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

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This report is also available in a Danish version.

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Section authors will welcome comments and feedback on the present report.

INTRODUCTION

In June 1995 the Minister of Health and the Minister of Agriculture and Fisheries initiated a programme to conduct a coordinated national surveillance and research programme to monitor resistance in bacteria from animals, foods and humans to antimicrobial agents used for therapy and/or growth promotion.

The surveillance programme was organized in collaboration between the Danish Veterinary Laboratory (DVL), the National Food Agency of Denmark (NFA) and Statens Serum Institut (SSI).

The present publication is the first joint reporting of results of the programme, however some of the results relating to resistance to antibiotic growth promoters among bacteria isolated from animals have previously been described in a separate report (in Danish) issued in July 1996.

Results of detailed studies, e.g. studies of resistance mechanisms will be published separately in scientific journals.

PROGRAMME OBJECTIVES

The surveillance programme has the following objectives:

- To monitor the occurrence of antimicrobial resistance among bacteria isolated from food animals, foods and from humans;
- To monitor the use of antimicrobial agents for treatment in humans and in animals and for growth promotion;
- To demonstrate associations between such use and the occurrence of resistance;
- To record trends in the above mentioned parameters.

The results are expected to provide knowledge about the public and animal health impact of resistance caused by the use of antimicrobial compounds in food animals and in humans and provide a basis for recommendations on the use of growth promoters and of therapeutic antimicrobials in the veterinary field and on the use of antimicrobials in human medicine. Furthermore, the surveillance is expected to result in an increased emphasis on research into antimicrobial resistance mechanisms.

INTERPRETATION AND USE OF RESULTS

It is a main forte of the surveillance programme that it is continuous so that the body of data will increase with time. The present report is the first in a series which will make it possible to monitor the trend in use of antimicrobials in food animals and in human medicine and changes in the occurrence of antimicrobial resistance.

It is also important to emphasize that the definition of a bacterial isolate as resistant or sensitive is associated with a degree of uncertainty. This uncertainty is not inconsiderable when the results are obtained in different laboratories and by different methods.

Ideally the methods used should be either identical or closely defined and calibrated against each other. Such calibration procedures will be carried out in the near future.

Only acquired resistance to compounds used in therapy are included in the calculation of multiresistance. As the panel of antimicrobials is extensive and includes substances that are not, for some bacterial species, therapeutically relevant, the proportion of multiresistant strains is comparatively high.

Therefore the occurrence of multiresistance as used in the present context cannot be used as a predictor of therapeutic success, but rather as a parameter which can facilitate the comparison of levels of resistance between bacterial species and between bacteria isolated from different sources.

SUMMARY OF RESULTS

Food animals

Use of antimicrobials. Unfortunately there are no official data available at present on the use of antimicrobials for treatment of animals. Unofficial figures indicate that the consumption decreased by about 20%, from 27,400 kg active compound in the first half of 1995, to 22,000 kg in the first half of 1996. Aminoglycosides, macrolides, penicillins and tetracyclines accounted for almost 80% of the total. In comparison in all of 1995 94,000 kg of active compounds were used for growth promotion. Tylosin, a macrolide used in pigs, accounted for more than 50% of this total.

Detailed recording of the use of antimicrobials in food animals needs to be established.

Antimicrobial resistance. Bacteria isolated as pathogens in animals, zoonotic bacteria and bacteria frequently occurring in healthy animals (indicator organisms) were isolated from pigs, cattle and broilers and their susceptibility to a panel of antimicrobial agents that included growth promoters and therapeutic substances were determined.

Resistance to all antimicrobials was observed. There was considerable variation in the occurrence of resistance between bacteria from different animals and between different bacterial species. Part of this variation could be explained by the pattern of antimicrobial use in the different animal groups. In other cases an association between antimicrobial use and occurrence of resistance was not apparent.

A high frequency of resistance to macrolides, sulphonamides, aminoglycosides, beta-lactam antibiotics and tetracycline was observed in several bacterial species from different animals.

Resistance to antimicrobials was most frequently observed in bacteria isolated from pigs compared to bacteria isolated from cattle and broilers. This pattern was also reflected in the finding that multiresistant bacteria were most frequently isolated from pigs.

Resistance to growth promoters was frequently observed among the indicator bacteria *Enterococcus faecium* and *Enterococcus faecalis* from all animals. Resistance was detected less frequently in zoonotic and pathogenic microorganisms. Co-resistance to therapeutic antimicrobials was frequently observed in bacteria resistant to growth promoters which are structurally related to therapeutic agents (avoparcin, virginiamycin, spiramycin and tylosin).

The finding of relatively high levels of resistant salmonella and Campylobacter, and the presence of multiresistant salmonella and Campylobacter in animals, notably pigs, is a cause of concern and needs further investigation.

The results show that the efficacious treatment of infection in food animals does not appear to be immediately at risk, and that use of narrow-spectrum antimicrobials will usually be effective. However, the high occurrence of resistance to tetracycline among some strains of bacteria from pigs indicates that a high selective pressure is exerted by this group of compounds. Co-resistance between tetracycline and other antimicrobial agents is common, at least in some bacteria. Therefore, narrow-spectrum antimicrobials should be used in preference to wide-spectrum substances such as tetracycline.

Resistance to the newer quinolones and to cephalosporins was rare in the animal isolates. However, the experience in other countries shows that resistance to these compounds may quickly become wide-spread with increasing consumption.

Foods

Antimicrobial resistance. The antimicrobial susceptibilities of 375 *Escherichia coli*, 210 *Enterococcus faecium*, 172 *Enterococcus faecalis* and 90 Campylobacter spp. isolates were tested against a range of therapeutic and growth promoting antimicrobial agents. Food samples were collected nationwide from different retail outlets. The foods sampled included the following groups: beef, pork, poultry, dairy products, fish, fruit and vegetables.

The results of the survey reflect the resistance status of bacteria that can be isolated from foods that are entering the households.

Resistance was demonstrated to all therapeutic antibiotics and most of the growth promoting antimicrobial agents. The only growth promoting agents, to which no resistance was recorded, were carbadox and salinomycin.

The highest frequencies of resistance from foods were recorded among bacteria isolated from poultry. Resistance to tetracycline was present in 41% of the *E. faecium* isolates and in 59% of the *E. faecalis* isolates from poultry. The frequencies of macrolide and streptogramin resistance were also high among enterococci isolated from poultry.

E. coli isolates from poultry had a remarkably high frequency of multiresistance (28%) compared to isolates from the other food categories (0% to 8%). This is probably caused by imported poultry constituting part of the retail samples.

Resistant bacteria commonly occurred in pork. However, the frequencies of resistance among isolates from pigs at slaughter were generally higher than among isolates from retail pork samples.

Bacteria from the other food groups exhibited lower levels of resistance, especially bacteria from vegetables.

Humans

Use of antimicrobials. Since January 1994 the Danish Medicines Agency has received monthly reports on the use of antibiotics from all Danish pharmacies. It is now possible to account for more than 95% of the use of antimicrobials in humans in Denmark.

The antibiotic consumption in Denmark is stable and low (13.8 defined daily doses/1000 inhabitants/day in 1995) compared to the consumption in other countries, notably the Scandinavian countries. Thus, Denmark has the lowest consumption per capita among the Nordic countries.

Antimicrobial resistance. There is generally a very low level of resistance in Denmark as compared to other countries. Among *Staphylococcus aureus* there is 86% resistance to penicillin and 0.5% resistance to methicillin. In *E. coli*, 33% of blood isolates are resistant to ampicilllin.

In a two year period only one isolate from an infected patient of vancomycin resistant enterococci has been found in Denmark.

Among *Salmonella* Typhimurium the highest level of resistance is against sulfamethizol and streptomycin (10%) and against ampicillin (around 20%).

Association between use of antimicrobials and occurrence of resistance

The occurrence of resistance against tetracycline among *E. coli* compared with estimates of the quantities used provides an example of the comparisons that will be possible within the integrated monitoring programme.

E. coli is a frequent cause of disease in humans, cattle and pigs and is also a part of the normal intestinal microflora. Table I shows the frequencies of resistance against tetracycline and ampicillin among *E. coli* from humans, food animals and from food. It is evident that for indicator *E. coli* the occurrence of resistance in slaughter animals and in beef and pork are very similar. Poultry represents an exception to this as the occurrence of resistance in retail samples is considerably higher than in broilers at slaughter. This difference is thought to be largely explained by the presence of imported poultry among the retail samples.

The consumption of tetracycline amount to 12% (or a total of 3,000 kg) of the total consumption of antibiotics by humans, measured in Defined Daily Doses (DDD). Tetracycline is mainly used outside hospitals for peroral treatment of chlamydia infections and in the treatment of acne. A minor part has been used in the treatment of upper respiratory infections. Accordingly it is minimal, what has been used in treating infections with *E. coli* in humans but nevertheless the resistance in *E. coli* isolated from human blood is as high as 22%.

As a consequence, other factors are suspected to contribute to this relatively high level of resistance. It is believed that around half of those *E. coli* causing bacteriaemia in humans originate from outside hospitals. There are not yet results of community studies of resistance among *E. coli* in Denmark, and the occurrence of tetracycline resistance at present in the general population is not known.

Studies have shown that within a few days of starting use of tetracycline to treat acne, tetracycline resistant *E. coli* will be present in the intestinal tract of the patient. The occurrence of resistance observed in this investigation therefore may reflect transmission of resistance determinators within the human population. Alternatively, if the planned studies show that the occurrence of tetracycline resistance among *E. coli* in the general population is lower than 22%, it raises the possibility that the occurrence results from, for example, co-selection in environments with intensive use of antimicrobials.

Finally, the levels of tetracyclin resistance among *E. coli* isolates causing human disease could be a result of resistance acquired before the bacteria colonized human intestinal tract. One possibility is that they are a result of zoonotic spread of indicator *E. coli* from animals via food.

The use of tetracycline as a therapeutic in veterinary medicine amounts to 9 tons, which corresponds to 20% of the total use, calculated by weight. Among the clinical isolates from animals there is a high degree of resistance against tetracycline. For *E. coli* isolated from cattle the percentage is 78% and for pigs 57%. A high frequency of resistance is also found for *E. coli* isolated as indicator bacteria from pigs. For bacteria isolated from live animals and food the resistance is 30% and 29%, respectively.

CONCLUSIONS

In general, efficaceous treatment of infections in humans and in animals is still possible, also when using compounds with a narrow spectrum of activity.

However, the widespread occurrence of resistance to tetracycline, in particular among bacteria isolated from pigs, is a cause of concern, because tetracycline has been shown to co-select resistance to a number of antimicrobials used in therapy. The occurrence of resistance to newer, broad-spectrum antibiotics such as quinolones was low among bacteria isolated as pathogens but widespread among enterococci and among *C. coli* from pigs. The experience from other countries show that resistance to these compounds rises rapidly with increasing use.

Detailed recording of the use of antimicrobial agents in food animals needs to be implemented so that meaningful comparisons between antimicrobial use and occurrence of resistance can be made.

In bacteria from foods various levels of resistance among isolates of indicator bacteria (*E. coli*, *E. faecium*, *E. faecalis*) were demonstrated to all therapeutic and most of the growth promoting antimicrobial agents.

Antibiotic resistance most frequently occurred among isolates from poultry, followed by isolates from pork. Indicator bacteria from the other food groups showed lower levels of resistance with bacteria isolated from vegetables possessing the lowest levels.

Generally the prevalence of resistance in the most common human pathogenic bacteria is low and does not give cause to concern. The level of resistance is very low in Denmark as compared to other countries. This is a result of the official policy to encourage use of antibiotics with a narrow spectrum of activity.

