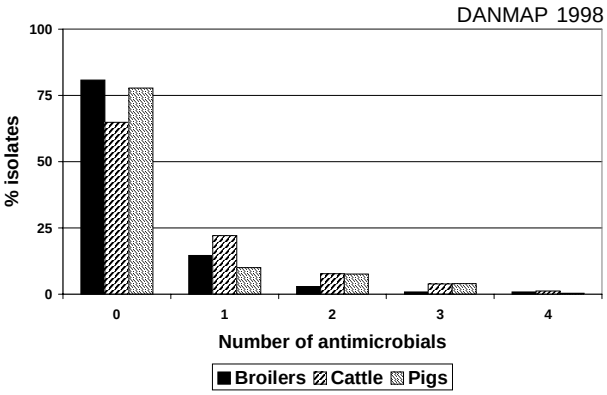


Figure 5 Distribution of resistance markers to ampicillin, gentamicin, streptomycin, tetracycline and nalidixic acid among all Salmonella *serotypes from food animals in Denmark in 1998*

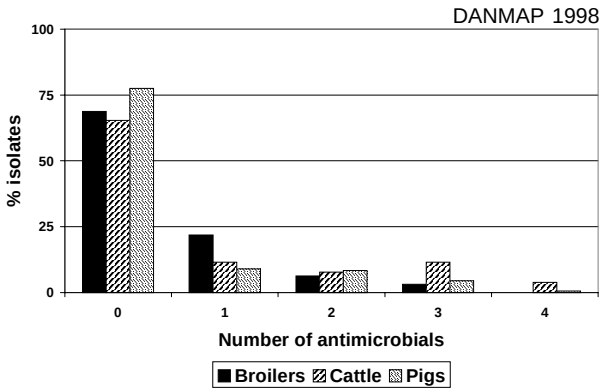


Figure 6 Distribution of resistance markers to ampicillin, gentamicin, streptomycin, tetracycline and nalidixic acid among Salmonella *Typhimurium from food animals in Denmark in 1998*

Antimicrobial resistance among all *Salmonella* from foods is presented in Table 13.

Salmonella from foods are frequently resistant to a number of different antimicrobials. Figure 7 shows the level of multiresistance in isolates from pork and broiler meat to 5 antimicrobials: ampicillin, streptomycin, tetracycline, nalidixic acid and gentamicin. Sixty-seven percent of the isolates from pork and 45% of the isolates from broiler meat were fully susceptible to these 5 antimicrobials. Twenty percent of the isolates from pork and 30% of isolates from broiler meat were resistant to one antimicrobial. Resistance to four antimicrobials was recorded in 5% of the isolates from pork and in 10% of the isolates from broiler meat. No isolate was resistant to all 5 antimicrobials.

The overall frequency of resistance in *Salmonella* from foods is similar to the levels recorded in 1997.

Salmonella in humans

There has been an increase in the frequencies of antimicrobial resistance among *Salmonella* Typhimurium in 1998 (Table 15). This increase is primarily due to the first Danish outbreak of *S.* Typhimurium DT104 caused by food of domestic origin (*Anon., Weekly Epidemiological Record, 1998, 73, 327).* This outbreak included 25 culture-confirmed cases. In total 7 percent of the *S.* Typhimurium cases were due to DT104 in 1997 compared with 13 percent in 1998. The outbreak strain exhibited the classic penta-resistance pattern and was also resistant to spectinomycin and to nalidixic acid as well.

A number of the patients involved in the outbreak were hospitalised and treated with ciprofloxacin. There were several treatment failures, even though

Table 13 Antimicrobial resistance (%) among Salmonella *from all serotypes isolated from food, Denmark,1998*

DANMAP 1998

Compound	Beef	Pork	Broiler	Other poultry	Other foodstuffs
Tetracycline	10	25	35	43	25
Chloramphenicol	10	6	3	13	8
Ampicillin	10	11	30	24	17
Trimethoprim	10	5	18	17	8
Gentamicin	0	1	2	0	0
Kanamycin	10	1	1	4	0
Streptomycin	20	13	18	34	11
Ciprofloxacin	0	0	0	0	0
Nalidixic acid	10	6	21	19	11
Colistin	0	0	0	0	0
Number of isolates	10	109	103	83	36

Screening of imported poultry meat

In 1997 the Danish Veterinary and Food Administration and the Danish Zoonosis Centre conducted a screening of imported poultry for *Salmonella*. This investigation resulted in the isolation of 207 different strains of *Salmonella* from different lots of poultry meat, including meat from broilers, hens, ducks and turkey.

The predominant serotypes of the import investigation were *S.* Typhimurium (15 percent) *S.* Enteritidis (13 percent) and *S.* Hadar (13 percent). The serotype distribution is presented in Table 14 below. A total of 8 penta-resistant *S.* Typhimurium Definitive Type 104 was isolated.

In general the resistance level in this screening of imported poultry is higher than seen among Danish isolates from routine monitoring of poultry in retail outlets. In imported poultry meat, 46 percent of the isolates were resistant to tetracycline and 30 and 44 percent to ampicillin and streptomycin, respectively. Forty-nine percent of the isolates were resistant to nalidixic acid and one isolate also to ciprofloxacin. Eight percent of the isolates were resistant to gentamicin. Multiple resistance was also more frequent among isolates from imported poultry products as compared with *Salmonella* from broiler meat of mainly domestic origin (Figure A and Figure 7).

Table 14 Distribution (%) of Salmonella *serotypes in a survey of imported poultry, Denmark, 1998*

	DANMAP 1998
Serotypes	%
Enteritidis	13
Hadar	13
Indiana	9
Infantis	5
Typhimurium	15
Wirchow	3
Other	42
Number of isolates	207

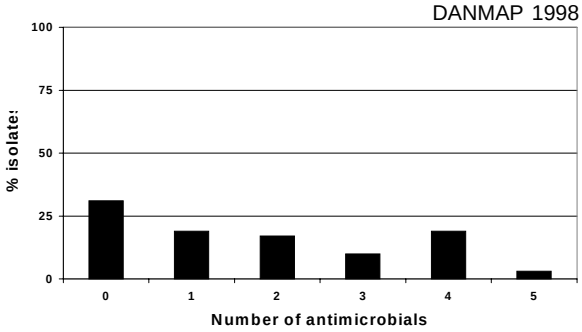


Figure A Distribution of resistance to ampicillin, gentamicin, streptomycin, tetracycline and nalidixic acid among all Salmonella *serotypes isolated from imported poultry, Denmark, 1998*

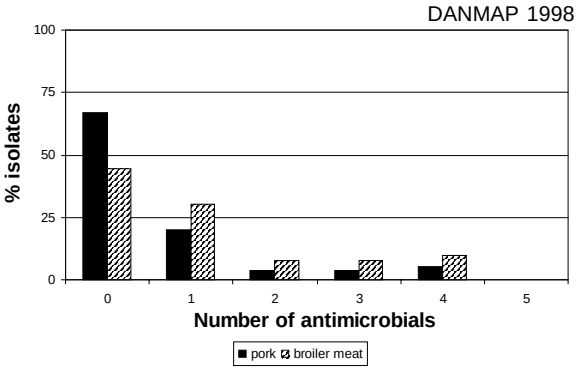


Figure 7 Distribution of resistance markers to ampicillin, gentamicin, streptomycin, tetracycline and nalidixic acid among all Salmonella *serotypes from pork and broiler meat, Denmark, 1998*

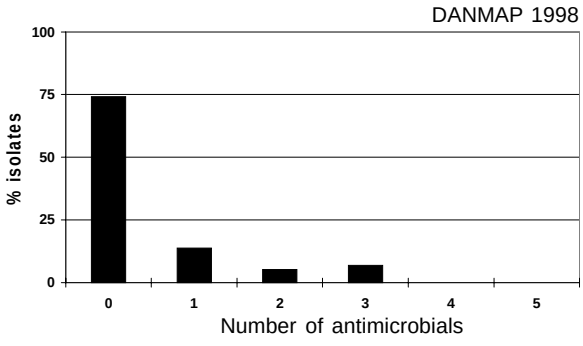


Figure 8 Distribution of resistance markers to ampicillin, gentamicin, streptomycin, tetracycline and nalidixic acid among Salmonella *Typhimurium from pork, Denmark, 1998*

the isolates were not resistant to fluoroquinolones according to the breakpoints used at present (corresponding to a MIC of 2 µg/ml). Statens Serum Institut is now reconsidering which breakpoint to recommend for use in hospital laboratories in Denmark. The deliberations have not yet been concluded, however, they may result in a change of breakpoint to 0.125 µg/ml.

Figure 9 shows the distributions of nalidixic acid and ciprofloxacin MIC values and inhibition zones- for *S.* Typhimurium from food animals, food and humans in 1997 and 1998. The population distributions indicate that a shift towards decreased susceptibility has occurred.

Table 16 compares resistance among *S.* Typhimurium from food animals, foods and humans and Table 17 resistance among *S.* Enteritidis from poultry and humans. Both tables also show the changes from 1997 to 1998.

Table 16 indicates that resistance has become more frequent among *S.* Typhimurium isolates from cattle in 1998 as compared with 1997. This is because in 1998 four of the 26 *S.* Typhimurium isolates were penta-resistant DT104 and the increased frequency was seen for the antimicrobials to which DT104 is resistant. These four isolates represent the only cattle herds where DT104 was found during 1998. In contrast, only 5 of 178 *S.* Typhimurium isolates from pigs were DT104.

Resistance to nalidixic acid among *S.* Typhimurium isolates from poultry increased from 1 percent in 1997 to 6 percent in 1998 (statistically non-significant). Among *S.* Typhimurium from pigs, antimicrobial resistance has remained almost unchanged as compared with 1997.

Among *S.* Enteritidis isolates from broilers and layers, resistance to nalidixic acid increased from 0 percent in 1997 to 7 percent in 1998. This increase is statistically significant.

Yersinia enterocolitica O:3

Yersinia from food animals

Since the mid-1980's the human incidence of yersiniosis has declined from about 30 cases per 100,000 to 9 per 100,000 in 1998 (*Annual Report on Zoonoses in Denmark 1998).* Pigs are often carriers of these bacteria and are considered the most important reservoir for human infections. Most *Yersinia enterocolitica* O:3 isolates were resistant to ampicillin (Table 11), but susceptible to almost all other antimicrobials included in the test panel.

Yersinia in foods

We susceptibility tested 6 strains of *Y.* enterocolitica that were isolated from pork. Except for resistance to ampicillin alle were fully susceptible.

Yersinia from humans

In 1998, 120 of 464 isolates were tested for antimicrobial susceptibility. Two isolates were resistant to streptomycin and spectinomycin. No resistance markers other than ampicillin were found and there has been no change in resistance frequencies since 1995.

Table 15 Occurrence of resistance (%) among Salmonella *Enteritidis and* Salmonella *Typhimurium isolated from humans, Denmark, 1995-1998*

DANMAP 1998

ATC-group	Compound	Salmonella Enteritidis				Salmonella Typhimurium		
		1995	1996	1997	1998	1995	1997	1998
J01A	Tetracycline	1	1	1	1	19	18	22
J01B	Chloramphenicol	1	0	0	0	8	8	18
J01C	Ampicillin	3	1	2	2	10	11	24
J01D	Ceftriaxone		0	1				
J01E	Sulfamethizol	3	1	1	1	18	19	30
	Trimethoprim	1	1	1	1	3	3	5
J01G	Apramycin	0	0	0	0	1	1	2
	Gentamicin	1	0	1	1	1	1	1
	Kanamycin		0	1	0		1	1
	Spectinomycin	2	1	1	1	9	10	20
	Streptomycin	3	0	1	1	19	19	32
J01M	Ciprofloxacin	0	0	0	0	0	0	1
	Nalidixic acid	1	1	1	2	1	1	9
J01X	Colistin	0	0	1	0	0	1	0
Number of isolates		384	206	658	466	398	644	624