Detailed recording of the antibiotic consumption in human medicine provided by the Danish Medicines Agency will make it possible to monitor changes in the consumption and to intervene if major shifts in the pattern of use should occur.

Public health aspect of antimicrobial resistance in food animals

It should be noted that the isolation of most human bacterial strains in the present report precedes the isolation of bacteria from animals and food.

Until now the levels of antimicrobial resistance in zoonotic microorganisms from human gastro-intestinal infections have not been monitored systematically in Denmark. The first results presented in this report do not cover all the zoonotic organisms and all the sources which in the future will be included in the surveillance programme. However, conclusions based on the present results are that the levels of antimicrobial resistance described in this first report do not give rise to immediate concern. With a few exceptions, which await further investigation, there was good agreement between the levels of resistance observed in isolates from animals and humans.

Pigs are generally assumed to be the only source of yersinia infections in Denmark. It is therefore interesting that none of human yersinia isolates were resistant to trimethoprim, compared with 45% of yersinia isolates from pigs. We can offer no explanation for this at present, however the difference may be a result of differences in the methods used.

The levels of resistance in salmonella isolated from animals have been found to increase in Denmark during recent years, possibly as a result of therapeutic use of antimicrobials in animals. The continued surveillance will show to what extent changes in levels of resistance in zoonotic bacteria from different animals and food stuffs affect the levels of resistance observed in the zoonotic bacteria isolated from human infections. The level of resistance among *Salmonella* Typhimurium isolated from humans is unchanged from 1993 to 1996.

It has been demonstrated that the use of avoparcin as a growth promoter is associated with the occurrence of vancomycin resistant *E. faecium* (VRE). The present surveillance found VRE in animals and in a retrospetive study of humans, and VRE were also detected among isolates from foods.

Table I. Resistance against ampicillin and tetracycline in *E. coli* from different sources.
Resistens overfor ampicillin og tetracyclin hos E. coli fra forskellige kilder.

	Pathogenic isolates			Indicator isolates								
	Human	Cattle	Pigs	Cattle		Pigs		Poultry		Dairy	Fish	Fruit
				Animals	Beef	Animals	Pork	Broilers	Retail			
Tetracycline	22%	78%	57%	6%	9%	30%	29%	9%	43%	4%	9%	7%
Ampicillin	33%	80%	16%	1%	5%	11%	11%	12%	17%	4%	4%	0%

Consumption of antimicrobials and occurrence of antimicrobial resistance in bacteria collected from broilers, cattle and pigs between October 1st 1995 and September 30th 1996

We would like to acknowledge the assistance of meat inspection staff and plant personnel in collecting the slaughter samples from broilers, cattle and pigs. We would also like to thank the Cattle Health Laboratory at Ladelund and the Laboratory of the Federation of Danish Pig Producers and Slaughterhouses in Kjellerup for making isolates of pathogenic bacteria available for the programme. We are very grateful to Niels Erik Rønn, the Federation of Danish Pig Producers and Slaughterhouses and Erik Jakobsen, the Danish Pharmaceutical Association, for providing data on the use of therapeutic antimicrobial agents.

We also thank Bent T. Viuf, Danish Plant Directorate, for making data on the use of antimicrobial growth promotors available.

CONCLUSIONS

The body of data included 350 isolates of enterococci, 785 *Escherichia coli*, 196 *Campylobacter coli/C. jejuni*, 371 coagulase negative staphylococci (CNS), 211 *Staphylococcus aureus*, 71 *Staphylococcus hyicus*, 66 *Actinobacillus pleuropneumoniae*, 73 *Yersinia enterocolitica*, and 248 *Salmonella enterica*.

Antimicrobial resistance was observed against most antibiotics, however, the occurrence varied considerably depending on the bacterial species and on the species of animal from which the isolates originated.

More than of 90% of all enterococcal isolates from all animals were resistant against four or more groups of antimicrobial agents (multiresistant).

Apart from enterococci, multiresistance among bacteria from broilers was only seen in 2% of indicator *E. coli*. Seventy-five per cent of pathogenic *E. coli* from cattle were multiresistant, compared with 27% and 30%, respectively, of *S. hyicus* and pathogenic *E. coli* from pigs.

For the growth promoters avoparcin, tylosin and virginiamycin high levels of co-resistance was observed to therapeutic glycopeptides, macrolides and streptogramins, respectively. In the case of streptogramins, the genetic basis for this resistance is not known.

Among the zoonotic bacteria and the indicator bacteria, the occurrence of resistance was generally highest in isolates from pigs.

In isolates from cattle and in pigs there was a statistically significant difference in occurrence of resistance among *E. coli* collected as indicator bacteria and pathogenic strains of *E. coli*. This suggests that therapeutic use of antimicrobials has had a significant impact on resistance levels.

Surveillance programmes designed to monitor possible spread of antimicrobial resistance to humans should, therefore, include bacterial isolates from the normal animal population whereas programmes to monitor the efficacy of therapeutic agents in diseased animals may be based on isolates from diagnostic submissions alone.

This part of the study provided little information on the risk of transmission of antimicrobial resistance to humans. Among *C. coli* from pigs 55% were resistant to erythromycin, most likely as a result of the use of tylosin as growth promoter. However, only a small proportion of the human campylobacter infections in Denmark are caused by *C. coli* (less than 10%).

In contrast, the occurrence of resistance in salmonella is unlikely to be a result of the use of growth promoting antibiotics. Only 2 of 11 growth promoting antimicrobials used are active against Gram negative bacteria and generally, less than 5% of the isolates were resistant to these 2 agents, carbadox and olaquindox.

The results show that the efficacious treatment of infection in food animals does not appear to be immediately at risk, and that use of narrow-spectrum antimicrobials will usually be effective. However, the high occurrence of resistance to tetracycline among some strains of bacteria from pigs indicates that a high selective pressure is exerted by this group of compounds. Co-resistance between tetracycline and other antimicrobial agents is common, at least in some bacteria. Therefore, narrow-spectrum antimicrobials should be used in preference to wide-spectrum substances such as tetracycline.

Resistance to the newer quinolones and to cephalosporins was rare in the animal isolates. However, the experience in other countries shows that resistance to these compounds may quickly become wide-spread with increasing consumption.

INTRODUCTION

A publication (in Danish) issued in July 1996 reported on the occurrence of resistance to antimicrobial growth promoters and related therapeutic agents in bacteria isolated from food animals during the last quarter of 1995 and the first quarter of 1996.

The present publication reports the occurrence of antimicrobial resistance among bacteria isolated from food animals during one year from October 1st 1995 through September 30th 1996. The results include resistance to antimicrobial growth promoters as well as therapeutic agents. For reference, data on the consumption of therapeutic agents between January 1995 and June 1996 as well as on the quantities of antimicrobial growth promoters used in 1995 have been included.

Caution is required in interpreting the results on resistance to growth promoting antimicrobials since at present no reference methods are established internationally for these substances. Similar precautions need to be taken for some of the therapeutic agents tested. Furthermore, so far the surveillance includes relatively few isolates.

In the future, when more isolates have been examined and the genetic basis for antimicrobial resistance has been clarified and combined with results of analyses of the trends in consumption of antimicrobial substances and in the occurrence of antimicrobial resistance, conclusions about causal relationships may be possible.

MATERIALS AND METHODS

Collection of bacterial isolates

The bacterial isolates are collected on a continuous basis and consist of representative samples of:

- Animal pathogens
- Zoonotic bacteria
- Indicator bacteria (bacteria commonly occurring in the intestinal tract of animals and in humans)

The animal pathogens have been chosen to represent some of the most common bacterial pathogens in pigs, cattle and broilers. They constitute a systematic random sample among isolates recovered from submissions to the Danish Veterinary Laboratory, to the laboratory of the Danish Pig Producers and Slaughterhouses in Kjellerup (pigs) and to the Cattle Health Laboratory at Ladelund (bovine mastitis).

Animal pathogens were isolates of *E. coli* from diagnostic submissions from pigs, cattle and broilers and *A. pleuropneumoniae* and *S. hyicus* from pigs, where the first 25 isolates each quarter were collected for testing (only one isolate per herd). For cattle, 50 isolates of *S. aureus* and 100 coagulase negative staphylococci are collected each quarter.

Salmonella enterica, *Campylobacter jejuni*, *Campylobacter coli* and *Y. enterocolitica* were chosen to represent zoonotic bacteria, because these are the most common causes of food borne infections in Denmark.

E. coli, *E. faecalis* and *E. faecium* were chosen to represent indicator bacteria, because they occur in the normal intestinal tract flora of most animals. Therefore, a direct comparison of the resistance levels observed in different species of animals is possible.

Indicator bacteria and zoonotic agents were isolated from samples of the intestinal contents collected at slaughter of pigs, cattle and broilers. The samples were collected monthly (for broilers: weekly) as a systematic random sample in all major slaughter plants. The number of samples from each plant was roughly proportional to the number of animals slaughtered. Salmonella from pigs were selected among isolates from samples submitted during the course of an existing surveillance programme. These samples include faecal material from pens in subclinically infected herds as well as pork examined for salmonella in slaughter plants.

The number of samples collected each quarter for isolation of zoonotic and indicator bacteria was

- 250 caecal samples from pigs
- 75 rectal samples from cattle (calves 8-9 months of age)
- 250 cloacal swabs from broilers

The samples were not examined for the presence of all the above bacterial agents each month, but the analyses were staggered over each quarter, to provide the required number of isolates.

The slaughter house samples were sent to the DVL for examination. Standard methods were used for the isolation of bacteria and all isolates were

identified to species level before being tested for antimicrobial resistance.

Determination of antimicrobial resistance

Bacterial isolates were tested for resistance against antimicrobial agents used for growth promotion and therapy. Susceptibility to antimicrobial agents used for growth promotion was mainly determined as minimum inhibitory concentrations (MIC's) by the agar dilution method of NCCLS, using spot inoculation of approximately 10^4 CFU (Figure 1). Susceptibility to therapeutic agents was determined as mm inhibition zone using a tablet diffusion method (Rosco Diagnostica) in a 14 cm dish with Müller-Hinton II agar, inoculated with approximately 10^5-10^6 CFU according to ICS (semi-confluent growth) (Figure 2). For Campylobacter all determinations of antimicrobial susceptibility were performed as MIC tests.

The antimicrobials tested, the diffusible amount and the break-points used to discriminate between resistant and susceptible isolates are shown in Table 1.1.

RESULTS

Table 1.2a shows the consumption of antimicrobials for therapy during 1995 and the first half of 1996, while Table 1.2b shows the use of antimicrobials for growth promotion during 1995. Table 1.2c shows the annual production of broilers, cattle and slaughter pigs in Denmark. Tables 1.3a and 1.3b present the resistance among pathogenic bacteria to antimicrobial growth promoters and therapeutic agents, respectively. Tables 1.4a and 1.4b show the results for zoonotic bacteria and Tables 1.5a and 1.5b for indicator bacteria. Table 1.6 shows the proportions of resistant and multiresistant isolates.

The occurrence of resistance is presented as per cent resistant isolates of a bacterial species among all isolates of that species. The occurrence therefore does not always reflect the proportion of animals harbouring that resistant bacterial species. In most instances the proportion of positive samples, is considerably less than unity, for example, *E. faecium* was found in only 8% of pig samples. The prevalence of animals carrying a resistant bacterial species, therefore, most often will be less than the figures shown.

Results for the individual antibiotics will be presented by group in alphabetical order. The groups of bacteria (pathogens, zoonotic agents and indicators) will be presented separately, as appropriate.

In broilers, only 6 pathogenic *E. coli* were recovered, while from pigs and cattle only 3 isolates of *C. jejuni* and *E. faecalis*, respectively, were recovered. Because of the small numbers these results have been excluded.

Aminoglycosides

This group includes apramycin, gentamicin, neomycin, spectinomycin and streptomycin. The antimicrobial spectrum ranges from narrow in streptomycin to broad in gentamicin. Aminoglycosides are used for therapy in animals and in humans. Enterococci are relatively resistant to normal therapeutic levels of aminoglycosides. High-level tablets were used to test for resistance of enterococci against streptomycin and gentamycin, however, for apramycin and neomycin only low-level tablets were available.

Pathogens. Among isolates of *S. aureus* from cattle and *A. pleuropneumoniae* from pigs 7% and 18%, respectively, were resistant to apramycin. Apramycin is not approved for use in animals in Denmark. Among *E. coli* 13% of isolates from

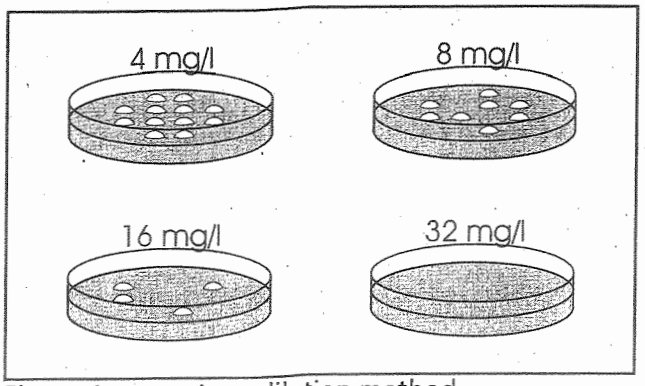

Figure 1. Agar dilution method.

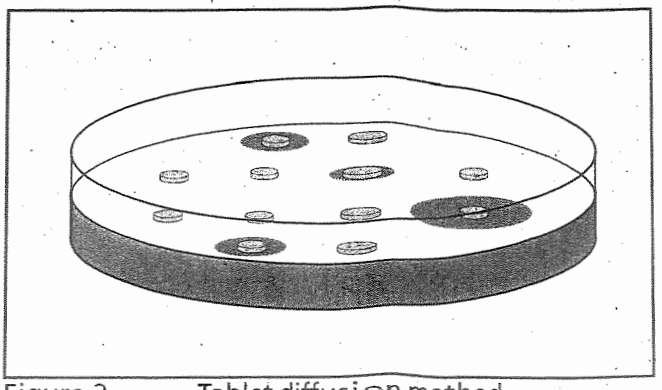
Figure 2. Tablet diffusion method.

diagnostic submissions from cattle and 1% from pigs were resistant to gentamicin, while 83% and 69%, respectively, were resistant to streptomycin. Among food animals, gentamicin is only approved for use in newborn piglets. Resistance to neomycin among *E. coli* from pigs was common (21%), compared with 6% in cattle. In isolates from pigs 27% of pathogenic *E. coli* were resistant to spectinomycin, while 30% of *S. hyicus* isolates were resistant to streptomycin and 10% to spectinomycin.

Zoonotic bacteria. The occurrence of resistance to aminoglycosides among zoonotic bacteria from broilers and cattle ranged from 0 to 13%. However, 44% of *C. coli* and 21% of salmonella from pigs were resistant to streptomycin, and 14% of *Y. enterocolitica* isolates from pigs were resistant to apramycin.

Indicator bacteria. Among *E. faecium* and *E. faecalis*, most isolates were resistant to normal therapeutic levels of apramycin. High-level resistance to gentamicin was not observed. Thirty-four per cent of *E. faecium* and 42% of *E. faecalis* from pigs were resistant to neomycin, compared with 9% of *E. faecium* from broilers, where neomycin is not used. Among isolates of *E. coli* and enterococci from broilers and cattle the occurrence of resistance to streptomycin was less than 6-8%, while in pigs, 55% of *E. coli*, and 40% of *E. faecium* were resistant. In pigs, between 8 and 26% of indicator bacteria isolates were resistant to spectinomycin.

Avilamycin

Avilamycin is an oligosaccharide antibiotic with activity only against Gram positive bacteria. In the EU it is approved for use as a growth promoter in broilers and pigs, but is used mainly in broilers.

Sixty-nine per cent of *E. faecium* isolates from broilers were resistant to avilamycin, compared with only 1-2 percent of *E. faecium* and *E. faecalis* from pigs. Resistant strains were not observed among staphylococci from cattle or pigs.

Bacitracin

Bacitracin is a polypeptide antibiotic approved for growth promotion in broilers, calves and pigs and for therapy. It is active against Gram positive bacteria only. The therapeutic use of bacitracin is very limited.

Forty-one per cent and 31% of *E. faecium* isolates from broilers and pigs, respectively, were resistant to bacitracin. Three per cent of *E. faecalis* and none of the staphylococcal isolates were resistant.

Beta-lactam antibiotics

a. Penicillins

This group includes penicillin and ampicillin, both of which are used for therapy only. Penicillin is active primarily against Gram positive bacteria, while ampicillin is active against both Gram positive and Gram negative bacteria. However, ampicillin is not normally used against staphylococci.

Pathogens. Resistance to ampicillin occurred at a high level (80%) only in *E. coli* from cattle, compared with 16% of *E. coli* from pigs. Resistance to penicillin ranged from 29% among *S. aureus* from cattle to 59% of *S. hyicus* isolates from pigs.

Zoonotic bacteria. Almost all isolates from cattle were susceptible to ampicillin, while 7% of *C. jejuni* and 17% of *C. coli* from broilers and pigs, respectively, were resistant. Eighteen per cent of salmonella from poultry and 9% of Salmonella from pigs were resistant to ampicillin. In contrast, almost all isolates of *Y. enterocolitica* (97%) were resistant to ampicillin.

Indicator bacteria. Among *E. faecium*, 33% of the isolates from broilers and 52% of the isolates from pigs were resistant to penicillin, while virtually all *E. faecalis* isolates from pigs were susceptible. Among *E. coli*, 12% and 10% of isolates from broilers and pigs, respectively, were resistant to ampicillin. In contrast, all enterococci tested were susceptible to ampicillin.

b. Cephalosporins

The cephalosporins are represented by ceftiofur, a third generation cephalosporin with activity against a wide spectrum of Gram negative bacteria but only moderate activity against Gram positives. Till now isolates of Gram negative bacteria, including zoonotic agents, have not been tested for resistance to ceftiofur but will be in the future.

Resistance to ceftiofur among staphylococci isolated from pigs and cattle occurred at a low frequency, less than 5%. Among *E. faecium* from broilers and pigs 80% and 84%, respectively, were resistant to ceftiofur, while 53% of *E. faecalis* from pigs were resistant.

Chloramphenicol

Chloramphenicol is an antibiotic with a wide spectrum of activity which includes Gram negative as well as Gram positive bacteria. It has not been used in food animals in Denmark since 1978, however, in late 1995 a related compound, florfenicol was approved and is presently marketed for therapy in cattle.

Pathogens. Resistance to chloramphenicol in isolates of *E. coli* from diagnostic submissions from cattle and pigs occurred at a frequency of 21% and 22%, while it was rare in Staphylococcus and in Actinobacillus (2% or less).

Zoonotic bacteria. Resistance to chloramphenicol was almost absent among isolates of zoonotic bacteria from broilers and cattle, however, 12% of *C. coli* and 20% of Salmonella from pigs were resistant. Only a single isolate of Y. enterocolitica was resistant to chloramphenicol.

Indicator bacteria. The occurrence of resistance among *E. coli* isolated from slaughterhouse samples was significantly lower (5% and 14% in cattle and pigs, respectively) than observed in isolates from diagnostic submissions.

Flavomycin

Flavomycin is an antibiotic, effective against Gram positive bacteria and approved for use as a growth promoter in broilers, cattle and pigs. Occurrence of resistance among *E. faecium* from all three species of animals was high (72% - 93%), while only a single resistant isolate was recovered among *E. faecalis* from pigs and none among staphylococci. Some reports indicate that *E. faecium* is not intrinsically resistant to flavomycin. Therefore, the occurrence may reflect acquired resistance. However, the genetic basis is not known at present.

Glycopeptides

This group includes the antibiotic growth promoter avoparcin, approved for use in broilers, calves and pigs until May 1995 and the therapeutic agents vancomycin and teicoplanin. Vancomycin and teicoplanin are approved for use in humans. Glycopeptide antibiotics are effective only against Gram positive bacteria.

Pathogenic bacteria. Resistance to vancomycin was not observed among staphylococci, however, 4% of coagulase negative staphylococci were moderately resistant to teicoplanin and 1% to avoparcin.

Indicator bacteria. Fifty-nine per cent of *E. faecium* isolates from poultry and 29 % from pigs were resistant to avoparcin, while none of *E. faecalis* isolates from pigs were resistant. The occurrence of resistance to vancomycin was high among isolates of *E. faecium* from poultry and pigs (56% and 24%, respectively), while the occurrence of resistance to teicoplanin among *E. faecium* from broilers was 17% and 7% in isolates from pigs.

Ionophores

This group includes monensin and salinomycin, both of which are approved for use as growth promoters in cattle and pigs, respectively, and as anticoccidials in poultry. They are active against Gram positive bacteria. The occurrence of resistance among isolates of enterococci and staphylococci was low, with 6% resistance among *S. hyicus* as the highest frequency.

Macrolide - lincosamide group

The macrolides include erythromycin which is used for therapy only (in humans and in small animal practice) and tylosin and spiramycin, which are used for therapy as well as for growth promotion. Macrolides are active against most Gram positive bacteria, but in general not against Gram negative. Lincomycin is a lincosamide, which is also mainly active against Gram positive bacteria. Lincosamides exhibit cross-resistance with macrolides and in the context of the present report they are treated as one group.

Pathogenic bacteria. Isolates were in most cases either simultaneously resistant or susceptible to all macrolide antibiotics tested. Only a limited number of the pathogenic bacteria *S. aureus* and coagulase negative staphylococci from cattle were resistant to macrolides (3% or less). In contrast 41% of *S. hyicus* from pigs were resistant to 1 or more antibiotics in this group.

Zoonotic bacteria. Sixty-seven per cent of *C. coli* from pigs were resistant to one or more of the macrolides, including 55% that were resistant to erythromycin. Most *C. jejuni* from broilers and cattle were susceptible to macrolides.

Indicator bacteria. Resistance to macrolides was widespread (from 54% to 91%) among the indicator bacteria *E. faecalis* and *E. faecium* from both pigs and broilers.

In general macrolide resistance was very common among both pathogenic, zoonotic and indicator bacteria from pigs. Macrolide resistance was also

commonly found among bacteria from broilers, but at levels lower than in pigs while the level among isolates from cattle was lower still.

Polymyxins

This group is represented by colistin, which is active against a rather narrow range of Gram negative bacteria only. Resistance to colistin was observed only in an isolate of *A. pleuropneumoniae* and a pathogenic *E. coli* strain, both from pigs.

Quinolones

This group includes enrofloxacin and nalidixic acid. These antibiotics are used for therapy only. While the former is effective against Gram negative as well as some Gram positive bacteria, the latter has a relatively narrow spectrum of activity against Gram negative bacteria. Enrofloxacin has been licensed for use in production animals in Denmark only since 1993.

Pathogens. While resistance to enrofloxacin was almost absent in the bacterial species tested, 6%-10% of *E. coli* from cattle and pigs and of *A. pleuropneumoniae* from pigs were resistant to nalidixic acid.

Zoonotic bacteria. Quinolone resistance occurred at relatively low levels (up to 3% of isolates tested were resistant to enrofloxacin and 15% to nalidixic acid) in zoonotic bacteria from broilers and cattle, however, 23% of *C. coli* isolates from pigs were resistant to enrofloxacin and 27% to nalidixic acid. The reason for this disparity is not known. It may be a result of the differences in predominance of Campylobacter species between domestic animals, or it may be a result of greater enrofloxacin use in pigs than in broilers and cattle.