DANMAP 1998

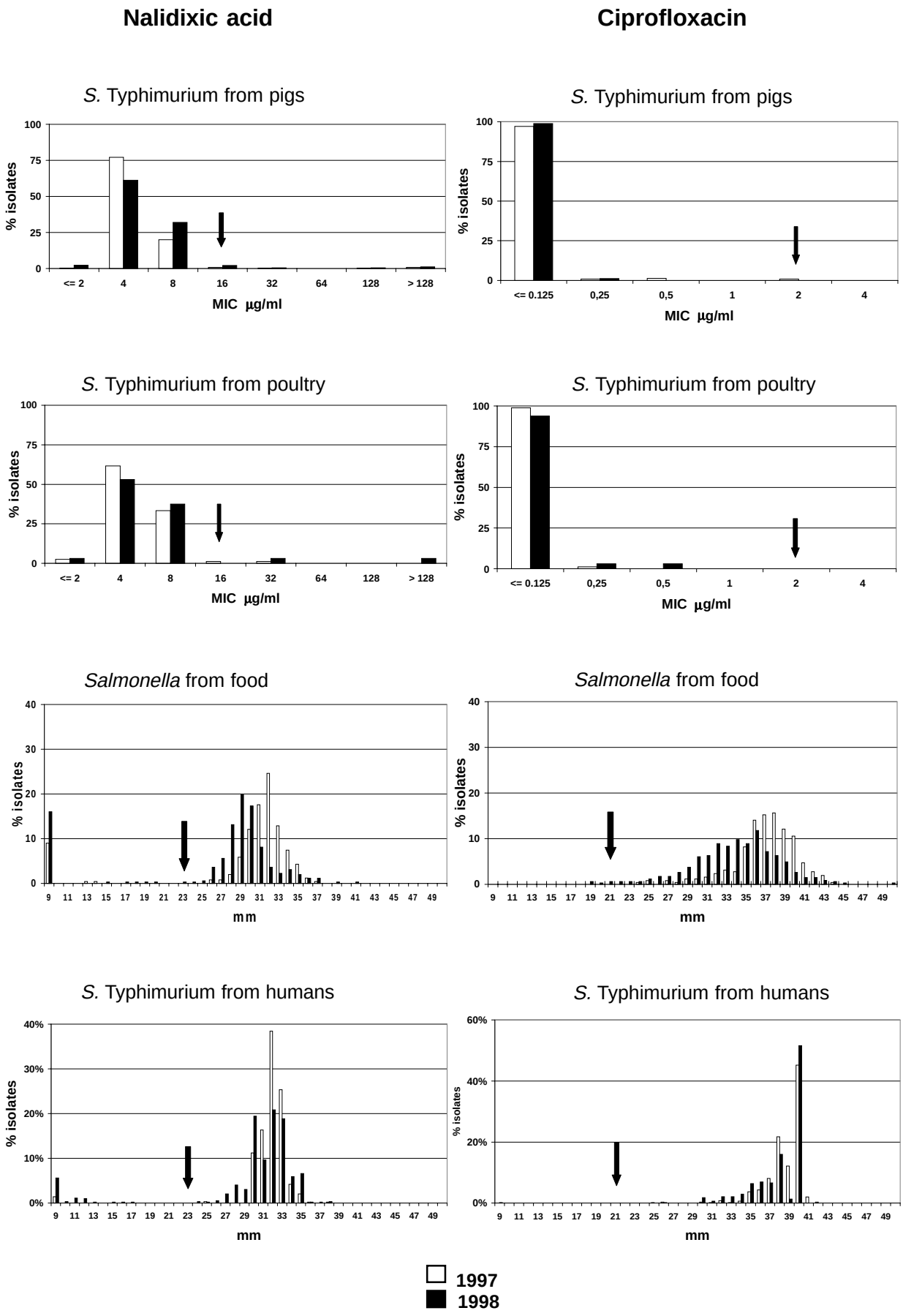


Figure 9 Distribution of nalidixic acid and ciprofloxacin, MIC values and inhibition zones for Salmonella *isolates, Denmark, 1997-1998. Arrows indicates the breakpoints used*

Table 16 Comparison of resistance (%) and percent change as compared to 1997 among Salmonella *Typhimurium from food animals, pork and humans, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Poultry		Cattle		Pigs		Pork		Humans	
		1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98
J01A	Tetracyclines	6	+ 6	23	+ 18	16	0	24		22	+ 4
J01B	Chloramphenicol	0	0	19	+ 19	6	+ 2	12	+ 12	18	+ 10
J01C	Ampicillin	9	0	19	+19	7	0	10	+ 8	24	+ 13
J01E	Sulfonamid	19	- 9	38	+ 33	29	+ 1			30	+ 11
	Trimethoprim	9	+ 8	0	0	11	+ 6	3	+ 3	5	+ 2
J01G	Apramycin	0	0	0	0	0	0			2	+ 1
	Gentamicin	0	0	0	0	0	0	2	+ 2	1	0
	Kanamycin	0	0	4	+ 4	6	+ 6	0	+ 0	1	0
	Streptomycin	22	+ 17	27	+ 22	17	- 9	10	+ 2	32	+ 13
J01M	Ciprofloxacin	0	0	0	0	0	0	0	0	1	+ 1
	Nalidixic acid	6	+ 5	8	- 2	2	0	0	- 2	9	+ 8
J01X	Colistin	0	- 3	0	0	0	- 1	0	0	0	0
Number of isolates		32		26		178		59		624	

Table 17 Comparison of resistance (%) among Salmonella *Enteritidis from poultry and humans and percent change as compared to 1998, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Poultry		Humans	
		1998	Change 97-98	1998	Change 97-98
J01A	Tetracyclines	2	+ 1	1	0
J01B	Chloramphenicol	0	0	0	0
J01C	Ampicillin	4	+ 4	2	0
J01E	Sulfonamid	3	+ 3	1	0
	Trimethoprim	3	+ 3	1	0
J01G	Apramycin	0	0	0	0
	Gentamicin	0	0	1	0
	Kanamycin	0	0	0	0
	Streptomycin	2	+ 1	1	0
J01M	Ciprofloxacin	0	0	0	0
	Nalidixic acid	7	+ 7	2	1
J01X	Colistin	0	0	0	-1
Number of isolates		113		446	

Campylobacter

Campylobacteriosis is the second most common foodborne zoonosis in Denmark. The number of reported human cases has tripled since 1992. The majority (90-95 percent) of human infections are caused by *Campylobacter jejuni*. It is estimated that approximately 80 percent of all human cases are acquired domestically (*Annual Report on zoonoses in Denmark 1998).*

There are no internationally agreed standards for susceptibility testing of *Campylobacter* and the institutes that take part in DANMAP applied different methods. We have compared the methods and the results have been shown to be highly comparable (*Engberg et al., Clin. Microbiol. Infect., in press)*.

Campylobacter from food animals

C. jejuni is the predominant species in broilers and cattle while *C. coli* is the most prevalent species in pigs. *Campylobacter* was detected in approximately 28 percent of the samples from broilers, whereas in cattle and pigs *Campylobacter* was detected in 46 percent and 58 percent, respectively (Table 18).

Although studies in a number of countries have reported high proportions of *C. jejuni* isolates resistant to quinolones, less than 5 percent of the *C. jejuni* isolates from cattle and broilers in 1998 were resistant to enrofloxacin and nalidixic acid. Resistance to macrolides was also rare (Table 19).

Among *C. coli,* antimicrobial resistance is more common. In 1997 and 1998, a high proportion of the isolates were resistant to macrolides (erythromycin, tylosin and spiramycin) (Table 19). The finding that one third of *C. coli* from broilers were resistant to macrolides is interesting since the use of macrolides in Danish broiler flocks is extremely limited. Among *C. coli* from pigs, resistance to enrofloxacin increased from 4 to 17 percent from 1997 to 1998 and resistance to nalidixic acid from 9 to 25 percent.

Campylobacter from foods

C. jejuni is the predominating species isolated from foods and the vast majority of strains were isolated from poultry. Consequently, only isolates belonging to this species were tested for antimicrobial susceptibility. All isolates originated from Danish or imported poultry. A total of 139 isolates were examined and the results are given in Table 20. Generally, the level of resistance was slightly higher in isolates from broiler meat than in isolates from other poultry meat.

In isolates from broiler meat, the frequencies of resistance to nalidixic acid and ciprofloxacin were 16 percent, and in meat from other poultry 10 and 7 percent, respectively.

Resistance to the macrolide erythromycin was also recorded at frequencies of 3 percent and 5 percent in meat from broilers and meat from other poultry, respectively. Approximately 10 percent of the isolates were resistant to tetracycline. When 1998 data are compared with 1997 data, there are only few changes. However, there is a trend towards an increased frequency of resistance in 1998.

Campylobacter from humans

Resistance frequencies in *Campylobacter jejuni* are shown in Table 21. There is no increase in resistance since 1996. In contrast to previous reports the results for 1998 represent domestically acquired infections only, while isolates from cases suspected of having been acquired abroad have been excluded.

Table 22 compares resistance between *C. jejuni* from broilers, broiler meat and humans, and also shows the changes in resistance between 1997 and 1998. There are obvious differences in the occurrence of resistance between isolates from broilers, broiler meat and humans in 1998; however, the differences are statistically non-significant. For the human isolates, it must be remembered that imported cases have been excluded from the 1998 data. This difference in sampling procedure precludes detailed comparisons between 1998 and previous years.

Figure 10 shows the distribution of MIC's, respectively inhibition zones of nalidixic acid and ciprofloxacin in *Campylobacter*. For *C. coli* from pigs there is a shift towards decreased susceptibility, while among *C. jejuni* from cattle and broilers the shift, if any, is towards increased susceptibility. Among *C. jejuni* from poultry meat there is an increase in the proportion of isolates completely resistant to quinolones. *C. jejuni* from humans show a shift towards increased susceptibility to quinolones.

Table 18 Prevalence of Campylobacter *in samples collected at slaughter of broilers, cattle and pigs, Denmark, 1998*

DANMAP 1998

	Number Samples	C. jejuni			C. coli		
		Number Positive	%	Number susceptibility tested	Number Positive	%	Number susceptibility tested
Broilers	930	262	28.2	71	33	3.6	15
Cattle	84	35	41.7	32	3	3.6	-
Pigs	194	12	6.2	-	100	51.6	87

Table 19 Susceptibility and occurrence of resistance in Campylobacter *from food animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	C. jejuni							
		Broilers N=930				Cattle N=84			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.125-32	0.25	0.25	1	0.25-0.5	0.25	0.25	0
J01B	Chloramphenicol	1-4	2	4	0	0.5-4	2	4	0
J01C	Ampicillin	1-64	8	16	4	0.5-32	4	8	3
J01E	Sulfonamide	8-512	64	256	1	4-256	32	256	0
	Trimethoprim	8-64	64	64	-	2-32	32	32	-
J01F	Erythromycin	0.25-32	1	2	1	0.25-2	0.5	2	0
	Spiramycin	0.25-32	1	4	1	0.5-2	1	2	0
	Tylosin	1-128	4	8	1	2-64	4	64	0
J01G	Apramycin	0.25-2	1	1	0	0.5-2	1	1	0
	Gentamicin	0.25-0.5	0.25	0.25	0	0.25-0.5	0.25	0.25	0
	Neomycin	0.5-1	0.5	1	0	0.5-1	0.5	0.5	0
	Spectinomycin	1-16	8	8	0	2-16	4	8	0
	Streptomycin	1-1	1	1	0	1-1	1	1	0
J01M	Enrofloxacin	0.031-4	0.25	0.25	3	0.031-4	0.25	0.25	3
	Nalidixic acid	4-256	4	8	4	1-128	4	8	3
J01X	Colistin	1-64	8	16	1	0.125-32	4	16	0
	Carbadox	0.063-4	0.063	0.125	0	0.063-32	0.063	8	0
	Olaquindox	0.125-16	1	1	0	0.25-16	1	4	0
Number of isolates					71				32

ATC-group	Compound	C. coli							
		Broilers N=930				Pigs N=194			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.25-1	0.25	0.25	0	0.25-64	0.25	1	1
J01B	Chloramphenicol	2-8	2	8	0	0.5-16	4	8	0
J01C	Ampicillin	2-64	4	16	7	0.5-64	8	32	11
J01E	Sulfonamide	16-256	32	256	0	0.25-1024	64	256	6
	Trimethoprim	32-64	64	64	-	32-256	64	64	-
J01F	Erythromycin	0.5-64	2	64	33	0.25-64	64	64	68
	Spiramycin	0.5-64	4	64	33	0.5-64	64	64	67
	Tylosin	4-256	8	256	33	2-256	256	256	66
J01G	Apramycin	1-2	1	2	0	0.25-2	1	2	0
	Gentamicin	0.25-0.5	0.25	0.5	0	0.25-4	0.25	0.5	0
	Neomycin	0.5-2	0.5	1	0	0.5-2	1	1	0
	Spectinomycin	1-16	8	8	0	1-64	8	16	6
	Streptomycin	1-256	1	64	13	1-256	4	128	33
J01M	Ciprofloxacin	0.031-4	0.063	4	13	0.031-16	0.25	4	17
	Nalidixic acid	4-128	8	128	13	0.5-256	8	128	25
J01X	Colistin	0.5-32	4	16	0	0.125-32	2	16	0
	Carbadox	0.063-1	0.25	1	0	0.063-4	0.5	1	0
	Olaquindox	0.5-2	1	2	0	0.25-8	1	4	0
Number of isolates					15				87

Table 20 Occurrence of resistance (%) among Campylobacter jejuni *from retail poultry meat, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Broiler meat	Other Poultry meat [a])
J01A	Tetracycline	11	8
J01B	Chloramphenicol	4	1
J01F	Erythromycin	5	3
J01G	Gentamicin	0	0
	Kanamycin	6	2
	Streptomycin	5	7
J01M	Ciprofloxacin	16	7
	Nalidixic acid	16	10
Number of isolates		93	46

a) Mainly turkey

Table 21 Occurrence of resistance (%) among Campylobacter *from humans, Denmark, 1996-1998*