Indicator bacteria. Resistance to enrofloxacin was widespread among enterococci from all 3 animal species. Among *E. coli* from broilers 15% were resistant to nalidixic acid, compared with 2% of isolates from pigs and none from cattle.

Quinoxalines

This group includes the growth promoting agents carbadox and olaquindox, effective against Gram negative bacteria. Both are approved for use in pigs only. The occurrence of resistance among the strains tested was generally low. However, 14% of salmonella from cattle were resistant to carbadox.

Streptogramins

This group includes virginiamycin, an antibiotic used for growth promotion in broilers, cattle and pigs, and pristinamycin which is used to treat infections in humans. Pristinamycin is not registered for use in Denmark. Virginiamycin is a mixture of two components, M1 and S1. Pristinamycin is also a mixture, consisting of the components pristinamycin IA, IB and IC as well as pristinamycin IIA and IIB, where IIA is identical with virginiamycin M1. Streptogramins are effective mainly against Gram positive bacteria. E. faecaelis is considered to be intrinsically resistant to pristinamycin and to virginiamycin.

The occurrence of resistance to pristinamycin and virginiamycin among the Gram positive pathogenic bacteria was 3% or less. Among the enterococci, 43% and 47% of isolates of *E. faecium* from broilers and pigs, respectively, were resistant to virginiamycin. Resistance to pristinamycin followed the same pattern.

Sulphonamides

Sulphonamides have a wide spectrum of antibacterial activity that includes both Gram positive and Gram negative bacteria, however enterococci are intrinsically resistant to sulphonamides. They are among the most commonly used antimicrobial agents. Sulphonamides are not used for growth promotion.

Pathogens. The occurrence of resistance among all pathogenic bacteria ranged from 20% among *Staphylococcus hyicus* to 93% among *E. coli* from cattle. These bacteria are not intrinsically resistant to sulphonamides, and the occurrence of resistance may therefore be a result of selective pressure by sulphonamides.

Zoonotic bacteria. Resistance to sulphonamides was widespread among Salmonella from all three species of animals (45% to 65%). Twenty-eight per cent of *C. jejuni* from cattle were resistant, compared with 8% of *C. coli* from pigs and none of campylobacters from broilers. The reason for this difference is not known at present.

Indicator bacteria. Among *E. coli* from broilers and pigs 34% and 37%, respectively, were resistant, compared with 5% of cattle isolates.

Tetracycline

Tetracyclines are approved for therapy only and are widely used in food animals. They have a broad spectrum of activity including both Gram positive and Gram negative bacteria.

Pathogens. The occurrence of resistance was high among *E. coli* from cattle and pigs (78% and 57%, respectively), while 14% of *A. pleuropneumoniae* and 34% of *S. hyicus* from pigs were resistant.

Zoonotic bacteria. Among the zoonotic isolates, the occurrence of resistance to tetracycline was 4% or less, except for salmonella in pigs where 22% were resistant.

Indicator bacteria. The occurrence of resistance among *E. coli* isolated from slaughterhouse samples from cattle was lower (6%) than among *E. coli* from diagnostic submissions (78%). In pigs 30% of indicator *E. coli* were resistant, compared with 57% of pathogenic *E. coli*. Resistance among enterococci ranged from 6% to 87%, again highest in pigs. The occurrence of resistance among pig isolates is most likely a result of a widespread use of tetracycline in pig farms.

Trimethoprim

Trimethoprim is most often used in combination with sulphonamides with which it exhibits synergy.

Pathogens. The occurrence of resistance to trimethoprim generally followed that to sulphonamides, although it tended to be lower. An exception to this was *S. hyicus*, where 44% were resistant to trimethoprim, compared with 20% to sulphonamides.

Zoonotic bacteria. Among the zoonotic bacteria Campylobacter are intrinsically resistant to trimethoprim. Salmonella had a low occurrence of resistance (up to 6% in isolates from pigs), while trimethoprim was one of the few antibiotics to which isolates of Y. enterocolitica were often resistant (45%).

Indicator bacteria. As was the case with sulphonamides, a high proportion of enterococcal isolates were resistant to trimethoprim. Resistance was significantly less common among *E. coli* from slaughter samples than among *E. coli* from diagnostic submissions and ranged between 1% and 8%.

Multiple antimicrobial resistance

The occurrence of multiple antimicrobial resistance to therapeutic agents (resistance to 4 or more groups of antimicrobials) is shown in Table 1.6. The zoonotic Campylobacters are regarded as intrinsically resistant to trimethoprim and enterococci to sulphonamides. Therefore, these observations were excluded from the calculations.

The occurrence of resistance among pathogenic bacterial isolates ranged from 64% in *A. pleuropneumoniae* from pigs to 100% in *E. coli* from cattle. The occurrence of multiresistance ranged from 1% of S. aureus to 75% of pathogenic *E. coli*, both from cattle.

In the group of zoonotic bacteria, 16% and 48% of *C. jejuni* isolates from broilers and cattle, respectively, were resistant, however none were multiresistant. In contrast, 88% of *C. coli* from pigs were resistant, while 10% were multiresistant. Among salmonella, the occurrence of resistance ranged from 53% in isolates from cattle to 75% of isolates from broilers. However, multiresistance was only observed among salmonella from pigs (9%). All Yersinia isolates were resistant, mainly to ampicillin, but only 1% were multiresistant.

While 2% or less of indicator *E. coli* from broilers and cattle were multiresistant, this proportion reached 9% in pigs. In cattle and pigs, where sufficient numbers of *E. coli* were available in the indicator and the pathogen groups of isolates, the proportions of resistant and multiresistant isolates were lower among indicator isolates than among pathogens. This difference was statistically highly significant.

DISCUSSION

In 1969, the Swann report recommended that antimicrobial agents that are used for therapy should not also be used for growth promotion. This recommendation has since formed the basis for approval of antimicrobial growth promoters within the EU. However, in spite of the use of tylosin and spiramycin as therapeutic agents these antibiotics are also approved for growth promotion. As part of requirements prior to approval of an antimicrobial growth promoter by the EU the compound must be tested for cross-resistance to therapeutic antimicrobial agents, and also for its ability to select for antimicrobial resistance. Should resistance factors be demonstrated it must be examined whether they confer multiresistance and

whether the resistance is transferable.

Until recent years possible resistance caused by the use of EU approved growth promoters was considered to have no public health implications. However, since 1994 studies in several European countries have demonstrated that use of avoparcin, a compound closely related to vancomycin (an antibiotic used for the treatment of infections with multiresistant Gram positive bacteria such as enterococci and staphylococci in humans) was associated with the occurrence of vancomycin-resistant *E. faecium* in food animals. Following this discovery, the use of avoparcin was banned in Denmark in May 1995, a ban that became EU wide in December 1996. The discovery also precipitated the initiation of the present integrated surveillance programme.

Since the first antimicrobials were used for the treatment of infections some 50 years ago, bacteria have become resistant to every antimicrobial developed.

Antimicrobial resistance in bacteria may be either intrinsic or acquired, however, for a number of bacteria it is uncertain whether resistance is one or the other. For example, virtually all *Y. enterocolitica* are resistant to ampicillin, mediated by beta-lactamase activity. This is an acquired trait, but extremely common so that it may be said to have become typical for *Yersinia enterocolitica*. Bacteria with acquired resistance may be present in animals as a direct result of use of antimicrobial agents or because they have been introduced from an environment where antimicrobials were used. Finally the resistant bacteria may owe their presence to the use of antimicrobials other than the one to which resistance was observed (co-selection).

Antimicrobial resistance is closely associated with antimicrobial consumption. Regrettably, there are no official statistics on the use of therapeutic antimicrobials. However, we have been provided with reliable estimates by industry sources (Table 1.2a). The estimates show that there was a decline from 27,400 kg active compound used during the first half of 1995 (data not shown) to 22,000 kg during the first half of 1996, a decrease of about 20%. This result must be interpreted with caution because the comparison is on a weight basis.

Aminoglycosides, macrolides, penicillins and tetracyclines accounted for 78% of the total quantity used. Most of the antimicrobials were used in pigs. An estimated 64% of aminoglycosides, 89% of macrolides, 31% of penicillins and 85% of tetracyclines were used in pig farms (data not shown). Pigs account for most of the production of food animals (Table 1.2c).

Table 1.2b shows the use of antimicrobial growth promoters in food animals in 1995. These are official figures provided by the Danish Plant Directorate. Figures for 1996 are not yet available. The total quantity used was 93,936 kg active compound. Of these, an estimated 80,784 kg, including 52,275 kg of tylosin, were used in pigs.

An objective of the present surveillance programme is to provide a basis to ensure that continued, cost-effective treatment of infections in animals and in humans will remain possible. This is achieved by adopting a policy on antimicrobial usage in an attempt, where possible, to counteract further problems of antimicrobial resistance. Such policy may be based on the results of the present surveillance programme.

More than a year after the ban on the use of avoparcin a high proportion of isolates of *E. faecium* from broilers and pigs were still resistant to glycopeptides.

Resistance to vancomycin and avoparcin in enterococci is normally determined by genes located on a transposon, a highly mobile genetic element. The same genes may confer resistance to teicoplanin, however the genetic basis for the teicoplanin resistance observed here is not known and will be the subject of further investigations.

Resistance to the growth promoter virginiamycin and the related antibiotic pristinamycin was observed among *E. faecium* from broilers and pigs.

The resistance may be acquired and result from virginiamycin use. However, the genetic basis for this possible cross-resistance is not known at present and needs to be investigated before firm conclusions can be drawn.

The observation that the occurrence of resistance is lower among *E. coli* isolated from animals at slaughter than among pathogen isolates is important. If it can be extrapolated to other bacterial species it shows that surveillance programmes based on monitoring resistance in bacteria isolated from diagnostic submissions will tend to overestimate the occurrence of antimicrobial resistance in the general animal population.

The occurrence of resistance to streptomycin in isolates from pigs of pathogenic *E. coli* , indicator

E. coli, *C. coli*, *S. hyicus* and salmonella ranged from 21% to 69% while in cattle and broilers it was high only among pathogenic *E. coli* from cattle (83%). This most likely reflects selective pressure.

Studies of the population distribution of inhibition zones indicate the breakpoint to streptomycin used for *E. coli*, salmonella and *Y. enterocolitica* may be too high, and that the reported occurrence of resistance is therefore also too high. Further studies may result in the breakpoint being changed.

A similar association between use of a compound and the occurrence of resistance is seen with avilamycin where 69% of *E. faecium* isolates from broiler were resistant, compared with 2% of *E. faecium* from pigs.

Among the indicator bacteria and among salmonella, resistance to tetracycline was most widespread among isolates from pigs.

For macrolides, 59% and 65% of *E. faecium* from broilers were resistant to tylosin and erythromycin, respectively, compared with 90% of isolates from pigs. Erythromycin is not used in food animals and it is most likely that the resistance is a result of the use of spiramycin and tylosin as growth promoters in broilers and in pigs, respectively. The therapeutic use of tylosin in pigs is also likely to have had an effect, which cannot be separated from the effect of its use as a growth promoter.

A high proportion (80%) of *E. coli* isolates as pathogens from cattle was resistant to ampicillin. The estimated consumption of ampicillin in cattle is high, compared with the size of the population (data not shown). Ampicillin is used in cattle mainly to treat mastitis, rather than for the treatment of enteric disease in young animals. The cattle isolates of pathogenic *E. coli* originate from the latter subpopulation.