DANMAP 1998

ATC-group	Compound	Campylobacter jejuni / coli		
		1996	1997	1998
J01A	Tetracycline	8	9	7
J01B	Chloramphenicol	1	2	0
J01F	Erythromycin	2	2	0
J01G	Apramycin	0	0	0
	Gentamicin	2	0	0
	Kanamycin		2	2
	Spectinomycin	2	2	0
	Streptomycin	3	7	1
J01M	Ciprofloxacin	13	12	11
	Nalidixic acid	14	14	11
J01X	Colistin	1	0	1
Number of isolates		93	111	117

Table 22 Comparison of resistance (%) and percent change as compared to 1997 among Campylobacter jejuni *from broilers, broiler meat and humans, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Broilers		Broiler meat		Humans	
		1998	Change 97-98	1998	Change 97-98	1998	Change 97-98
J01A	Tetracycline	1	- 2	11	+ 3	7	- 2
J01B	Chloramphenicol	0	0	4	+ 4	0	- 2
J01F	Erythromycin	1	0	5	+ 2	0	- 2
J01G	Apramycin	0	0			0	0
	Gentamicin	0	0	0	0	0	0
	Streptomycin	0	- 7	5	+ 2	1	- 6
J01M	Ciprofloxacin	3	+ 2	16	+ 11	11	- 1
	Nalidixic acid	4	+ 1	16	+ 11	11	- 3
J01X	Colistin	1	+ 1			1	+ 1
Number of isolates		71		93		117	

The data for broiler meat includes samples of imported products

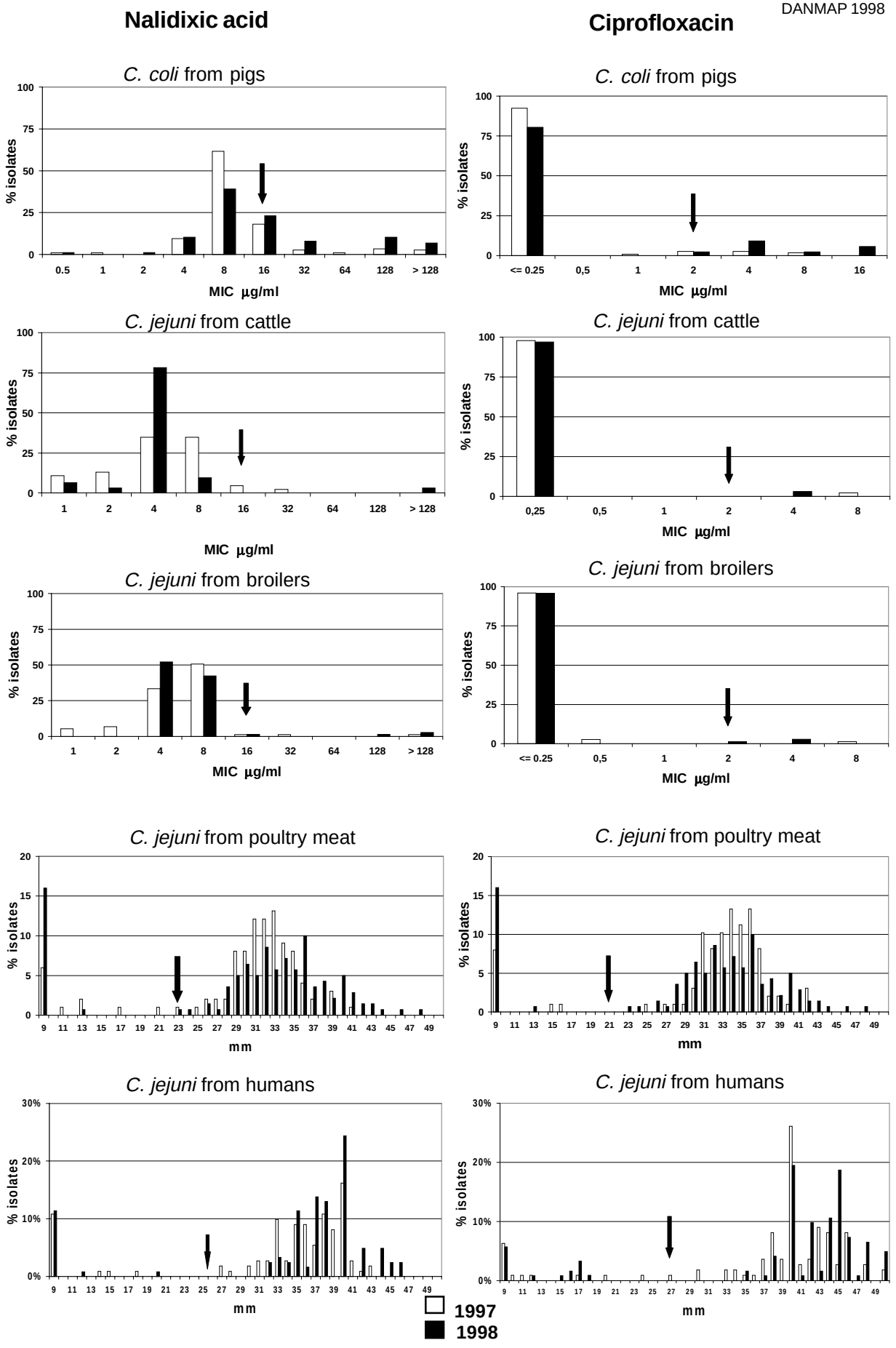


Figure 10 Distribution of nalidixic acid and ciprofloxacin, MIC values and inhibition zones for Campylobacter *isolates, Denmark, 1997-1998. Arrows show breakpoint.*

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 and because they respond to selective pressure by antimicrobial*s.* Accordingly, they provide a measure of the occurrence of resistance in the population as a whole.

In 1998 community isolates of enterococci or *E. coli* from humans were not collected.

Enterococci from food animals

The indicator bacteria from food animals are isolated from faecal samples from cattle and pigs and from cloacal swabs from broiler*s.* The samples are collected at slaughter and represent the normal populations of the respective animal species. As far as possible each sample represents a herd or flock and the number of samples from each slaughter plant is determined in proportion to the throughput of the plant.

For pigs, a total of 914 samples were collected. Eight hundred and three farms were sampled once during 1998, 42 were sampled twice, 6 three times and 2 farms 4 and 5 times, respectively. For 3 samples, the farm of origin could not be determined. For broilers, 1,096 samples were received from 282 out of 342 farms with a commercial production. Most of the farms sampled were represented by 1 to 6 flocks, however a small number had more than 6 flocks sampled. For cattle, the herd distribution of samples has not been determined.

In 1998, *E. faecalis* was isolated from samples from broilers in addition to *E. faecium*. Furthermore the isolation procedure used for samples from cattle and pigs was optimised to improve the recovery of *E. faecium*. Table 23 shows the rates of recovery and the proportion of isolates tested for antimicrobial susceptibility.

Table 24 and 25 presents the occurrence of resistance among enterococci from food animals.

The occurrence of resistance to antimicrobial growth promoters among *E. faecium* from broilers and pigs has been monitored since the last quarter of 1995 when the DANMAP programme was initiated. Glycopeptide resistance (measured as resistance to avoparcin) among isolates from broilers has declined from 82 percent in 1995, the year avoparcin was banned in Denmark, to 9 percent in 1998 (Figure 11). This decline is statistically significant. In contrast, the occurrence of resistance among *E. faecium* from pigs has remained essentially unchanged during the same time period. Possible explanations for this disparity include differences in production systems and differences in co-selection by other growth promoters or by therapeutic agents. A more detailed discussion may be found in *Bager et al., 1998* (see Appendix 2).

As a result of the food animal producers' voluntary stop of growth promoter use, avilamycin has not been used in broiler flocks hatched after February 15 1998. Figure 12 shows that the occurrence of avilamycin resistance among *E. faecium* from broilers declined from 79 percent in 1996 to 29 percent in 1998. This reflects a decline by 75 percent in the consumption of avilamycin since 1996. It seems likely that there is an association between the reduced consumption and the decrease in resistance. Unlike the situation for isolates from broilers, there is almost no resistance to avilamycin among *E. faecium* from slaughter pigs where avilamycin has been used in minimal amounts.

The most widely used growth promoter in recent years has been the macrolide tylosin. It was used in pigs, mainly in those above 35 kg live weight. The enterococci are tested for susceptibility to erythromycin as representatives of the macrolide group. Erythromycin is not used in food animals, however we have previously found a high degree of cross-resistance between erythromycin and tylosin

Table 23 Prevalence of enterococci in samples collected from broilers, cattle and pigs at slaughter, Denmark, 1998

DANMAP 1998

	Number	E. faecium			E. faecalis		
	Samples	Number positive	%	Number susceptibility tested	Number Positive	%	Number susceptibility tested
Broilers	1096	155	14.1	151	178	16.2	167
Cattle	251	25	10.0	22	10	4.0	-
Pigs	914	177	19.4	153	225	24.6	136

(*DANMAP 97*). As in 1997, the majority of *E. faecium* isolates from both broilers and pigs in 1998 were resistant to erythromycin. The macrolides are also used therapeutically and therefore a selective pressure is still present. Figure 13 shows the trend in resistance to erythromycin among *E. faecium* from pigs and broilers.

According to industry sources, macrolides are used minimally in broilers in Denmark and the frequent occurrence of macrolide resistance among *E. faecium* from broilers is most likely the result of selection by the B component of the growth promoter virginiamycin. The consumption of virginiamycin increased by 400 percent from 1995 to 1997.

Table 24 Susceptibility and occurrence of resistance among Enterococcus faecium *from food animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	E. faecium											
		Broilers N= 1096				Cattle N=251				Pigs N=914			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.25-32	0.5	32	31	0.25-16	0.5	1	5	0.25-64	32	32	57
J01B	Chloramphenicol	2-32	8	8	1	4-8	8	8	0	1-64	8	8	6
J01C	Penicillin	1-32	4	16	16	1-8	2	4	0	1-32	4	16	16
J01F	Erythromycin	0.25-32	32	32	72	0.25-16	2	8	18	0.25-64	32	32	71
J01G	Gentamicin	16-16	16	16	0	16-32	16	16	0	16-32	16	16	0
	Kanamycin	64-2048	256	1024	3	64-1024	512	1024	0	64-2048	512	2048	25
	Streptomycin	32-2048	128	256	5	32-256	128	256	0	32-2048	128	2048	24
J01X	Avoparcin	0.25-256	4	4	9	1-8	4	4	0	0.125-256	4	64	16
	Teicoplanin	0.5-32	0.5	2	8	0.5-1	1	1	0	0.5-32	0.5	32	16
	Vancomycin	0.5-32	1	2	9	0.5-4	1	2	0	0.5-32	1	32	15
	Synercid	0.25-32	8	16	74	0.5-4	2	4	14	0.25-32	2	8	46
	Virginiamycin	1-128	16	64	60	2-32	4	32	45	1-128	8	16	56
	Avilamycin	0.5-256	2	32	28	0.5-2	2	2	0	0.125-2	1	2	0
	Bacitracin	2-128	128	128	77	16-128	64	128	45	2-256	64	128	43
	Flavomycin	0.125-256	8	256	46	4-256	128	256	95	0.25-256	128	256	95
	Nitrofurantoin	32-256	32	128	11	32-256	64	128	23	32-128	64	64	6
	Monensin	0.25-4	2	2	0	2-4	4	4	0	0.125-4	2	2	0
	Salinomycin	0.25-4	1	2	0	0.5-0.5	0.5	0.5	0	0.125-2	0.5	0.5	0
Number of isolates					151				22				156

Table 25 Susceptibility and occurrence of resistance among Enterococcus faecalis *from food animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	E. faecalis							
		Pigs N=914				Broilers N=1096			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.25-32	32	32	81	0.25-32	32	32	61
J01B	Chloramphenicol	4-64	8	16	4	2-64	8	16	3
J01C	Penicillin	1-8	2	4	0	1-128	2	2	1
J01F	Erythromycin	0.25-32	32	32	79	0.25-32	4	32	43
J01G	Gentamicin	16-2048	16	16	1	16-2048	16	16	1
	Kanamycin	64-2048	64	2048	27	64-2048	64	64	1
	Streptomycin	64-2048	256	2048	37	32-2048	256	1024	10
J01X	Avoparcin	0.125-8	4	4	0	0.25-8	4	4	0
	Teicoplanin	0.5-4	0.5	0.5	0	0.5-2	0.5	0.5	0
	Vancomycin	0.5-4	1	2	0	0.5-4	1	2	0
	Avilamycin	0.125-128	2	2	1	0.125-4	1	2	0
	Bacitracin	2-128	32	128	10	2-128	128	128	57
	Flavomycin	0.125-128	0.25	0.5	3	0.125-16	0.25	0.5	1
	Nitrofurantoin	32-256	32	32	1	32-256	32	32	4
	Monensin	0.125-2	2	2	0	0.250-4	2	2	0
	Salinomycin	0.125-0.5	0.5	0.5	0	0.125-2	0.25	0.5	0
Number of isolates					136				168

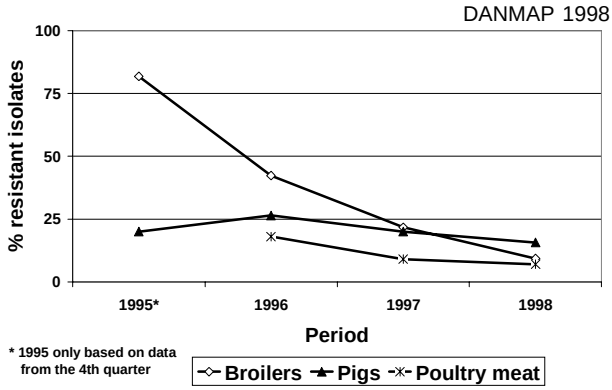


Figure 11 Trend in occurrence of resistance to avoparcin among Enterococcus faecium *from broiler, pigs and poultry meat, Denmark,1995-1998*

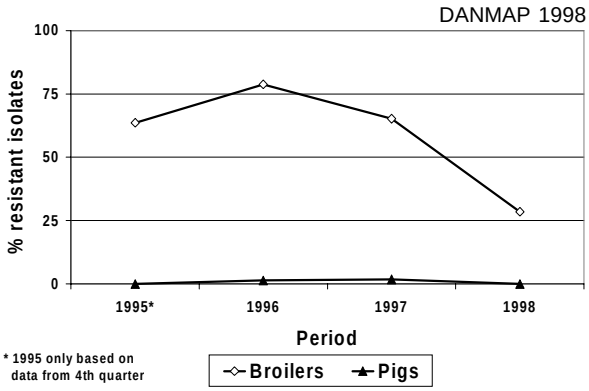


Figure 12 Trend in occurrence of resistance to avilamycin among Enterococcus faecium *from broiler and pigs, Denmark, 1995-1998*

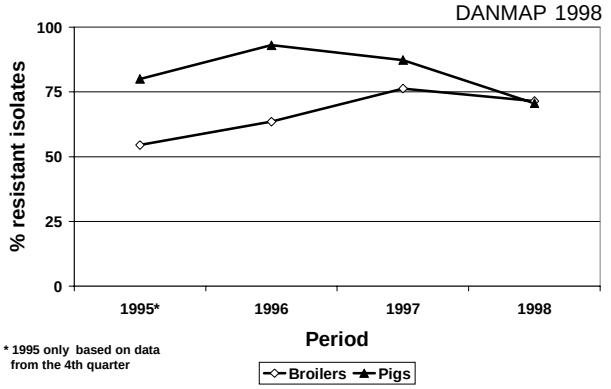


Figure 13 Trend in occurrence of resistance to erythromycin among Enterococcus faecium *from broiler and pigs, Denmark, 1995-1998*

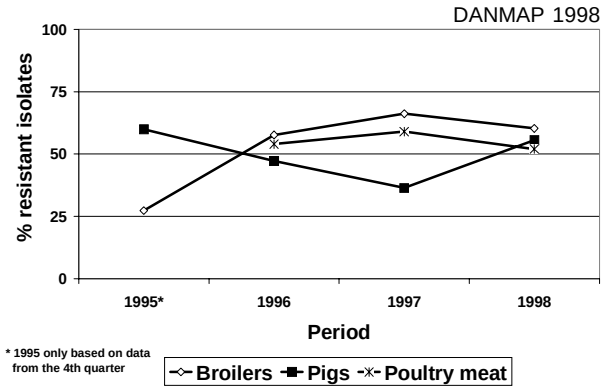


Figure 14 Trend in occurrence of resistance to virginiamycin among Enterococcus faecium *from broiler, pigs and poultry meat, Denmark, 1995-1998*

Between 1996 and 1998, about 2/3 of *E. faecium* isolates from broilers were resistant to virginiamycin, compared with between 36 and 56 percent of isolates from slaughter pigs (Figure 14). The consumption of virginiamycin in 1998 was reduced by 90 percent compared with 1997. In isolates from broilers, we expect a decrease in frequency of resistance to virginiamycin, although we cannot predict the rate of decline. For pigs, because of MLS-resistance and because of the therapeutic use of macrolides, it can not be predicted how the occurrence of virginiamycin resistance will evolve.

Enterococci from food

The bacteria present in a food sample are the cumulated result of all the regimes to which the foodstuff has been subjected and tracing the history of a bacterial strain isolated from a retail sample is therefore difficult. One of the problems about determining the origin of a bacterial isolate is the possibility of cross contamination and possibly growth during production or distribution. In the present survey the majority of the samples examined were from raw untreated foods like minced meat (beef or pork), and vegetables. However, despite reservations about the origin of the bacterial isolates, the results of the survey, reflect the resistance status of bacteria that can be isolated from foods entering the households.

A total of 455 food samples were investigated for presence of enterococci by the method described in Appendix 1. These investigations resulted in the isolation of a total of 262 strains of *E. faecium* and 106 strains of *E. faecalis.* It was possible to isolate *E. faecium* and *E. faecalis* from all food categories, and the overall prevalence was 81 percent. The detailed results of the investigations are given in Tables 26 and 27. The majority of the samples in the category other poultry meat is turkey meat, and the category vegetables consisted mainly of different types of sprout products.

Resistance to tetracycline was primarily recorded in isolates of enterococci from broiler meat with 28 percent and 34 percent of *E. faecium* and 59 percent and 75 percent of *E. faecalis* being resistant to tetracycline (Table 27).

Table 26 Prevalence of enterococci in food samples collected from retail outlets, Denmark, 1998

DANMAP 1998

	Number samples	E. faecium		E. faecalis	
		Number positive	%	Number positive	%
Pork	72	28	39	40	56
Beef	56	31	55	17	30
Broiler meat	94	60	64	17	18
Other poultry	128	76	59	20	16
Vegetables	105	67	64	12	11
Total	455	262	58	106	23

A relatively high frequency of resistance to the macrolide erythromycin was recorded for both *E. faecium* and *E. faecalis.* Thus between 43 and 58 percent of isolates of *E. faecium* and between 8 and 70 percent of isolates of *E. faecalis* from pork, beef, broiler meat, other poultry meat and vegetables (sprouts) were resistant to erythromycin (Table 27). It is interesting to see the high frequency of resistance in isolates from vegetables (sprouts). It is likely that the majority of these strains originate from the processing environment of the sprouting facilities. In other words, the enterococcal clones prevalent in sprouts are different from the clones in e.g. pigs and pork.

All *enterococcus* isolates from pork, beef, other poultry and vegetables were susceptible to the glycopeptide vancomycin and resistance was only recorded for 7 percent of *E. faecium* from broiler meat.

Generally, a low frequency of resistance was recorded to the ionophores monensin and salinomycin in isolates from all food categories.

Resistance to bacitracin was mainly observed in enterococci from poultry. Sixty-two percent of *E. faecium* and 65 percent of *E. faecalis* from broiler meat, and 29 percent of *E. faecium* and 35 percent of *E. faecalis* from other poultry meat were resistant to bacitracin. Bacteria from the other food categories were generally susceptible to bacitracin.

Resistance to avilamycin was observed in all food categories. Twelve percent of both *E. faecium* and *E. faecalis* isolates from broiler meat were resistant to avilamycin. Avilamycin resistance among isolates from pork and beef may be a result of cross-contamination as avilamycin has had little or no use in pigs and cattle in Denmark. Flavomycin resistance was frequent among *E. faecium*. The lowest frequency (46 percent) was recorded in pork, whereas bacteria from all other food categories had resistance frequencies over 50 percent.

Among *E. faecium* from most of the food categories there was a moderate frequence of resistance, around 10 percent, to virginiamycin. However, over 50 percent of the isolates from broiler meat and from other poultry were resistant. *Enterococcus faecalis* exhibits natural resistance to streptogramins.

Tables 28 and 29 compare resistance among *E. faecalis* and *E. faecium*, respectively, from food animals and the foods derived from them.

For *E. faecalis,* there were big differences in occurrence of resistance to tetracycline, erythromycin and streptomycin among isolates from pigs and pork (Table 29). Similar differences were observed for *E. faecium*. The most likely explanation is that the enterococci found in pork in retail outlets reflects cross-contamination from other sources as much as it reflects the reservoir represented by the gastro-intestinal tract of the live pig.

Table 27 Occurrence of resistance (%) among enterocci from food collected from retail outlets, Denmark, 1998

DANMAP 1998

ATC group	Compound	Pork N=72		Beef N=56		Broiler meat N=94		Other poultry N=128		Vegetables N=105	
		E. faecium	E. faecalis	E. faecium	E. faecalis	E. faecium	E. faecalis	E. faecium	E. faecalis	E. faecium	E. faecalis
J01A	Tetracycline	4	10	3	18	28	59	34	75	15	0
J01B	Chloramphenicol	7	20	6	41	7	35	9	55	13	42
J01C	Penicillin	0	0	0	0	0	0	0	0	0	0
J01F	Erythromycin	43	8	39	18	58	47	61	70	69	33
J01G	Gentamicin	0	0	0	0	0	0	0	5	0	0
	Streptomycin	4	0	0	6	0	12	8	50	0	0
J01X	Vancomycin	0	0	0	0	7	0	0	0	0	0
	Virigiamycin	7	-	16	-	52	-	58	-	12	-
	Avilamycin	0	5	13	6	12	12	4	25	7	8
	Bacitracin	7	0	6	0	62	65	29	35	3	0
	Flavomycin	46	0	61	6	55	0	74	0	69	8
	Monensin	7	5	3	6	12	18	9	15	10	8
	Salinomycin	7	5	10	0	12	12	4	5	7	17
Number of isolates		28	40	31	17	60	17	76	20	67	12

Table 28 Occurrence of resistance (%) among Enterococcus faecium *from animals and food, Denmark, 1998, and percent change as compared to 1997*

DANMAP 1998

ATC group	Compound	Pigs		Pork		Cattle		Beef		Broilers		Broiler meat	
		1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98
J01A	Tetracycline	57	- 12	4	- 1	5	- 14	3	0	31	+ 11	28	+ 13
J01B	Chloramphenicol	6	+ 2	7	+ 2	0	0	6	- 2	1	+ 1	7	+ 1
J01C	Penicillin	16	- 30	0	0	0	- 13	0	0	16	- 3	0	- 2
J01F	Erythromycin	71	- 16	43	+ 17	18	- 1	39	+ 3	72	- 4	58	- 13
J01G	Gentamicin	0	0	0	0	0	0	0	- 3	0	0	0	0
	Streptomycin	24	0	4	+ 4	0	- 13	0	- 3	5	0	0	- 3
J01X	Vancomycin	15	- 5	0	0	0	0	0	0	9	- 12	7	- 2
	Virginiamycin	56	+ 20	7	- 3	45	+ 32	16	+ 13	60	- 6	52	- 7
	Avilamycin	0	- 2	0	0	0	- 6	13		28	- 37	12	- 38
	Bacitracin	43	+ 12	7	+4	45	- 18	6	+ 1	77	- 8	62	- 9
	Flavomycin	95	+ 11	46	- 48	95	+ 7	61		46	- 19	55	- 6
	Monensin	0	0	7	+ 7	0	0	3		0	0	12	+ 12
	Salinomycin	0	0	7	+ 7	0	0	10		0	0	12	+ 12
Number of isolates		153		28		22		31		151		60	

Table 29 Occurrence of resistance (%) among Enterococcus faecalis *from animals and food, Denmark, 1998, and percent change as compared to 1997*

DANMAP 1998

ATC group	Compound	Pigs		Pork		Broilers	Broiler meat	
		1998	Change 97-98	1998	Change 97-98	1998[a)]	1998	Change 97-98
J01A	Tetracycline	81	- 4	10	- 9	61	59	+ 6
J01B	Chloramphenicol	4	- 6	20	+ 16	3	35	+ 24
J01C	Penicillin	0	- 1	0	0	1	0	0
J01F	Erythromycin	79	- 12	8	+ 1	43	47	+ 20
J01G	Gentamicin	1	- 1	0	0	1	0	- 2
	Streptomycin	37	- 4	0	- 5	10	12	+ 1
J01X	Vancomycin	0	0	0	0	0	0	0
	Avilamycin	1	- 3	5	+ 5	0	12	+ 12
	Bacitracin	10	+ 4	0	0	57	65	+ 18
	Flavomycin	3	+ 3	0	- 10	1	0	0
	Monensin	0	0	5	+ 5	0	18	+ 18
	Salinomycin	0	0	5	+ 5	0	12	+ 12
Number of isolates		136		40		168	17	

a) Not susceptibility tested in 1997

Escherichia coli from food animals

In addition to enterococci, the samples from slaughter animals were also examined for *E. coli*. The recovery of *E. coli* is shown in Table 30 and the results of the susceptibility testing are given in Table 31.