The results show that the efficacious treatment of infection in food animals does not appear to be immediately at risk, and that use of narrow-spectrum antimicrobials will usually be effective. However, the high occurrence of resistance to tetracycline among some strains of bacteria from pigs indicates that a high selective pressure is exerted by this group of compounds. Co-resistance between tetracycline and other antimicrobial agents is common, at least in some bacteria. Therefore, narrow-spectrum antimicrobials should be used in preference to wide-spectrum substances such as tetracycline.

Resistance to the newer quinolones and to cephalosporins was rare in the animal isolates. However, the experience in other countries shows that resistance to these compounds may quickly become wide-spread with increasing consumption.

Table 1.1 Breakpoints used. Isolates were considered resistant if they exhibited MIC's higher or inhibitions zones less than shown in the table.
Anvendte breakpoints. Isolater betragtes som resistente hvis deres MIC-værdi er højere eller hæmningszone mindre end vist i tabellen.

	Bacterial genera				
Antimicrobial agent 1)	*A. pleuro-pneumoniae*	*C. jejuni C. coli*	*E. coli S. Enterica Y. enterocolitica*	*Enterococci*	*Staphylococci*
Ampicillin (T) 33 ug	17 mm	16 ug/ml	23 mm	18 mm	
Apramycin (T) 40 ug	23 mm	16 ug/ml	23 mm	23 mm	23 mm
Avilamycin (G)	-	-	-	8 ug/ml	8 ug/ml
Avoparcin (G)	-	-	-	8 ug/ml	8 ug/ml
Bacitracin (G+T) 40 units	-	-	-	17 mm	17 mm
Carbadox (G)	64 ug/ml	64 ug/ml	64 ug/ml	-	-
Ceftiofur (T) 30 ug	20 mm	23 mm	23 mm	23 mm	23 mm
Chloramphenicol (T) 60 ug	24 mm	16 ug/ml	24 mm	24 mm	24 mm
Colistin (T) 150 ug	18 mm	32 ug/ml	18 mm	-	-
Enrofloxacin (T) 10 ug	17 mm	2 ug/ml	21 mm	21 mm	21 mm
Erythromycin (T) 78 ug	23 mm	16 ug/ml	-	23 mm	23 mm
Flavomycin (G)	-	-	-	8 ug/ml	8 ug/ml
Gentamicin (T) 40 ug 2)	23 mm	16 ug/ml	23 mm	15 mm	23 mm
Lincomycin (T) 19 ug	-	-	-	23 mm	23 mm
Monensin (G+T)	-	-	-	8 ug/ml	8 ug/ml
Nalidixic acid (T) 130 ug	23 mm	16 ug/ml	23 mm	-	-
Neomycin (T) 120 ug	20 mm	16 ug/ml	20 mm	20 mm	20 mm
Olaquindox (G)	64 ug/ml	64 ug/ml	64 ug/ml	-	-
Penicillin (T) 5 ug 3)	10 mm	-	-	10 mm	24 mm
Pristinamycin (T) 30 ug	-	-	-	23 mm	23 mm
Salinomycin (G+T)	-	-	-	8 ug/ml	8 ug/ml
Spectinomycin (T) 200 ug	20 mm	16 ug/ml	20 mm	20 mm	20 mm
Spiramycin (G+T) 200 ug	23 mm	16 ug/ml	-	23 mm	23 mm
Streptomycin (T) 100 ug 4)	23 mm	32 ug/ml	24 mm	15 mm	24 mm
Sulfonamide (T) 240 ug.	20 mm	256 ug/ml	24 mm	-	24 mm
Teicoplanin (T) 60 ug	-	-	-	14 mm	14 mm
Tetracycline (T) 80 ug	20 mm	4 ug/ml	24 mm	24 mm	24 mm
Trimethoprim (T) 5.2 ug	16 mm	-	16 mm	17 mm	17 mm
Tylosin (G+T) 150 ug	19 mm	64 ug/ml	-	21 mm	21 mm
Vancomycin (T) 5 ug	-	-	-	11 mm	11 mm
Virginiamycin (G) 30 ug	-	-	-	23 mm	23 mm

1) (T) indicates use for therapy, (G) indicates use for growth promotion, (G+T) indicates growth promotion and therapy. Figures indicate diffusible amount of antimicrobial in tablets.
(T) indikerer brug til behandling, (G) indikerer brug til vækstfremme, (G+T) indikerer brug til behandling og vækstfremme. Tal viser diffundérbare mængder antimikrobielt stof i tabletterne.

2) For enterococci: 250 µg
For enterokokker: 250 µg

3) For enterococci: 62.5 µg
For enterokokker: 62,5 µg

4) For enterococci: 500 µg
For enterokokker: 500 µg

Table 1.2a. Estimated consumption of therapeutic antimicrobials in all domestic animals in 1995 and during the first 6 months of 1996 (kg. active compounds)
Anslået forbrug af terapeutiske antibiotika til alle husdyr i 1995 og første halvår af 1996 (kg. aktivt stof) 1)

Therapeutic group *Terapeutisk gruppe*	1995, total *1995 totalt*	1. half, 1996 *1. halvdel af 1996*
Aminoglycosides	7600	3800
Macrolides	9500	4300
Penicillins	8800	4300
Semisynthetic penicillins (e.g. ampicillin, amoxicillin, ceftiofur)	4500	2000
Sulfa-TMP	6300	2300
Other sulphonamides	1800	400
Tetracyclines	9000	4700
Other antimicrobials (e.g. demetridazol, colistin, enrofloxacin)	2100	200
Total	49600	22000

1) Source: N.E. Rønn, Federation of Danish Pig Producers and Slaughterhouses; and E. Jacobsen, Danish Pharmaceutical Association.
Kilde: N.E. Rønn, Danske Slagterier; og E. Jacobsen, Danmarks Apotekerforening

Table 1.2b. Consumption of antimicrobial growth promoters during 1995.
Forbrug af antibiotiske vækstfremmere i 1995.

Antimicrobial group	Growth promotor *Vækstfremmere*	Kg. active compounds 1) *kg. aktivt stof* Total	Pigs *Svin*	Cattle *Kvæg*	Broilers *Slagtekyllinger*
Flavofosfolipol	Flavomycin	48	-	-	48
Glycopeptide	Avoparcin	5.690	2.500	2.090	1.100
Ionophor	Monensin	5.007	-	5.007	(2.463)
Ionophor	Salinomycin	850	850	-	(3.758)
Macrolide	Spiramycin	507	-	-	507
Macrolide	Tylosin	52.275	52.275	-	-
Oligosaccharid	Avilamycin	1.665	265	-	1.400
Polypeptid	Bacitracin 2)	7.910	6.000	-	610
Quinoxalines	Carbadox	1.181	1.181	-	-
Quinoxalines	Olaquindox	16.213	16.213	-	-
Streptogramines	Virginiamycin	2.590	1.500	-	1.090

1) Source: The Danish Plant Directorate. Distribution among animal species are estimates only.
Kilde: Plantedirektoratet. Fordeling på dyrearter er baseret på skøn
2) Approximately 1300 kg. used in mink
Ca. 1300 kg. brugt til mink
() Used as anticoccidials
Brugt som coccidiostatika

Table 1.2c. The approximate number of animals slaughtered in 1995 1).
Det omtrentlige antal dyr der er slagtet i 1995 1).

	Number (1995)	Tonnes carcase weight (1994)
Broilers	113 million	150000
Cattle	700000	190000
Slaughterpigs	19 million	1600000

1) Source: Danmarks Statistik, 1994, 1995.
Kilde: Danmarks Statistik, 1994, 1995.

Table 1.3a. Percent resistance to growth promoting antimicrobial agents among selected pathogenic bacteria
Procent resistens overfor vækstfremmende antibiotika blandt udvalgte patogene bakterier

	Cattle *Kvæg*			Pigs *Svin*		
Growth promoters *Vækstfremmere*	CNS 1) N=n=371 2)	E. coli N=n=80	S. aureus N=n=211	A. pleuro-pneumoniae N=n=66	E. coli N=n=67	S. hyicus N=n=71
Avilamycin	0	-	0	-	-	0
Avoparcin	1	-	0	-	-	0
Bacitracin	0	-	0	-	-	0
Carbadox	-	1	-	0	0	
Flavomycin	0	-	0	-	-	0
Monensin	0	-	0	-	-	6
Olaquindox	-	0	-	0	0	
Salinomycin	0	-	0	-	-	0
Spiramycin	1	-	1	5	-	38
Tylosin	2	-	1	0	-	34
Virginiamycin	1	-	0	-	-	1

1) Coagulase negative staphylococci
Koagulasenegative staphylokokker
2) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n=antal positive prøver

Table 1.3b Percent resistance to therapeutic agents among selected pathogenic bacteria
Procent resistent overfor terapeutiske antibiotika blandt udvalgte patogene bakterier

Therapeutic agents *Terapeutika*	Cattle *Kvæg*			Pigs *Svin*		
	CNS 1) N=n=371 2)	E. coli N=n=80	S. aureus N= n=211	A. pleuro-pneumoniae N=n=66	E. coli N=n=67	S. hyicus N=n=71
Ampicillin	-	80	-	2	16	-
Apramycin	0	3	7	18	3	1
Ceftiofur	2	-	4	-	-	1
Chloramphenicol	1	21	2	2	22	0
Colistin	-	0	-	2	1	-
Enrofloxacin	0	1	0	0	1	0
Erythromycin	3	-	1	0	-	37
Gentamicin	1	13	0	0	1	1
Lincomycin	3	-	1	-	-	41
Nalidixic acid	-	6	-	6	10	-
Neomycin	0	6	0	0	21	1
Penicillin	31	-	29	0	-	59
Pristinamycin	0	-	1	-	-	3
Spectinomycin	0	1	1	0	27	10
Streptomycin	6	83	5	0	69	30
Sulphonamides	33	93	26	53	85	20
Teicoplanin	4	-	0	-	-	0
Tetracycline	5	78	1	14	57	34
Trimethoprim	40	69	7	6	25	44
Vancomycin	0	-	0	-	-	0

1) Coagulase negative staphylococci
Koagulasenegative staphylokokker
2) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n=antal positive prøver

Table 1.4a. Percent resistance to growth promoting antimicrobial agents among selected zoonotic bacteria
Procent resistens overfor vækstfremmende antibiotika blandt udvalgte zoonotiske bakterier

Growth promoters *Vækstfremmere*	Broilers *Slagtekyllinger*			Cattle *Kvæg*		Pigs *Svin*		
	C. coli	C. jejuni	S. enterica	C. jejuni	S. enterica	C. coli	S. enterica	Y. enterocolitica
	N=890 n=13 1) 2)	N=890 n=55 3)	N=598 n=40	N=82 n=29	N=308 n=59	N=320 n=99	N=n=150	N=420 n=73
Carbadox	0	0	0	0	14	0	3	0
Olaquindox	8	0	3	0	0	0	0	0
Spiramycin	38	0	-	3	-	60	-	-
Tylosin	38	0	-	0	-	67	-	-

1) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n=antal positive prøver
2) An additional 28 isolates not tested
Yderligere 28 isolater ikke undersøgt
3) An additional 191 isolates not tested
Yderligere 191 isolater ikke testet

Table 1.4b. Percent resistance to therapeutic agents among selected zoonotic bacteria
Procent resistens overfor terapeutiske antibiotika blandt udvalgte zoonotiske bakterier