Among indicator *E. coli* from broilers, cattle and pigs resistance to enrofloxacin was not observed in 1997 and 1998. The proportion of *E. coli* from broilers resistant to nalidixic acid increased from 8 percent in 1997 to 26 percent in 1998. In 1998, 4 percent and 1 percent of the *E. coli* isolates from cattle and pigs, respectively, were resistant to nalidixic acid while in 1997 nalidixic acid resistance was not observed. In addition to quinolone resistance, *E. coli* from broilers also showed a statistically significant increase in resistance to sulfonamides, trimethoprim and streptomycin from 1997 to 1998. This is in contrast with *E. coli* from cattle and pigs, where the percentage of resistant isolates has shown little change from 1997 to 1998. In 1997 and especially in 1998 the broiler industry experienced some disease problems with a particularly resistant clone of *E. coli*. Often, enrofloxacin was used to treat these outbreaks of *E. coli* septicaemia. As we do not serotype our *E. coli* isolates from the normal population we do not know to which extent this particularly resistant strain may have affected the results for 1998.

Table 30 Prevalence of Escherichia coli *in samples from broilers, cattle and pigs at slaughter, Denmark, 1998*

DANMAP 1998

	Number samples	Number positive	%	Number susceptibility tested
Broilers	724	512	70.7	202
Cattle	114	103	90.4	101
Pigs	339	298	87.9	298

Escherichia coli from food

A total of 505 food samples were examined for *E. coli* with the method described in Appendix 1.These analyses resulted in isolation of 444 (88 percent) strains. The prevalence in the 5 different food categories is presented in Table 32. It should be noted that the high prevalence of *E. coli* in vegetables reflects that this category primarily consists of different types of sprout products, e.g. bean-, alfalfa-, azuki-, and radish sprouts.

The majority of the strains from beef and pork were from food of Danish origin. All vegetables (sprouts) tested were also of Danish origin, however most of the sprouts were produced from imported seed*s.* In the category of broiler meat, 62 percent of the samples were of Danish origin, 22 percent were imported and for 16 percent no origin was recorded. In the category “other poultry meat”, 23 percent of the samples were from imported products, and information about origin was missing for 21 percent of the samples.

The results of susceptibility testing are presented in Table 33. Generally, the highest level of resistance was found among isolates from poultry. In the category “other poultry” (mainly turkey), 52 percent of the strains were resistant to tetracycline, 24 percent to ampicillin and 21 percent to trimethoprim. Nineteen percent of the strains were resistant to nalidixic acid and 3 percent also to ciprofloxacin. For isolates from broiler meat resistance to tetracycline was the most frequent (19 percent) followed by resistance to nalidixic acid (18

Table 31 Susceptibility and occurrence of resistance among Escherichia coli *isolated from animals at slaughter, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	E. coli (from healthy animals)											
		Broilers N=724				Cattle N=114				Pigs N=339			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracyclines	0.5-32	2	32	18	0.5-32	2	16	12	0.5-32	2	32	37
J01B	Chloramphenicol	1-16	4	8	0	2-16	8	8	0	1-64	4	8	6
J01C	Ampicillin	0.5-32	4	32	16	2-32	4	4	2	1-32	4	32	10
J01E	Sulfonamid	8--512	32	512	38	8-512	16	512	23	8-512	16	512	41
	Trimethoprim	0.5-32	0.5	32	13	0.5-32	0.5	0.5	3	0.5-32	0.5	32	10
J01G	Apramycin	2-16	4	8	2	1-16	4	8	3	1-16	4	8	1
	Gentamicin	0.5-2	0.5	2	0	0.5-2	0.5	1	0	0.5-4	0.5	1	0
	Kanamycin	1-64	4	8	0	1-64	4	8	2	1-64	4	8	7
	Streptomycin	4-128	8	64	19	2-128	8	16	6	2-128	32	128	61
J01M	Ciprofloxaxin	0.125-8	0.125	0.25	0	0.125-0.25	0.125	0.125	0	0.125-0.25	0.125	0.125	0
	Nalidixic acid	2-128	4	128	26	2-128	4	8	4	2-128	4	4	1
J01X	Colistin	1-64	1	1	2	1-1	1	1	0	1-8	1	1	0
	Carbadox	8-64	8	8	0	8-32	8	8	0	8-32	8	8	0
	Olaquindox	4-32	16	16	0	8-32	16	16	0	2-64	16	16	0
	Nitrofurantoin	32-128	32	32	1	32-32	32	32	0	32-128	32	32	0
Number of isolates					202				101				298

percent) and ampicillin (11 percent). Widespread resistance against tetracycline (29 percent) and ampicillin (11 percent) was also recorded in strains from pork. *Escherichia coli* from vegetables (sprouts) were generally susceptible to all antimicrobials tested with the exception of tetracycline (26 percent resistance).

In general, the levels of resistance in 1998 were similar to those seen in 1997. There was, however, an increase in resistance to nalidixic acid among isolates from broiler meat, for which the figures for 1997 and 1998 were 6 percent and 18 percent, respectively.

Table 32 Prevalence of Escherichia coli *in food, Denmark, 1998*

DANMAP 1998

	Number samples	Number positive	%
Other poultry	132	128	97
Broiler	119	110	92
Beef	87	72	83
Pork	84	77	92
Vegetables	83	57	69
Total	505	444	88

Table 33 Occurrence of resistance (%) among Escherichia coli *from food, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Pork N=84	Beef N=87	Broiler meat N=119	Other poultry N=132	Vegetables N=83
J01A	Tetracycline	29	11	19	52	26
J01B	Chloramphenicol	5	3	5	7	2
J01C	Ampicillin	16	11	11	24	2
J01E	Trimethoprim	3	7	10	21	0
J01G	Gentamicin	0	1	1	1	0
	Kanamycin	4	2	3	5	0
	Streptomycin	23	8	11	23	4
J01M	Ciprofloxacin	1	0	0	3	0
	Nalidixic acid	3	1	18	19	0
J01X	Colistin	0	0	0	0	0
	Carbadox	0	0	3	2	0
	Olaquindox	0	0	0	0	0
Number of isolates		77	72	110	128	57

Table 34 Occurrence of resistance (%) among Escherichia coli *from Danish and imported poultry meat other than from broilers, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Denmark	Imported a)
J01A	Tetracycline	39	80
J01B	Chloramphenicol	4	9
J01C	Ampicillin	13	43
J01E	Trimethoprim	28	57
J01G	Gentamicin	0	0
	Kanamycin	3	11
	Streptomycin	13	46
J01M	Ciprofloxacin	1	0
	Nalidixic acid	19	11
J01X	Colistin	0	0
	Carbadox	1	0
Number of isolates		69	35

a) 32 isolates from French poultry, 2 isolates from Italian poultry, and one from German poultry.

The low frequency of resistance in *E. coli* from vegetables (sprouts) is interesting, because these strains are isolated from an environment different from the environment of fresh meat. Sprouts are usually produced on a fairly large scale, where seeds are allowed to germinate under humid and warm conditions. The *E. coli* isolates from sprouts may originate from faecally contaminated seeds, but the sprouts may also be contaminated during production and handling.

Table 34 shows the frequencies of resistance in *E. coli* from Danish and imported poultry meat other than broiler meat. The resistance frequency in *E. coli* from imported poultry is generally higher than in the isolates from Danish poultry. Resistance to nalidixic acid, with 19 percent in strains isolated from Danish poultry meat and 11 percent in imported poultry represents an exception to this general observation.

Table 35 compares the occurrence of resistance for *E. coli* strains from healthy animals and food for 1997 and 1998. The figures are very similar for broilers and broiler meat, and cattle and beef. However, when comparing the levels of resistance for strains from pigs and pork, it is notable that the level of resistance to streptomycin is higher in pigs than in pork.

Generally, there were no major differences in the frequency of resistance in *E. coli*, when comparing the results of 1998 with the results of 1997.

Table 35 Occurrence of resistance (%) among Escherichia coli *from animals and food, Denmark, 1998, and percent change as compared to 1997*

DANMAP 1998

ATC-group	Compound	Broilers		Broiler meat		Cattle		Beef		Pigs		Pork	
		1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98	1998	Change 97-98
J01A	Tetracycline	18	+ 7	19	0	12	+ 2	11	- 4	37	0	29	+ 1
J01B	Chloramphenicol	0	0	5	+ 2	0	0	3	0	6	- 3	5	0
J01C	Ampicillin	16	+ 5	11	- 3	2	- 2	11	+ 6	10	0	16	- 14
J01E	Trimethoprim	13	+ 10	10	-3	3	0	7	+ 4	10	+ 4	3	0
J01G	Gentamicin	0	0	1	+ 1	0	- 1	1	+ 1	0	0	0	- 2
	Kanamycin	0	- 1	3	0	2	+ 1	2	+ 2	7	0	4	- 4
	Streptomycin	19	+ 10	11	+ 3	6	- 9	8	+ 4	61	- 3	23	+ 8
J01M	Ciprofloxacin	0	0	0	- 1	0	0	0	0	0	0	1	+ 1
	Nalidixic acid	26	+ 18	18	+ 12	4	+ 4	1	+ 1	1	+ 1	3	+ 2
J01X	Colistin	2	+ 2	0	0	0	0	0	0	0	0	0	0
	Carbadox	0	0	3	+ 3	0	0	0	0	0	0	0	- 4
	Olaquindox	0	0	0	0	0	0	0	0	0	0	0	- 2
Number of isolates		202		110		101		72		298		77	

Resistance in bacteria from diagnostic submissions

Bacteria from food animals

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

Bacteria from diagnostic submissions are more often resistant than similar bacterial species from healthy animals. The increased resistance frequency may mean that *E. coli* O149 F4 in general are more often resistant than is the case with other *E. coli* subtypes. This could be the case because they are often the cause of disease and therefore frequently subjected to selective pressure by antimicrobials. Alternatively, it is possible that submission of samples to the laboratory does not happen before antimicrobial treatment has been attempted, and failed. This would imply that the resistant strains of, for example, *E. coli* O149 F4 represent a subpopulation among all *E. coli* O149 F4 in pigs.

Escherichia coli

Generally, *E. coli* from broilers (mainly serotypes O2 and O78) were less often resistant than *E. coli* from cattle (all isolates were F5) and pigs (serotype O149 F4) (Table 36). Among *E. coli* from broilers, none were resistant to ciprofloxacin in 1998 whereas only one of 28 isolates tested was resistant in 1998. In contrast, 38 percent and 51 percent of the isolates were resistant to nalidixic acid in 1997 and 1998, respectively. Additionally, resistance to tetracycline, ampicillin, sulfonamide and streptomycin was more frequent in 1998 as compared to 1997.

Among isolates from cattle, resistance to fluoroquinolones (ciprofloxacin) and to nalidixic acid was recorded in 8 percent and 17 percent of isolates, respectively. It is worthy of note that 16 percent of the isolates were resistant to gentamicin, even though gentamicin is not registered for use in cattle. This may be an example of co-selection of a resistance marker through the use of other antibacterial agents.

In 1998, 12 percent of the *E. coli* isolates from pigs were resistant to ciprofloxacin (up from 2 percent in

Table 36 Susceptibility and occurrence of resistance among Escherichia coli *isolated from diagnostic submissions from animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	E. coli (from diagnostic submissions)											
		Poultry				Cattle				Pigs			
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	0.5-32	32	32	55	1-32	32	32	78	0.5-32	32	32	64
J01B	Chloramphenicol	2-8	4	8	0	4-64	8	64	21	2-128	8	64	38
J01C	Ampicillin	2-32	8	32	46	1-32	32	32	84	1-32	4	32	42
J01E	Sulfonamide	8-512	512	512	75	8-512	512	512	91	8-512	512	512	81
	Trimethoprim	0.5-32	0.5	1	1	0.5-32	32	32	66	0.25-32	1	32	44
J01G	Apramycin	2-8	4	4	0	1-8	4	8	0	1-8	2	8	0
	Gentamicin	0.5-32	0.5	1	3	0.5-32	1	16	16	0.25-32	0.5	1	1
	Kanamycin	1-64	2	4	3	2-64	8	64	38	1-64	4	64	31
	Streptomycin	4-128	64	128	54	4-128	128	128	91	2-256	64	128	79
J01M	Ciprofloxaxin	0.125-0.5	0.125	0.25	0	0.125-8	0.125	1	8	0.125-8	0.125	4	12
	Nalidixic acid	2-128	32	128	51	2-128	4	128	17	2-128	8	128	42
J01X	Colistin	1-2	1	1	0	1-4	1	1	0	1-4	1	1	0
	Carbadox	8-8	8	8	0	8-16	8	8	0	4-32	8	32	0
	Olaquindox	8-32	16	16	0	8-32	16	32	0	8-128	32	64	2
	Nitrofurantoin	32-32	32	32	0	32-32	32	32	0	32-64	32	32	0
Number of isolates					67				101				108

Table 37 Susceptibility and occurrence of resistance among staphylococci *and* Actinobacillus pleuropneumoniae *from diagnostic submissions from animals, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Staphylococci												A. pleuropneumoniae			
		Cattle								Pigs				Pigs			
		CNS				S. aureus				S. hyicus							
		Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant	Range	MIC50	MIC90	% resistant
J01A	Tetracycline	1-32	1	1	4	1-1	1	1	0	0.5-32	1	32	29	12-34	27	32	7
J01B	Chloramphenicol	2-16	4	8	0	4-16	8	8	0	4-16	8	16	0	29-45	36	40	0
J01C	Ampicillin	-	-	-	-	-	-	-	-	0.25-32	2	32	-	26-42	32	38	0
	Oxacillin	0.5-1	0.5	0.5	0	0.5-1	0.5	0.5	0	0.5-4	0.5	0.5	1	-	-	-	-
	Penicillin	0.063-2	0.063	0.25	16	0.063-2	0.063	2	21	0.063-16	1	2	66	17-37	22	25	0
J01D	Ceftiofur	-	-	-	-	-	-	-	-	1-1	1	1	0	-	-	-	-
J01E	Sulfonamide	16-512	32	512	16	16-512	64	512	28	8-512	32	512	29	11-32	21	26	39
	Trimethoprim	1-32	2	16	13	1-8	2	4	0	1-32	8	32	39	9-39	27	33	6
J01F	Erythromycin	0.25-16	0.25	0.5	3	0.25-16	0.25	1	2	0.25-32	0.5	16	27	23-32	27	30	0
	Lincomycin	-	-	-	-	-	-	-	-	2-32	16	32	60	-	-	-	-
	Spiramycin	-	-	-	-	-	-	-	-	4-32	8	32	40	20-28	24	26	23
	Tylosin	-	-	-	-	-	-	-	-	1-32	1	32	40	19-32	22	25	0
J01G	Apramycin	-	-	-	-	-	-	-	-	2-4	2	4	0	18-29	24	27	1
	Gentamicin	1-4	1	1	0	1-2	1	1	0	0.25-4	1	1	0	24-34	29	32	0
	Kanamycin	4-128	4	4	2	4-8	4	4	0	4-64	4	4	1	-	-	-	-
	Neomycin	-	-	-	-	-	-	-	-	0.5-16	0.5	16	20	21-32	26	30	0
	Spectinomycin	-	-	-	-	-	-	-	-	32-32	32	32	100	23-37	31	34	0
	Streptomycin	2-64	2	8	6	2-64	8	16	2	2-128	32	64	54	23-36	30	33	0
J01M	Ciprofloxacin	0.25-0.5	0.25	0.25	0	0.25-1	0.25	0.5	0	0.25-8	0.25	0.5	5	-	-	-	-
	Enrofloxacin	-	-	-	-	-	-	-	-	0.125-0.25	0.125	0.25	0	23-36	30	34	0
	Nalidixic acid	-	-	-	-	-	-	-	-	-	-	-	-	9-38	29	33	1
J01X	Avoparcin	0.25-8	2	4	0	0.25-128	2	4	1	0.25-4	2	4	0	-	-	-	-
	Teicoplanin	-	-	-	-	-	-	-	-	0.5-0.5	0.5	0.5	0	-	-	-	-
	Vancomycin	0.5-4	1	1	0	0.5-2	0.5	1	0	0.5-2	1	1	0	-	-	-	-
	Colistin	-	-	-	-	-	-	-	-	-	-	-	-	19-26	21	24	0
	Pristinamycin	-	-	-	-	-	-	-	-	0.25-4	0.5	4	20	-	-	-	-
	Synercid	0.5-1	0.5	0.5	0	0.5-2	0.5	0.5	0	0.5-4	0.5	1	1	-	-	-	-
	Virginiamycin	0.25-8	1	4	2	0.125-16	2	4	9	0.125-64	2	4	3	-	-	-	-
	Avilamycin	0.125-4	2	2	0	0.125-4	2	2	0	0.25-2	2	2	0	-	-	-	-
	Carbadox	-	-	-	-	-	-	-	-	-	-	-	-	2-32	4	8	0
	Olaquindox	-	-	-	-	-	-	-	-	-	-	-	-	4-32	8	16	0
	Bacitracin	2-128	32	128	15	2-128	4	32	2	2-128	32	64	2	-	-	-	-
	Flavomycin	0.125-1	0.125	0.25	0	0.125-256	0.125	0.25	1	0.125-256	0.125	0.125	1	-	-	-	-
	Nitrofurantoin	32-128	32	32	1	32-256	32	32	1	32-32	32	32	0	-	-	-	-
	Monensin	0.25-8	2	4	0	0.125-4	1	2	0	0.25-4	2	2	0	-	-	-	-
	Salinomycin	0.125-2	1	2	0	0.125-2	1	2	0	0.125-2	1	2	0	-	-	-	-
	Fusidic acid	0.5-4	0.5	1	5	0.5-4	0.5	0.5	1	0.5-0.5	0.5	0.5	0	-	-	-	-
Number of isolates					100				200				93				99

1997) and 42 percent to nalidixic acid. The latter represents an increase of 19 percent from 1997. Apart from quinolone resistance, resistance to chloramphenicol, ampicillin, sulfonamide, trimethoprim and streptomycin was more frequent among pig isolates in 1998 as compared to 1997.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae is a common cause of respiratory disease in pigs. From 1997 to 1998, the percentage of isolates resistant to sulfonamides decreased from 69 percent to 39 percent. *Actinobacillus pleuropneumoniae* was susceptible to most antimicrobials in the test panel (Table 37).

Staphylococci

Coagulase negative staphylococci (CNS) and *Staphylococcus aureus* were isolated from bovine mastitis. In general the isolates were susceptible to most antimicrobials in the test panel (Table 37). From 1997 to 1998, the percentage of *S. aureus* and CNS isolates resistant to sulfonamides decreased significantly. Among CNS, there has also been a significant decrease in the frequency of isolates resistant to trimethoprim.

In general, most of the isolates were susceptible to antimicrobials used as growth promoters. This is in accordance with the fact that growth promoters have not been used in dairy cattle.

Among *Staphylococcus hyicus* from pigs, the occurrence of resistance was widespread (Table 37). In 1998, 27 percent of the isolates were resistant to erythromycin. This represents a significant decrease as compared to 1997 where 62 percent of the isolates were resistant, and may be associated with the decline in consumption of tylosin for growth promotion. It has previously been shown that exposure to tylosin has an immediate effect on the frequency of macrolide resistance among *S. hyicus* (*Aarestrup and Carstensen, 1998).*

Bacteria from food

Staphylococcus aureus

Fifty-one isolates of *S. aureus* from various food categories were tested for antimicrobial susceptibility and the results are presented in Table 38. Approximately half of the isolates were resistant to penicillin.

Listeria monocytogenes

In 1998, 135 *L. monocytogenes* isolates were tested for antimicrobial susceptibility as part of the surveillance program. The isolates came from different sources, e.g. vegetables, fish and pork. Nine of the isolates were resistant to penicillin. The results of the susceptibility testing of *L. monocytogenes* are presented in Table 39.

Bacteria from humans

This report includes resistance data from four counties representing approximately 25 percent of the Danish population. Earlier DANMAP reports only included data from the clinical microbiological laboratories in Aarhus and Roskilde counties. This year, we present data from two additional counties: Nordjylland county and Copenhagen Municipality. The participating laboratory in Copenhagen represents one third of the total number of bed days in this municipality. More information on demographics is presented in Table 2.

Table 38 Occurrence of resistance (%) among Staphylococcus aureus *from food, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	% resistant
J01A	Tetracycline	14
J01B	Chloramphenicol	0
J01C	Penicillin	53
J01E	Trimethoprim	0
J01F	Erythromycin	8
J01G	Gentamicin	0
	Kanamycin	0
	Streptomycin	4
J01M	Ciprofloxacin	2
J01X	Bacitracin	0
	Vancomycin	0
	Virginiamycin	0
Number of isolates		51

Table 39 Occurrence of resistance (%) among Listeria monocytogenes *from food, Denmark, 1998*

DANMAP 1998

ATC group	Compound	% resistant
J01A	Tetracycline	1
J01B	Chloramphenicol	3
J01C	Penicillin	7
J01E	Trimethoprim	0
J01F	Erythromycin	0
J01G	Gentamicin	0
	Kanamycin	0
	Streptomycin	3
J01X	Bacitracin	1
	Vancomycin	0
	Virginiamycin	1
Number of isolates		135

The results from the participating laboratories have been made available to us as data, categorised as resistant or susceptible, and include hospital isolates as well as isolates from general practice. In some instances the case definition varies among the laboratories, i.e. in some of the laboratories all blood isolates of coagulase negative staphylococci are tested for antimicrobial susceptibility while others only test isolates of clinical significance. Relevant differences are described below.

Escherichia coli

The results for a 4-year period are presented in Figure 15 and Table 40. The left-hand side of Figure 15 shows resistance to selected antibacterials among *E. coli* blood isolates. After a 3-year decrease in ampicillin resistance, there was a significant increase from 1997 to 1998, bringing the level back to around 34 percent, similar to the level observed in 1995. It is striking that the trend appears to be similar for three of the laboratories. The fluctuations are temporally related to fluctuations in the overall use of penicillins with extended spectrum. This may represent a causal association, although we would normally expect increases in the occurrence of resistance to be delayed with respect to increases in consumption. The laboratory in Aarhus had the highest frequency of resistance to ampicillin, 41 percent. The laboratory process samples from a university hospital where the patients treated generally represent more severe clinical cases. The proportions of isolates resistant to gentamicin and cefuroxime are low, and the worrying increase in cefuroxime resistance in Aarhus from 1995 to 1997 has stopped and show a decrease from 9 percent in 1997 to 4 percent in 1998 ($p<0.0009$).

The right hand side of Figure 15 shows resistance to selected antibacterials among isolates of *E. coli* from urine samples. The results are shown separately for isolates from primary health care and from hospitals. Sulfamethizol is the drug of choice in primary health care for this type of infection in Denmark in spite of a resistance level of 35 percent. For two laboratories, the data represent samples from both the primary health care sector and from hospitals. A significant proportion of the urine isolates originating from primary health care are submitted because of treatment failure and therefore represents a selected population. Resistance in the normal flora of *E. coli* is probably somewhat lower, as illustrated in the DANMAP 97 report where we presented the results of a community study on resistance among *E. coli.*

Sulfamethizol resistance is more frequent among isolates from primary health care than among hospital isolates, which is in accordance with the fact that there is a rather limited use of sulfamethizol in hospitals. More isolates are resistant in Copenhagen, where they used 310 DDD sulfamethizol/1,000 inhabitants x year compared with Aarhus which used 231 DDD/1,000 inhabitants x year. However, the changes in resistance over time in each county are parallel.

For resistance against ampicillin, the relationship is somewhat different. Isolates from hospitals are more frequently resistant than isolates from primary health care. In both Aarhus and Copenhagen counties, about 18 percent are used in hospitals which is a higher fraction than for sulfamethizol. The total use of antibacterials in extended spectrum penicillins is about 19 percent higher in Copenhagen than in Aarhus. However, the differences in the percentage of resistance are very small. Finally, there is a very low level of quinolone resistance in *E. coli* isolates, about 2 percent, in all participating counties.