Therapeutic agents / *Terapeutika*	Broilers / *Slagtekyllinger*			Cattle / *Kvæg*		Pigs / *Svin*		
	C. coli	C. jejuni	S. enterica	C. jejuni	S. enterica	C. coli	S. enterica	Y. enterocolitica
	N=890 n=11 1) 2)	N=890 n=55 3)	N=598 n=40	N=82 n=29	N=308 n=58	N=320 n=99	N=n=150	N=420 n=73
Ampicillin	8	7	18	3	0	17	9	97
Apramycin	0	0	0	0	2	0	1	14
Chloramphenicol	0	5	0	0	2	12	20	1
Colistin	0	0	0	0	0	0	0	0
Enrofloxacin	0	0	0	3	0	23	0	0
Erythromycin	38	4	-	3	-	55	-	-
Gentamicin	0	0	0	0	0	0	1	1
Nalidixic acid	15	2	0	14	5	27	3	5
Neomycin	0	0	0	0	0	0	2	0
Spectinomycin	0	0	0	0	0	4	3	1
Streptomycin	8	2	13	7	5	44	21	5
Sulphonamides	0	0	65	28	45	8	50	3
Tetracycline	0	4	0	0	3	1	22	0
Trimethoprim	-	-	0	-	0	-	6	45

1) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n= antal positive prøver
2) An additional 28 isolates not tested
Yderligere 28 isolater ikke undersøgt
3) An additional 191 isolates not tested
Yderligere 191 isolater ikke testet

Table 1.5a. Percent resistance to growth promoting antimicrobial agents among selected indicator bacteria
Procent resistens overfor vækstfremmende antibiotika blandt udvalgte indikatorbakterier

Growth promoters *Vækstfremmere*	Broilers *Slagtekyllinger*		Cattle *Kvæg*		Pigs *Svin*		
	E. coli N=870 n=172 1)	E. faecium N=940 n=54	E. coli N=274 n=140	E. faecium N=260 n=13	E. coli N=560 n=327	E. faecalis N=944 n=225 2)	E. faecium N=944 n=58 3)
Avilamycin	-	69	-	0	-	1	2
Avoparcin	-	59	-	0	-	0	29
Bacitracin	-	41	-	8	-	3	31
Carbadox	0	-	1	-	0	-	-
Flavomycin	-	72	-	85	-	0	93
Monensin	-	0	-	0	-	3	2
Olaquindox	0	-	4	-	3	-	-
Salinomycin	-	0	-	0	-	0	2
Spiramycin	-	54	-	8	-	89	88
Tylosin	-	59	-	8	-	91	90
Virginiamycin	-	43	-	8	-	-	47

1) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n= antal positive prøver
2) An additional 70 isolates not tested
Yderligere 70 isolater ikke testet
3) An additional 13 isolates not tested
Yderligere 13 isolater ikke testet

Table 1.5b. Percent resistance to therapeutic agents among selected indicator bacteria
Procent resistens overfor terapeutiske antibiotika blandt udvalgte indikatorbakterier

Therapeutic agents / *Terapeutika*	Broilers / *Slagtekyllinger*		Cattle / *Kvæg*		Pigs / *Svin*		
	E. coli	E. faecium	E. coli	E. faecium	E. coli	E. faecalis	E. faecium
	N=870 n=172 1)	N=940 n=54	N=274 n=140	N=260 n=13	N=560 n=327	N=944 n=225 2)	N=944 n=58 3)
Ampicillin	12	0	1	0	10	0	0
Apramycin	3	93	0	85	10	97	90
Ceftiofur	-	80	-	92	-	53	84
Chloramphenicol	3	11	5	15	14	24	7
Colistin	0	-	0	-	0	-	-
Enrofloxacin	1	50	0	38	0	10	43
Erythromycin	-	65	-	38	-	91	91
Gentamicin	1	0	0	0	0	0	0
Lincomycin	-	91	-	77	-	100	95
Nalidixan	15	-	0	-	2	-	-
Neomycin	0	9	0	15	2	42	34
Penicillin	-	33	-	0	-	1	52
Pristinamycin	-	37	-	15	-	-	53
Spectinomycin	0	2	1	8	8	9	26
Streptomycin	6	0	5	8	55	29	40
Sulphonamides	34	-	5	-	37	-	-
Teicoplanin	-	17	-	0	-	1	7
Tetracycline	9	19	6	8	30	87	69
Trimethoprim	6	76	1	100	8	44	97
Vancomycin	-	56	-	0	-	0	24

1) N=number of samples examined, n=number of samples positive
N=antal undersøgte prøver, n=antal positive prøver
2) An additional 70 isolates not tested
Yderligere 70 isolater ikke testet
3) An additional 13 isolates not tested
Yderligere 13 isolater ikke testet

Table 1.6. Occurrence of antimicrobial resistance and multiresistance to therapeutic agents in bacterial isolats from broilers, cattle and pigs. Multiresistance was defined as resistance to 4 or more groups of antimicrobials.
Forekomst af antibiotikaresistens og multiresistens overfor terapeutiske antibiotika i bakterieisolater fra slagtekyllinger, kvæg og svin. Multiresistens er defineret som resistens overfor 4 eller flere stofgrupper.

	Broilers *Slagtekyllinger*			Cattle *Kvæg*			Pigs *Svin*		
Bacteria	n	%R	%MR	n	%R	%MR	n	%R	%MR
A. pleuropneumoniae	-	-	-	-	-	-	66	64	3
C. coli	13	46	8	-	-	-	99	88	10
C. jejuni	55	16	0	29	48	0	-	-	-
CNS	-	-	-	371	76	2	-	-	-
E. coli (indicator)	172	56	2	140	14	0	327	73	9
E. coli (pathogen)	-	-	-	79	100	75	67	99	30
E. faecalis	-	-	-	-	-	-	225	100	63
E. faecium	54	100	93	13	100	92	58	100	98
S. enterica	40	75	0	58	53	0	150	64	9
S. aureus	-	-	-	211	54	1	-	-	-
S. hyicus	-	-	-	-	-	-	71	92	27
Y. enterocolitica	-	-	-	-	-	-	73	100	1

n= number of isolates
%R= per cent resistant
%MR=per cent multiresistant

Antimicrobial resistance among bacteria isolated from food between July 1st 1996 and November 30th 1996

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CONCLUSIONS

In general it was possible to isolate the indicator bacteria, *Escherichia coli*, *Enterococcus faecium* and *E. faecalis* from raw foods of animal origin.

The results of the survey reflect the resistance status of bacteria that can be isolated from foods that are entering the households.

The antimicrobial susceptibilities of 375 *E. coli*, 210 *E. faecium*, 172 *E. faecalis* and 90 Campylobacter spp. isolates were tested against a range of therapeutic and growth promoting antimicrobial agents. The food samples belonged to six different groups: beef, pork, poultry, dairy products, fish, fruit and vegetables.

Resistance was demonstrated to all therapeutic and most of the growth promoting antimicrobial agents. The only growth promoting compounds to which no resistance was recorded were carbadox and salinomycin.

The bacterial isolates from poultry were most frequently resistant, followed by isolates from pork. Bacteria from the other food groups showed lower levels of resistance and bacteria isolated from vegetables were least frequently resistant.

E. coli isolates from poultry had a remarkably high frequency of multiresistance (28%) compared to isolates from the other food categories (0% to 8%).

Among Campylobacter isolates from poultry a small proportion was resistant to nalidixic acid and enrofloxacin. Approximately half of the Campylobacter isolates from poultry were resistant to ampicillin.

The data from this study represent the first results of the surveillance programme. The accumulation of resistance data in the coming years will enable a firmer determination of the resistance levels and trends in bacteria from foods.

INTRODUCTION

The present report from the National Food Agency of Denmark (NFA) is the first presentation of results from the national surveillance of foods. The report presents the results of antimicrobial susceptibility testing of bacteria isolated from foods collected between July 1st 1996 and November 30th 1996.

The programme is based on the surveillance of known zoonotic and ubiquitous human bacterial pathogens and indicator organisms. The human pathogenic bacteria to be monitored are salmonella, Campylobacter, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus*. *Escherichia coli* and *Enterococcus faecium* and *E. faecalis* are chosen as indicator bacteria. These genera are known to potentially serve as a reservoir of resistance to different antibiotics. Furthermore *E. coli* and enterococci are a natural part of the normal faecal flora of humans and warm blooded animals, thereby also serving as an indicator of faecal contamination of foods.

To fulfil the commitments of the national surveillance programme, the NFA established a collaboration with the Municipal Food and Environmental Laboratories (MFEL) in order to investigate foods from the retail level in all parts of Denmark. The antimicrobial resistance pattern of the strains isolated at the MFEL were determined against therapeutic agents and to antimicrobials used for growth promotion.

The report gives the antimicrobial susceptibility results of 375 *E. coli*, 210 *E. faecium*, 172 *E. faecalis* and 90 Campylobacter isolates. The results for salmonella, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* are not included in the present report because of insufficient size of data.

MATERIALS AND METHODS

Collection of food samples

All food samples were collected nationwide at retail outlets by The National Food Agency and 11 Municipal Food and Environmental Laboratories. The material represented food samples taken as part of the routine-control programmes of the MFEL and samples that were collected for the surveillance programme.

The collection of food samples for analyses of indicator bacteria was planned at the NFA and representatives of six different groups of foods, beef, pork, poultry, dairy products, fish, fruit and vegetables were analyzed. The material consisted of both Danish and imported foods.

Isolation of bacterial strains

The primary isolation of indicator organisms from food samples was performed by the MFEL. Strains were subsequently sent to the NFA in standard transport media for verification of identity and for antimicrobial susceptibility testing. Only one strain of *E. coli* and/or enterococci from each food sample was tested for antimicrobial resistance.

The isolation method for *E. coli* employed 5 grams of food that were incubated at 44°C in for 18-24 hours in 45 ml of MacConkey- or laurylsulfate-broth. The broth was streak-inoculated onto violet red bile agar and incubated for 48 hours at 44°C. Presumptive *E. coli* were subcultured onto blood agar, transferred to standard transport medium and shipped to the NFA.

Five grams of foods were analyzed for the presence of *E. faecium* or *E. faecalis* by adding 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 purified on blood agar and transferred to transport medium and shipped to the NFA.

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

Thermotolerant Campylobacter were isolated according to NMKL No. 119, 2nd ed. 1990.

All strains submitted by the MFEL were identified to species level at the NFA, using standard morphological examinations and biochemical tests.

Antimicrobial susceptibility tests

All isolates were tested for susceptibility to a range of different therapeutic and growth promoting antimicrobial agents at the NFA.

The sensitivity testing was performed by tablet diffusion tests (Rosco Diagnostica) on Müller-Hinton II agar, where sensitivity is monitored as a diameter of the inhibition zone (mm) or by determination of minimum inhibitory concentration (MIC) by the agar dilution method of NCCLS (Müller-Hinton II agar). For enterococci, resistance against gentamicin and streptomycin was carried out using low-level concentrations of the compounds, rather than high-level concentrations as recommended by the manufacturer (Rosco Diagnostica).

The susceptibility of Campylobacter was determined by the MIC method or by a modified tablet diffusion method, where Campylobacter were added to Blood agar Base No. 2 supplemented with 5% horseblood (final concentration of approximately 10^4 CFU/ml) before the agar was allowed to solidify. Tablets were subsequently placed on the agar surface. Plates were incubated at 42°C for 48 hours.

The breakpoints used to discriminate between sensitive and resistant isolates for the different antimicrobial agents are shown in Table 2.1.

RESULTS

The results of the susceptibility testing of the isolated strains are given for each species as the percentage of strains resistant to an antimicrobial agent. The results for the different food groups are presented in Tables 2.2 (*E. coli*), 2.3 (Enterococci) and 2.4 (Campylobacter). In the tables capital "N" represents the number of food samples analyzed for each genus while "n" represents the number of isolates recovered.

The indicator bacteria, *E. coli*, *E. faecium* and *E. faecalis*, were isolated from the six specified food groups: beef, pork, poultry, dairy products, fish, fruit and vegetables, whereas the 90 Campylobacter spp. isolates all originate from poultry samples.