Table 40 Occurrence of resistance (%) among Escherichia coli *from diagnostic submissions from humans in 4 counties, Denmark, 1998*

DANMAP 1998

ATC-group	Compound	Hospitals						General Practice		
		Blood				Urine		Urine		
		1 a)	5 a)	14 a)	16 a)	1	14	1	14	16
J01A	Tetracycline	18		23	24					
J01C	Ampicillin	36	34	41	37	37	41	36	37	38
	Mecillinam	7			5	6	9	4	7	4
J01D	Cefuroxime	1	2	4	2	1	8		6	
J01E	Sulfamethizol			34	35	37	32	39	34	40
	Trimethoprim			15	17	21	8	24	21	23
J01G	Gentamicin	1	0	1	1	1	2	1	1	1
J01M	Ciprofloxacin	1		5	3	2	2	2	2	2
	Nitrofurantoin					3	1	3	1	8
Number of isolates		200	130	532	282	2,900	4,837	4,000	3,380	1,874

a) County number: 1, Copenhagen Municipality; 5, Roskilde County; 14, Århus County; 16, Nordjylland County

Figure 15 Resistance (%) to selected antibacterial agents in Escherichia coli *from human blood and urine samples, Denmark, 1994-1998*

DANMAP 1998

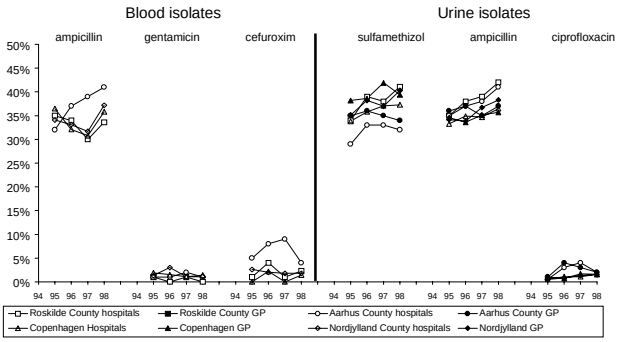


Figure 16 Resistance (%) to selected antibacterials agents in Streptococcus pneumoniae *from human blood and spinal fluid samples, Denmark, 1990-1997*

DANMAP 1998

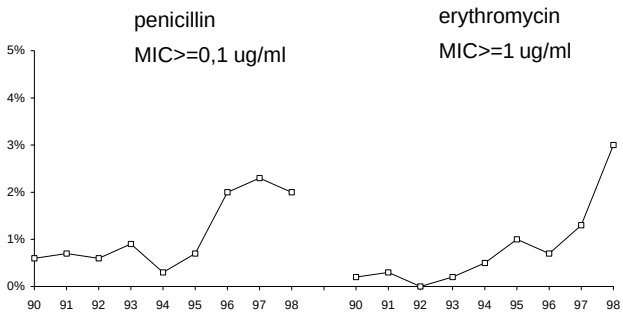


Figure 17 Resistance (%) to selected antibacterial agents in coagulase negative staphylococci *from human blood samples, Denmark, 1992-1998*

DANMAP 1998

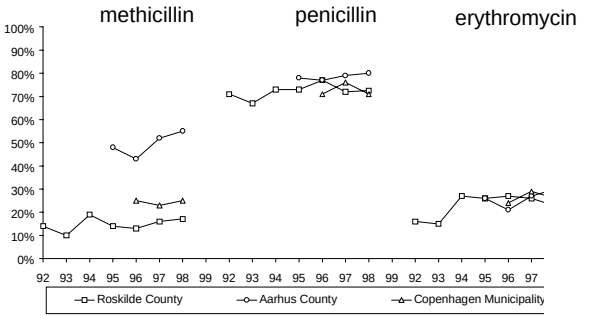
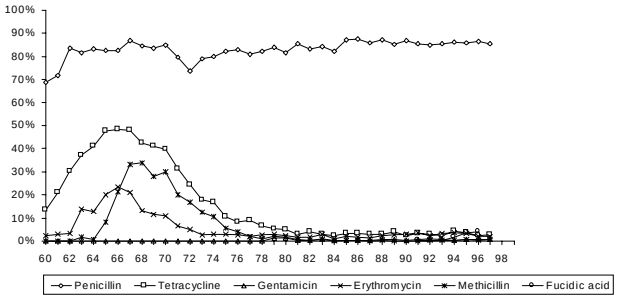


Figure 18 Resistance (%) to selected antibacterials agents in human blood isolates (N>25,000) of Staphylococcus aureus*, Denmark, 1960-1997*

DANMAP 1998



Streptococcus pneumoniae

Resistance to penicillin in *Streptococcus pneumoniae* is an increasing problem worldwide. In Denmark, this type of resistance has been rare. However, from 1995 to 1997, we observed a small but significant increase to more than 2 percent penicillin resistant isolates among *S. pneumoniae* (Figure 16). This was followed by a small decrease from 1997 to 1998. However, the frequency of resistance to macrolides continues to increase and has reached 3 percent in 1998. Even though some of the isolates represent infections acquired abroad, the trend is worrying. The problem must be seen in the light of the increased consumption of macrolides since 1994 as described in the section on antimicrobial consumption in this report. The pattern of consumption will now be analysed in detail and evaluated in relation to the recommended indications for use of macrolides in Denmark.

Coagulase negative staphylococci

Figure 17 shows resistance to selected antibacterials among clinical isolates of coagulase negative staphylococci from three of the participating counties. In general there is little increase in resistance, although a tendency towards increasing resistance to methicillin in Aarhus county is apparent. This must be seen in the light of an increase by about 70 percent in all Danish counties in the use of beta-lactamase resistant penicillins from 1994 to 1998.

Aarhus county has the highest percentage of resistance to methicillin (about 55 percent) followed by Copenhagen Municipality **(**about 25 percent). These counties both have university hospitals. This disparity in resistance frequency may be explained by differences in the procedures for selection of isolates for susceptibility testing. In Aarhus, only isolates with clinical relevance are tested, while in Copenhagen and Roskilde all isolates (including possible contaminants) are tested. This could explain a higher resistance level in Aarhus. Resistance to penicillin is, as expected, high, about 70 percent, and stable. For erythromycin the resistance level is about 25 percent and also stable.

Staphylococcus aureus

The *Staphylococcus* Laboratory at Statens Serum Institute has a very long tradition for phage typing of *Staphylococcus aureus* and the isolates have also been tested for antimicrobial susceptibility. Figure 18 shows the trend in resistance to selected antibacterials in *S. aureus* blood isolates since 1960. The isolates have also been tested for antimicrobial susceptibility. More than 25,000 isolates from all of Denmark are included.

Penicillin resistance has increased from about 70 percent in 1960 to about 85 percent in the 1990s. In the beginning of the period penicillin resistance in staphylococci was much more prevalent in hospital isolates than in community-acquired strains. Today, the antibiotic resistance pattern is almost identical in strains from the two sources. Studies on *S. aureus* coming from the normal Danish population find that about 75 percent of the isolates are resistant to penicillin. When looking at all clinical isolates of *S. aureus* there has been an increase in resistance to macrolides.

Figure 18 illustrates that Denmark experienced an epidemic of multi-resistant *S. aureus,* including methicillin resistant (MRSA) strains, during the end of the 1960s and the beginning of the 1970s. The reason for the disappearance of the multi-resistant strains is unknown. However, it paralleled a marked reduction in the consumption of broad spectrum antibiotics such as tetracycline and streptomycin. The changes in resistance also coincided with the development of clinical microbiology as a medical discipline and with an increasing awareness of the importance of hospital hygiene. Furthermore, there was an intensive campaign to teach Danish physicians the principles of prudent use of antimicrobials. At present, Denmark has a very low prevalence of MRSA, i.e. less than 0.5 percent and more than half of the infections are acquired abroad.

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Appendix 1
Materials and methods

Data on consumption of antimicrobials

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

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

The data on the consumption of therapeutics presented in this report comes from 2 sources. Data for the period until 1995 has been collated by section head N. E. Rønn, the Federation of Danish Pig Producers and Slaughterhouses and by E. Jacobsen, the Danish Pharmacy Association. The results are based on voluntary reporting to Danish Medical Statistics and may be incomplete. These results should therefore be regarded as estimates although they probably reflect rather accurately the true trend in consumption. Results for 1996 onwards are based on feedback on quantities sold provided by the pharmaceutical industry to the Danish Medicines Agency. 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 are 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
The Danish Medicines Agency is by law obliged to monitor the consumption of all medicinal products in humans. This is done by regular reporting from all pharmacies, including hospital pharmacies, to the DMA. Data for primary health care have been collected since 1994, while valid data on the consumption in hospitals are available from 1997 onwards.

In Denmark all antibacterials for use in humans are prescription-only medicine. All antibacterials are sold by pharmacies in defined packages. Each package is uniquely identified by a code which can be related to the size of package (in Defined Daily Doses DDD and by content), the Anatomical Therapeutic Chemical (ATC) classification system code of the product as well as the name of the producer. For each transaction the following information is collected in addition to the product code: social security number (CPR-number) of the patient, the date and place (pharmacy, hospital pharmacy, institution, etc.) and on reimbursement of cost as applicable. A code identifying the prescribing doctor is also included, however information on the indication for prescribing is not yet available. The data are transferred monthly to the Danish Medicines Agency in an electronic format. On-line transfer of the transactions in real time is being established.

This report includes data on the consumption of antibacterials for systemic consumption, on ATC-group J01, in the primary health care sector and in hospitals. The consumption is shown for the national as well as the regional (county) level. Denmark consists of 16 counties (excluding Greenland and the Faeroe Islands) with quite

different demographic and social structures. Throughout this report each county is identified by a code as shown in the legends for Figures 1-3. University hospitals are located in counties 1, 3, 9, 14 and 16, while counties 6, 7, 8, 10, 11, 13, 15 and 16 represent rural areas with a low population density.

For comparison between the counties we have chosen to present the amount of antibacterials used in DDD/1,000 inhabitants x year using the ATC-codes at level three and four. Hence, the denominators for the use in primary health care and for hospital use are the same, and it makes it possible to add numbers. It has not been possible to present the hospital consumption in relation to the number of bed days because this information was not available for 1998 at the time of writing. Table 2 shows the number of bed days in 1997 so that the data presented may be re-calculated using this other denominator.

Collection of bacterial isolates

Isolates from animals

Bacterial isolates included in the monitoring programme are collected from animals at slaughter (*Escherichia coli,* enterococci, *Campylobacter* and *Yersinia enterocolitica*), as well as from diagnostic submissions *(Actinobacillus pleuropneumoniae* and *Staphylococcus hyicus* from pigs, 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, feed 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 requested specifically for the DANMAP programme. Isolates of *Salmonella, Campylobacter, Listeria monocytogenes, Yersinia enterocolitica,* and *Staphylococcus aureus* were collected by all 32 MFELs.

The Danish Veterinary and Food Administration (DVFA) planned the collection of isolates of indicator bacteria (enterococci and *E. coli*) and 21 of the MFELs contributed samples of both Danish and imported foods. The food categories included were beef, pork, broiler meat, other poultry meat, and vegetables. The vegetable category primarily consisted of different types of sprout products, e.g. bean-, alfalfa-, azuki-, and radish sprouts.

Part of the *Salmonella* isolates from poultry originated from a survey of imported products carried out during the final 2 months of 1997 and the first half of 1998 in collaboration between the DVFA and the Danish Zoonosis Centre. These samples were collected by MFELs or DVFA staff according to a particular sampling plan. Most of the samples were taken at wholesale cold-stores, but a few of the samples were collected in retail outlets. The *Salmonella* analyses were performed by MFELs or by the Microbiological Section, Division of Food Analysis, Institute of Food Research and Nutrition, DVFA.

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-Vassi-liadis broth with 0.1 ml pre-enrichment broth and incubati-on 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 a 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.

Yersinia enterocolitica. The samples were examined for *Yersinia* by non-selective pre-enrichment of 20 g caecal contents in 180 ml of PSB, which was incubated for 3 hours at room temperature followed by 3 days at 4°C. Two ml of pre-enrichment culture was transferred to 100 ml of ITC (Irgasan-Ticarcillin-potassium Chlorate) broth, which is incubated for 2-4 days at 24°C. Ten ml of enrichment culture was inoculated onto CIN (Cefsulodin-Irgasan-Novobiocin) agar. Following incubation overnight at 30°C, red colonies with a red centre were sub-cultivated on BS (Brilliant gren sorbitol) agar. Sorbitol positive colonies were examined for urease activity and identified bioche-mically. Identification of strains suspected to belong to *Y. enterocolitica* O:3 was carried out by slide agglutination with type specific sera.

Escherichia coli. The material was inoculated directly onto Drigalski agar and incubated at 37°C overnight. One yellow colony was sub-cultivated on blood agar. 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 dissolved in 2 ml sodium chloride (0.9%) was spread on Slanetz agar and incubated for 2 days at 42°C. Up to three colonies showing a morphology typical of *E. faecalis* and *E. faecium* were re-inoculated on Slanetz agar and incubated for 2 days at 37°C. The isolates were then sub-cultivated onto aesculine agar. Aesculine positive, white colonies were identified according to the following criteria: motility, arginine dihydrolase and the ability to ferment mannitol, ribose, sorbitol, arabinose, raffinose and melibiose.

Enterococci from broilers were isolated and identified as follows. Cloacal swabs were incubated overnight at 42°C in Enterococcus selective broth, prepared with a composition identical to that of Enterococcosel broth (Becton Dickinson). Cultures were streaked on Slanetz agar and incubated for 48 h at 37°C. Colonies that morphologically resembled *E. faecium* and *E. faecium* were identified to species level by using standard biochemical and physiological tests as described above. All isolates that were verified as *E. faecium* and *E. faecium* were subjected to antimicrobial susceptibility testing.

Pathogens. The diagnostic submissions were examined for according to the standard procedures employed by the participating laboratories.

All isolates included in DANMAP 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. Strains were subsequently shipped to the DVFA in standard transport media. Verification of identity and antimicrobial susceptibility testing was performed by the Division of Microbiological Safety, Institute of Food Safety and Toxicology at DVFA. Only one strain of each species from a food sample was tested for antimicrobial susceptibility.