The antimicrobial resistance pattern was established for a total of 375 *E. coli*, 210 *E. faecium*, 172 *E. faecalis* and 90 Campylobacter spp. isolates. The results for the individual antimicrobial agents were as follows. A description of the antimicrobial groups and their antibacterial spectra may be found in section 1 of this report.

Aminoglycosides

E. coli isolates were generally sensitive to the aminoglycosides with the exception of resistance to streptomycin. The highest frequency of streptomycin resistant *E. coli* was found in poultry (38%) and in pork (25%). In the other food categories streptomycin resistance occurred in 7-12% of the *E. coli* isolates.

The Enterococcus isolates exhibited relatively low frequencies of resistance to the aminoglycosides with the exception of apramycin and neomycin. The majority of enterococci from beef, pork and poultry were resistant to apramycin. The lowest percentage of apramycin resistance in enterococci was found in dairy products (50%). Approximately half of the *E. faecalis* isolates exhibited resistance to neomycin.

Avilamycin

Sixteen percent of *E. faecium* and 9% of *E. faecalis* from poultry were resistant to avilamycin. Resistance to avilamycin was not observed in beef, pork or fruit and vegetables, whereas a few resistant isolates were found in dairy products and fish.

Bacitracin

Resistance to bacitracin was mainly observed in enterococci from poultry. Twenty-eight percent of *E. faecium* and 19% of *E. faecalis* from poultry were resistant to bacitracin. Bacteria from the other food categories were generally susceptible to bacitracin.

Beta-lactam antibiotics

In general, all enterococci were sensitive to beta-lactams with only 4 isolates resistant.

Resistance to ampicillin was found in some *E. coli* isolates. Eleven percent of *E. coli* from pork and 17% of *E. coli* from poultry were ampicillin resistant. Approximately half of Campylobacter isolates from poultry were resistant to ampicillin.

The cephalosporins were represented by ceftiofur. The prevalence of ceftiofur resistant enterococci was generally high, and varied between 55% and 90% depending on the food category.

Chloramphenicol

Isolates have not been tested for chloramphenicol resistance in this study, but chloramphenicol will be included in the future surveillance of resistance.

Flavomycin

Flavomycin resistance is frequent among *E. faecium*. The lowest frequency (50%) was recorded in poultry whereas isolates from all other food categories had resistance frequencies close to 100%. In general *E. faecalis* exhibited lower resistance frequencies ranging from 0% in poultry to 28% in fish.

Glycopeptides

The determination of resistance to vancomycin was carried out using tablet with a high concentration of vancomycin. Therefore, only high-level resistance to vancomycin was detected. All Enterococcus isolates from beef, pork, fish and fruit and vegetables were sensitive to glycopeptides, and only one isolate from dairy products was resistant to avoparcin. In poultry glycopeptide resistance was found among *E. faecium*, while all *E. faecalis* were sensitive. Seventeen percent of *E. faecium* isolates from poultry exhibited avoparcin resistance and 18% and 15% of the isolates were resistant to vancomycin and teicoplanin, respectively.

Ionophores

All isolates tested were sensitive to salinomycin. Resistance to monensin was recorded for enterococci from all food categories with frequencies varying from 20% in poultry to 82% in vegetables.

Macrolide/Lincosamide group

Low frequencies of tylosin resistance were recorded in enterococci from beef, pork and dairy products. In poultry tylosin resistant enterococci were more frequent, 48% of *E. faecium* and 31% of *E. faecalis* from poultry exhibited resistance to tylosin. One Enterococcus isolate from fish was resistant to tylosin while none of the isolates from vegetables were resistant.

The same pattern was observed for spiramycin. In poultry 48% of *E. faecium* and 30% of *E. faecalis* exhibited resistance to spiramycin. In beef, pork and dairy products resistance frequencies were

between 0 and 13%. None of the isolates from fish and vegetables were resistant.

Campylobacter from poultry were tested against erythromycin and all isolates were sensitive.

Lincomycin resistance was recorded among enterococci in all food categories. Almost all *E. faecalis* isolates were resistant to lincomycin, with percentages ranging from 91% to 100%, and approximately half of the *E. faecium* isolates were lincomycin resistant with percentages ranging from 43% to 82%.

Polymyxins

All *E. coli* isolates were sensitive to colistin, with the exception of one isolate from beef and one isolate from poultry.

Quinolones

Resistance to nalidixic acid was present in 23% of *E. coli* isolates from poultry, in all other foods the occurrence of resistance was zero or close to zero. Among Campylobacters from poultry a small proportion (6% and 9% of *C. coli* and *C. jejuni* respectively) was resistant to nalidixic acid.

Resistance to enrofloxacin was frequent among the enterococci. In beef, dairy products and vegetables half of the *E. faecium* isolates exhibited resistance to enrofloxacin. In poultry 28% of *E. faecium* isolates were resistant to enrofloxacin. For *E. faecalis* the frequencies of resistance varied between 8% and 33%.

All *E. coli*, with the exception of one isolate from poultry, were sensitive to enrofloxacin.

Among Campylobacters from poultry a small proportion (11% of *C. jejuni* and 6% of *C. coli*) was resistant to enrofloxacin.

Quinoxalines

Resistance to carbadox was not found in any isolates. Olaquindox resistance was found in 7%-14% of *E. coli* isolates from all food categories with the exception of vegetables.

Streptogramins

A general pattern for all food categories is the moderate resistance, around 10%, in *E. faecium*. However, in poultry 54% and 42% of *E. faecium* isolates exhibited resistance to virginiamycin and pristinamycin, respectively. *E. faecalis* exhibits natural resistance to streptogramins.

Sulphonamides

Among *E. coli* isolates from beef, pork and dairy products the sulphonamide resistance was about 12%, while in poultry 59% of *E. coli* isolates were resistant. Twenty-two percent of *E. coli* isolates from fish and none of the isolates from vegetables were sulphonamide resistant.

Tetracyclines

Tetracycline resistance was found in all food categories, usually in the range of 10% with the following exceptions: In pork 29% and in poultry 43% of *E. coli* isolates were tetracycline resistant. Also the enterococci from poultry exhibited high frequencies of tetracycline resistance. The highest percentage of tetracycline resistance, 59%, was recorded among *E. faecalis* from poultry.

Trimethoprim

All isolates from dairy products, fish and vegetables were sensitive to trimethoprim. Only a few isolates from beef were resistant. In pork and poultry, 11% and 25% of *E. coli* isolates were resistant, respectively.

Multiresistance

Multiresistance was defined as resistance to four or more groups of therapeutic antibiotics. The percentage of multiresistance (%MR) was calculated for each food category. The results are presented in Table 2.5.

In poultry 28% of *E. coli* isolates were multiresistant. Lower frequencies of multiresistance were recorded in pork (8%), dairy products (4%), fish (4%), and beef (2%). In vegetables no multiresistant *E. coli* isolates were found.

For enterococci, the occurrence of multiresistance was not calculated.

DISCUSSION

This report presents the antimicrobial resistance pattern of 375 *E. coli*, 210 *E. faecium*, 172 *E. faecalis* and 90 Campylobacter spp. strains isolated from six different food categories: beef, pork, poultry, dairy products, fish, fruit and vegetables.

As evident from the tables both *E. coli* and enterococci can often be isolated from raw foods of animal origin. The survival of *E. coli* outside its natural environment is poor and its presence is an indication of faecal contamination. The preva-

lences of *E. faecium* and *E. faecalis* in processed products and vegetables are generally higher than the prevalence of *E. coli*, indicating that the Gram positive enterococci are better suited to persist under the environmental conditions provided by foodstuffs.

The microbiology of a food sample is always a result of all the regimes that the foodstuffs have encountered under their way to the private households. Because of the extensive processing, handling and distribution of foods, tracing the origin of a bacterial strain isolated from a retail sample is difficult.

One of the main reasons is the possibility of cross contamination and growth during wholesale and retail handling. In the present survey the majority of the analyzed samples are raw untreated foods such as minced meat (beef and pork), fish and vegetables. Complex foods have in general been avoided because it is impossible to determine from which part of the food the isolate originated.

Both *E. coli* (N=78 and n=23) and enterococci (N=96 and n=46) were isolated from fish. However, *E. coli* and enterococci are not a normal part of the microbial flora of fish. It is, therefore, likely that the majority of the isolated faecal bacteria originate from the environment of the production facilities. This includes the possibility of direct contamination of the fish from the foodhandlers. Another possibility is that the fish were caught in faecally contaminated fresh- or coastal waters.

In general, the meat consumed in Denmark is of Danish origin. An important exception from this is poultry. About one third of the consumed poultry is imported, primarily from other European countries. This is also reflected in the poultry samples that have been included in this study. The poultry samples consisted primarily of broilers, but some samples were turkey, duck or other types of poultry.

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 Danish households.

The interpretation of disk inhibition zones and minimal inhibitory concentrations for the indicator bacteria have been guided by approved standards given by NCCLS. Because of the importance of therapeutic use of antibiotics in humans and animals the designations sensitive (or susceptible) and resistant usually reflect a therapeutically efficient concentration of the antibiotic.

For campylobacters there are no recognized standards for the interpretation of resistance. It should be noticed that the resistant Campylobacter isolates all belonged to sub-populations that exhibited small or no inhibitions zones, while the susceptible isolates exhibited large zones of inhibition.

Currently there are no internationally recognized procedures or breakpoints for the determination of resistance to most antibiotic growth promoters. The breakpoints used therefore have to be taken into account if the data for resistance to growth promoters are compared with other studies.

In the present study resistance was demonstrated to all therapeutic and most of the growth promoting antimicrobial agents. The only growth promoting compounds to which resistance was not recorded were carbadox and salinomycin.

In general the highest frequencies of resistance among indicator bacteria were found in poultry, pork and beef. Isolates from poultry were among the most often resistant. The isolates from fish, dairy products and vegetables showed lower levels of resistance. This picture is also reflected in the frequencies of multiresistance in *E. coli*.

In general there is a good correspondence between the observed resistance patterns and known cross-resistance to different compounds. Examples of this are the streptogramins, pristinamycin and virginiamycin, and the glycopeptides, avoparcin and vancomycin.

Cross-resistance between avoparcin and vancomycin is the reason why similar resistance frequencies were found for avoparcin and vancomycin in *E. faecium* isolates from poultry. A single avoparcin resistant *E. faecium* was isolated from dairy products, and this isolate was sensitive to vancomycin. A probable explanation for this is that the current study has used tablets containing 70 µg vancomycin. With this concentration of vancomycin it is possible to detect high-level vancomycin resistance only.

Future studies will include the examination of resistance mechanisms.

Campylobacter isolated from poultry had a high frequency of resistance to ampicillin, particularly when compared with the findings of DVL from broiler faecal samples. However, direct comparison is not possible at present due to the use of different methods, a problem which will be solved when planned calibrations have been carried out.

There was a good correspondence between the susceptibility results of NFA and DVL for *E. coli* isolated from beef and from cattle at slaughter. The number of *E. faecium* samples from cattle is low and comparisons to beef samples is therefore difficult.

The isolates from slaughter pigs exhibited more resistance than the isolates from pork. For *E. coli* from pork all frequencies of resistance, except streptomycin and sulphonamide, were higher among isolates from slaughter pigs. For *E. faecium* it is remarkable that the pig isolates showed resistance to avoparcin, while none of the pork isolates from foods were resistant to avoparcin. The frequencies of resistance to spiramycin, tylosin, tetracycline, and trimethoprim were also higher in slaughter pig isolates than in pork samples. It is remarkable that this study found a higher frequency of resistance to tylosin among *E. coli* form broilers than among isolates from pork.