The isolation method for *E. coli* used 5 g of food that were incubated at 44°C for 18-24 hours in 45 ml of MacConkey- or laurylsulfate-broth. The broth culture

was streak-inoculated onto violet red bile agar and incubated for 48 hours at 44°C. Presumptive *E. coli* were sub-cultured onto blood agar, transferred to standard transport medium and shipped to DVFA. The isolates were identified as *E. coli* by standard morphological examinations and biochemical tests, including Api 20E test (bioMérieux, France).

Analysis for enterococci was carried out by adding 5g of the sample to 45 ml of azide dextrose broth followed by incubation at 44°C for 18-24 hours. This was followed by streak inoculation onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours the plates were examined for growth and typical red colonies were sub-cultured on blood agar, then transferred to transport medium and shipped to the DVFA. The isolates were identified as *E. faecium* or *E. faecalis* by standard morphological examinations and biochemical tests, including 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, Y. enterocolitica and *S. aureus* were isolated as specified by DVFA in “Cirkulære om mikrobiologiske undersøgelser af levnedsmidler af 3. december 1997” (ISBN: 87-601-3122-5). According to this circular, *Salmonella* must be isolated according to NMKL no. 71, 4th ed. 1991. Thermotolerant *Campylobacter* were isolated and identified according to NMKL no. 119, 2nd ed., 1990. *Yersinia enterocolitica* were isolated according to NMKL no. 117, 3rd ed., 1996. *Staphylocooccus aureus* were isolated according to NMKL no., 66, second ed., 1992, and *L. monocytogenes* according to NMKL no. 136, 1990. All strains submitted by the MFEL were identified to the species level at the DVFA, using standard morphological examinations and biochemical tests. Sero- and phage typing of *Salmonella* was performed by the Danish Veterinary Laboratory.

Susceptibility testing

Isolates from animals

Susceptibility to the growth promoters avoparcin, avilamycin, flavomycin, monensin, salinomycin and olaquindox was determined as MIC (minimum inhibitory concentration) by plate dilution according to NCCLS guidelines. Plate dilution was also used to test the susceptibility of *Campylobacter* isolates to all animicrobials included in the panel. Isolates of *Actinobacillus* were examined using tablet diffusion on Müller-Hinton II agar, except for susceptibility to carbadox and olaquindox.

All other susceptibility testing was done with Sensititre (Trek Diagnostic Systems Ltd.), a commercially available MIC technique using dehydrated media 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 where there was no visible growth. The breakpoints used are shown in Table A1.

The following strains were used for quality control: *Staphylococcus aureus ATCC 25923, 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 food

All strains were tested for susceptibility to a range of different therapeutic and growth promoting antimicrobial agents.

Susceptibility testing was performed using two different methods. Tablet diffusion (Rosco Diagnostica) on Müller-Hinton II agar was used for therapeutic agents. For growth promoters (avilamycin, flavomycin, monensin, salinomycin, carbadox, and olaquindox) we determined the MIC by agar dilution method on Müller-Hinton II agar according to NCCLS guidelines.

The susceptibility of *Campylobacter* was determined by a modified tablet diffusion method *(Engberg et al., Clin. Microbiol. Infect., in press*), where *Campylobacter* were added to Blood agar Base No. 2 supplemented with 5% horse blood (final concentration of approximately 10^4 CFU/ml) before the agar was allowed to set. Tablets were subsequently placed on the agar surface. Plates were incubated at 42°C for 48 hours.

The susceptibility of *Salmonella* isolated from imported poultry was determined using the Etest (AB Biodisk) on Müller-Hinton II agar.

The following strains were used for quality control: *S.*

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

DANMAP 1998

Antimicrobial agent	Salmonella, Yersinia, E. coli		Staphylococci		Enterococci		Campylobacter		A. pleuropneumoniae (tablet diffusion)	
	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints µg/ml	Range	Breakpoints	Range
Ampicillin	16	0.5-32					16	0.25-32	17 mm	
Apramycin	8	1-64					16	0.25-32 c)	18 mm	
Avilamycin			8	0.12-128 b)	8	0.12-128 b)				
Avoparcin			8	0.25-128	8	0.25-128				
Bacitracin			64	2-128	64	2-128				
Carbadox	64	8-128					64	0.06-128 d)	64 µg/ml	0.12-256
Chloramphenicol	16	1-64	16	1-64	16	1-64	16	0.25-32 e)	24 mm	
Ciprofloxacin	2	0.12-8	2	0.25-8	2	0.12-16				
Colistin	8	1-64					32	0.12-256	18 mm	
Enrofloxacin							2	0.25-32 f)	17 mm	
Erythromycin			4	0.25-16	4	0.25-32	16	0.25-32	23 mm	
Flavomycin			8	0.12-128	8	0.12-128				
Fusidate			2	0.5-4						
Gentamicin	8	0.5-32	8	1-32	512 a)	16-2048	16	0.25-32	23 mm	
Kanamycin	32	1-64	32	4-128	1024	64-2048				
Monensin			16 a)	0.12-128	16	0.12-128				
Nalidixic acid	16	2-128					16	1-128	23 mm	
Neomycin							8	0.5-64	20 mm	
Nitrofurantoin	64	32-256	64	32-256	64	32-256				
Olaquindox	64	0.12-256					64	0.06-128 d)	64 µg/ml	0.12-256
Oxacillin			2	0.5-4						
Penicillin			0.12	0.06-2	8	1-128			10 mm	
Salinomycin			8	0.12-128	8	0.12-128				
Spectinomycin							16	0.25-32	20 mm	
Spiramycin							16	0.25-32	23 mm a)	
Streptomycin	16	2-128	16	2-64	1024 a)	32-2048	32	1-128	23 mm	
Sulfonamide	256 a)	8-512	256	16-512			256	4-512	20 mm	
Synercid			2	0.5-16	2	0.25-32				
Teicoplanin					16	0.5-32				
Tetracycline	8	0.5-32	8	1-32	8	0.25-32	4	0.25-32	20 mm	
Trimethoprim	8	0.5-32	8	1-32					16 mm	
Tylosin							64	0.5-128	19 mm	
Vancomycin			16	0.5-16	16	0.5-32				
Virginiamycin			4	0.12-128	4	0.12-128				

a) New breakpoints compared to 1998

b) In 3rd and 4th quarter 1998: 0.12-32

c) Left out in 3rd and 4th quarter 1998

d) In 3rd and 4th quarter 1998 the range was changed to 0.06-256

e) In 3rd and 4th quarter 1998 the range was changed to 0.25-64

f) In 3rd and 4th quarter 1998 the range was changed to 0.03-16

Table A2 Breakpoints used for bacteria from food. Isolates were considered resistant if they had a MIC higher or an inhibition zone less than shown in the table.

DANMAP 1998

Antimicrobial agent	Bacterial genera						
	E. coli	Salmonella Enterica		Yersinia enterocolitica	Campylobacter	Listeria / S. aureus	Enterococci
Ampicillin	23 mm	23 mm	16 µg/ml*	-	-	-	-
Apramycin	23 mm	23 mm	-	23 mm	23 mm	23 mm	-
Avilamycin	-	-	-	-	-	-	8 µg/ml
Bacitracin	-	-	-	-	-	17 mm	17 mm
Carbadox	64 µg/ml	-	-	-	-	-	-
Ceftiofur	23 mm	23 mm	-	23 mm	-	23 mm	-
Chloramphenicol	24 mm	24 mm	16 µg/ml*	24 mm	24 mm	24 mm	24 mm
Colistin	18 mm	18 mm		18 mm	18 mm	-	-
Ciprofloxacin	21 mm	21 mm	2 µg/ml*	21 mm	21 mm	21 mm	21 mm
Erythromycin	-	-	-	-	23 mm	23 mm	23 mm
Flavomycin	-	-	-	-	-	-	8 µg/ml
Fusidin	-	-	-	-	-	23 mm	-
Gentamicin	23 mm	23 mm	8 µg/ml*	23 mm	23 mm	23 mm	15 mm
Kanamycin	23 mm	23 mm	32 µg/ml*	23 mm	23 mm	23 mm	-
Lincomycin	-	-	-	-	-	23 mm	-
Methicillin	-	-	-	-	-	20 mm	-
Monensin	-	-	-	-	-	-	16 µg/ml
Nalidixic acid	23 mm	23 mm	16 µg/ml*	23 mm	23 mm	-	-
Nitrofurantoin	23 mm	23 mm	-	23 mm	-	-	20 mm
Olaquindox	64 µg/ml	-	-	-	-	-	-
Penicillin	-	-	-	-	-	24 mm	10 mm
Pristinamycin	-	-	-	-	-	23 mm	23 mm
Salinomycin	-	-	-	-	-	-	8 µg/ml
Spectinomycin	20 mm	20 mm	-	20 mm	20 mm	-	-
Streptomycin	24 mm	24 mm	16 µg/ml*	24 mm	24 mm	24 mm	15 mm
Sulfonamid	24 mm	-	-	24 mm	-	24 mm	-
Tetracyclin	24 mm	23 mm	8 µg/ml*	24 mm	24 mm	24 mm	24 mm
Trimethoprim	16 mm	16 mm	8 µg/ml*	16 mm	-	17 mm	-
Tylosin	-	-	-	-	-	21 mm	21 mm
Vancomycin	-	-	-	-	-	11 mm	11 mm
Virginiamycin	-	-	-	-	-	23 mm	23 mm

* Breakpoints used for the E-test.

aureus ATCC 25923, E. coli ATCC 25922, Pseudumonas aeruginosa ATCC 27853 and *E. faecalis ATCC 29212.*

The breakpoints used to discriminate between susceptible and resistant strains for the different antimicrobial agents are presented in Table A2.

Isolates from humans
In Aarhus county susceptibility testing is performed using the disk-diffusion method. The other three counties use the tablet diffusion method (Neo-sensitabs, Rosco, Roskilde, Denmark) and their results are therefore highly comparable.

For most of the pathogens, only the total number of isolates and the proportion resistant has been reported.

Each of the participating laboratories takes part in national and international quality assurance collaborations.

Performance test

In order to ascertain the comparability of susceptibility results obtained by the DANMAP participants, we carried out a performance test among the laboratories. The test included four laboratories, including two 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 enterobacteria (7 strains of *E. coli* and 1 *Serratia marscecens*) and one consisting of enterococci (8 strains - *E. faecalis, E. faecium, E. casseliflavus* and *E. gallinarum*).

Table A3 Results of performance testing (correct result/no. of tests) among laboratories participating in the DANMAP-programme. "S" denotes susceptible and "R" resistant

DANMAP 1998

	Enterobacteria		Enterococci		Total
	S	R	S	R	
Penicillin	-	-	-	36/36	36/36
Ampicillin	14/16	15/16	18/18	9/9	56/59
Cefuroxim	32/32	-	-	35/36	67/68
Erythromycin	-	-	24/24	8/8	32/32
Tetracycline	16/16	16/16	20/20	15/16	67/68
Gentamicin	32/32	-	28/28	8/8[#]	68/68
Nalidixic acid	24/24	8/8	-	-	32/32
Ciprofloxacin	28/28	4/4	-	-	32/32
Sulfonamide	12/12	19/20	-	-	31/32
Trimethoprim	12/12	19/20	-	-	31/32
Vancomycin	-	-	15/16	15/20	30/36
Teicoplanin	-	-	24/24	3/3	24/27
Polymyxin B	-	4/4 [*]	-	-	4/4
Nitrofurantoin	32/32	-	-	-	32/32
Total	202/204	85/88	129/130	129/136	545/558

[*] polymyxin B was tested with a Serratia strain

[#] gentamicin resistance for the enterococci denotes a MIC > 1024 mg/l

The results are presented in Table A3, which shows the gross results as the number of tests found either S (susceptible) or R (resistant) in relation to the total number of tests performed for each antimicrobial. Overall, the results showed agreement for 97.7 percent of the tests. The only major problem was found with vancomycin resistant enterococci and was due to difficulties categorising two vanB positive strains with a rather low vancomycin MIC. This type of resistance is known to cause problems when using the disc/tablet diffusion test.

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 datasheets. The results of all susceptibility tests were saved in a Paradox database as inhibition zones (mm) or MIC-values (µg/ml). For each bacterial isolate, information was 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 make it possible to obtain further information about the isolate from the laboratory. Information about country of origin was recorded whenever possible. The resistance data were transferred from the Paradox database to an Excel spreadsheet, where the data were processed.

The results of the susceptibility testing of the isolated strains are shown for each species as the percentage of strains resistant to an antimicrobial. The number of strains tested is represented by "n" whereas the total number of food samples analyses in each category is represented by a capital "N".

Appendix 2

This bibliography contains papers that present results of research carried out as part of the DANMAP programme. Only papers in refereed journals have been included. In addition, DANMAP results have been presented in a large number of abstracts, congress proceedings, and non-refereed publications.

1996

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1998

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