In *E. coli* from poultry there was correspondence between the findings of DVL and the findings of NFA, with the exception, that the isolates from the retail level had higher levels of resistance to streptomycin and tetracycline.

The resistance frequencies in *E. faecium* isolated from broilers at slaughter were generally higher than the resistance levels in isolates from the retail level. An exception to this was the resistance to tetracycline among *E. faecium*, where 41% of the isolates from retail poultry exhibited resistance compared to 19% in isolates from broilers at slaughter.

The data in this report represent the first results of a surveillance programme that will be continued in the coming years. The accumulation of further Danish results in addition to results of similar surveys in other countries will make an important contribution to an understanding of the public health implications of occurrence of antimicrobial resistance among food borne bacteria.

Table 2.1. Breakpoint values for determination of resistance. Isolates were considered resistant if they exhibited MIC-values higher than or inhibitions zones less than the breakpoint values.
Breakpoint-værdier til bestemmelse af resistens. Isolater betragtes som resistente ved MIC-værdier højere end eller hæmningszone mindre end den angivne værdi.

Antimicrobial agent	E. coli	Campylobacters	Enterococci
Ampicillin	23 mm	23 mm	18 mm
Apramycin	23 mm	23 mm	23 mm
Avilamycin	-	-	8 ug/ml
Avoparcin	-	-	8 ug/ml
Bacitracin	-	-	17 mm
Carbadox	64 ug/ml	64 ug/ml	-
Ceftiofur	-	-	23 mm
Chloramphenicol	-	-	-
Colistin	18 mm	-	-
Enrofloxacin	21 mm	21 mm	21 mm
Erythromycin	-	23 mm	-
Flavomycin	-	-	8 ug/ml
Gentamicin	23 mm	23 mm	15 mm 1)
Lincomycin	-	-	23 mm
Monensin	-	-	8 ug/ml
Nalidixic acid	23 mm	23 mm	-
Neomycin	20 mm	20 mm	20 mm
Olaquindox	64 ug/ml	64 ug/ml	-
Penicillin	-	-	10 mm
Pristinamycin	-	-	23 mm
Salinomycin	-	-	8 ug/ml
Spectinomycin	20 mm	20 mm	20 mm
Spiramycin	-	23 mm	23 mm
Streptomycin	24 mm	24 mm	14 mm 1)
Sulphonamide	24 mm	-	-
Teicoplanin	-	-	14 mm
Tetracycline	24 mm	24 mm	24 mm
Trimethoprim	16 mm	-	17 mm
Tylosin	-	21 mm	21 mm
Vancomycin	-	-	11 mm 1)
Virginiamycin	-	-	25 mm

1) For susceptibility testing of enterococci the amount of antibiotics in these tablets was different from what was used at DVL. Tablets with the following content were used: Gentamicin 40 µg, Streptomycin 100 µg, Vancomycin 70 µg.
Ved resistenstest af enterokokker var mængden af antibiotika i tabletterne forskellig fra mængden brugt ved SVS. Tabletter med følgende indhold blev brugt: gentamycin 40 µg, streptomycin 100 µg, vancomycin 70 µg.

Table 2.2 Percent resistant strains of *E. coli* in beef, pork, poultry, dairy products, fish, and vegetables and fruit
Procent resistente stammer af E. coli i oksekød, svinekød, fjerkræ, mælkeprodukter, fisk og frugt og grøntsager

Antimicrobial agent	Beef/oksekød	Pork/svinekød	Poultry/ Fjerkræ 1)	Dairy products/ mejeriprodukter	Fish/fisk	Vegetables and fruit/ grønsager og frugt
E. coli	N=122 n=101	N=95 n=65	N=174 n=145	N=81 n=26	N=78 n=23	N=115 n=15
Ampicillin	5	11	17	4	4	0
Apramycin	5	0	8	0	0	0
Carbadox	0	0	0	0	0	0
Colistin	1	0	1	0	0	0
Enrofloxacin	0	0	1	0	0	0
Gentamicin	0	3	4	4	0	0
Nalidixic acid	1	3	23	0	0	0
Neomycin	0	0	1	0	0	0
Olaquindox	14	12	7	8	13	0
Spectinomycin	1	3	1	0	0	0
Streptomycin	10	25	38	12	9	7
Sulphonamide	12	11	59	12	22	0
Tetracycline	9	29	43	4	9	7
Trimethoprim	2	11	25	0	0	0

1) Among the 145 *E. coli* isolates from poultry the majority, 113 isolates originated from broilers, 18 isolates from turkey, 5 isolates from duck, and the last 9 isolates belonged to different types of other poultry.
Blandt de 145 E. coli-isolater fra fjerkræ stammede 113 isolater fra slagtekyllinger, 18 isolater kom fra kalkun, 5 isolater fra and og de resterende 9 kom fra andre typer fjerkræ.

Table 2.3 Percent resistant strains of *E. faecium* and *E. faecalis* in beef, pork, poultry, dairy products, fish, and vegetables and fruit
Procent resistente stammer af E. faecium og E. faecalis i oksekød, svinekød, fjerkræ, mælkeprodukter, fisk og frugt og grøntsager

	Beef/oksekød		Pork/svinekød		Poultry/Fjerkræ 1)		Dairy products/ mejeriprodukter		Fish/fisk		Vegetables and fruit/ grøntsager og frugt	
	Enterococcus N=92		Enterococcus N=83		Enterococcus N=176		Enterococcus N=93		Enterococcus N=96		Enterococcus N=104	
Antimicrobial agent	E. faecium n=40	E. faecalis n=21	E. faecium n=23	E. faecalis n=38	E. faecium n=71	E. faecalis n=54	E. faecium n=28	E. faecalis n=25	E. faecium n=28	E. faecalis n=18	E. faecium n=20	E. faecalis n=16
Ampicillin	0	0	0	0	0	0	0	0	0	6	0	0
Apramycin	63	90	65	97	86	94	50	96	54	83	60	94
Avilamycin	0	0	0	0	16	9	4	0	0	7	0	0
Avoparcin	0	0	0	0	17	0	4	0	0	0	0	0
Bacitracin	0	5	0	3	28	19	4	0	4	0	0	0
Ceftiofur	80	90	74	55	55	69	57	72	68	83	75	56
Enrofloxacin	53	14	39	8	28	19	54	8	36	33	55	13
Flavomycin	95	19	87	6	50	0	96	12	93	28	100	9
Gentamicin	0	0	0	8	0	0	4	0	0	0	0	0
Lincomycin	45	95	78	91	82	98	43	100	54	94	56	91
Monensin	78	38	57	39	23	20	59	44	57	56	67	82
Neomycin	3	48	4	34	4	28	4	68	7	67	0	38
Penicillin	0	0	0	0	1	0	4	0	0	0	0	6
Pristinamycin	8	-	13	-	42	-	14	-	7	-	5	-
Salinomycin	0	0	0	0	0	0	0	0	0	0	0	0
Spectinomycin	3	10	9	15	3	2	0	0	11	6	0	0
Spiramycin	0	5	9	13	48	30	4	8	0	0	0	0
Streptomycin	0	5	4	8	8	15	0	16	0	0	0	13
Teicoplanin	0	0	0	0	15	0	0	0	0	0	0	0
Tetracycline	10	14	9	13	41	59	11	20	4	17	10	19
Trimethoprim	0	0	0	3	4	6	0	0	0	0	0	0
Tylosin	3	5	4	8	48	31	7	8	0	6	0	0
Vancomycin	0	0	0	0	18	0	0	0	0	0	0	0
Virginiamycin	10	-	22	-	54	-	7	-	7	-	10	-

1) Only a few of the enterococcus isolates belonged to poultry other than broilers.
Kun få enterkok-isolater var fra fjerkræ andet end slagtekyllinger.

Tabel 2.4. Percent of resistant strains of *Campylobacter* in poultry
Procent resistente stammer af Campylobacter i fjerkræ

	C. jejuni n=74	C. coli n=16
Ampicillin	47	63
Apramycin	0	0
Carbadox	0	0
Enrofloxacin	11	6
Erythromycin	0	0
Gentamicin	0	0
Nalidixic acid	9	6
Neomycin	1	0
Olaquindox	0	0
Spectinomycin	0	0
Spiramycin	0	0
Streptomycin	11	6
Tetracycline	19	6
Tylosin	0	0

Table 2.5: Occurence of resistance (R) to therapeutic antibiotics in *E. coli* from food. Multiresistance (MR) is defined as resistance to 4 or more groups of antibiotics.
Forekomsten af resistens (R) overfor terapeutiske antibiotika hos E. coli fra levnedsmidler. Multiresistens (MR) defineres som resistens overfor 4 eller flere grupper af antibiotika.

	n	%R	%MR
Beef/Oksekød	101	20	2
Pork/Svinekød	65	40	8
Poultry/Fjerkræ	145	77	28
Dairy products/ Mælkeprodukter	26	19	4
Fish/Fisk	23	35	4
Vegetable Fruit/ Grøntsager frugt	15	20	0

Antimicrobial resistance among human bacterial isolates and the use of antibiotics in Denmark in 1995

We greatly appreciate the assistance of the following: Pharmacist Kirsten Schaefer, Department of Health, County of Roskilde, Pharmacist Susanne Foss, The pharmacy at Roskilde Amtssygehus, MD Jens K. Moeller, Department for Clinical Microbiology, Aarhus Kommunehospital and Sociologist Karin Hovgaard, Danish Medicines Agency.

The section author may be contacted for a list of references.

CONCLUSIONS

The antibiotic consumption in Denmark is stable and low (13.8 defined daily doses/1000 inhabitants/day in 1995) compared to the consumption in other countries. Denmark has the lowest consumption per capita among the Nordic countries.

Generally the prevalence of resistance in the most common human pathogenic bacteria is low and does not give cause for concern. The level of resistance is low in Denmark compared to other countries.

In light of the rapidly increasing occurrence of antimicrobial resistance in most of the world it is of utmost importance that we continue and intensify the co-ordinated monitoring of antibiotic resistance. This will make it possible to take measures against incipient resistance problems.

Seen in international perspective it is of great importance that the Danish results are published in international journals especially because of the unique co-operation between the three institutes. International research and monitoring of antibiotic use and of the development of resistance must be strengthened.

INTRODUCTION

Statens Serum Institut (SSI) has for many years had a central position in the supervision of the epidemiology of human infectious diseases in Denmark. This supervision has included monitoring of antibiotic resistance in those bacteria which for various purposes have been sent to SSI. In 1995 increased funding was provided to strengthen the activity in this area. This lead to an intensified collaboration between the Danish Veterinary Laboratory (DVL), the National Food Agency (NFA) and Statens Serum Institut (SSI). The task for SSI in this connection is to collect data on antibiotic susceptibility in human bacterial pathogens from all over the country. In addition SSI in co-operation with the Danish Medicines Agency should acquire valid data on the antibiotic consumption in humans in Denmark.

MATERIALS AND METHODS

Bacterial isolates

Table 3.1 gives an overview of the bacterial species which are or will be included in the surveillance programme. With data on the antibiotic susceptibility in these bacteria it is possible to provide a general view of the prevalence of antibiotic resistance in the most important pathogenic bacteria in Denmark. The bacteria can be divided in three main groups: 1. clinical isolates; 2. bacteria from persons in the community and 3. indicator bacteria.

Clinical isolates. The major part of the material concentrates on clinical isolates (i.e. bacteria isolated from patients with infections). *Escherichia coli* is the most common cause of infection in humans, e.g. urinary tract infection, wound infection and septicaemia. *E. coli* belongs to the Enterobacteriaceae which are naturally occurring in the human gut. Other bacteria from this family are *Enterobacter cloacae* and *Klebsiella pneumoniae* which have been shown to rapidly develop antibiotic resistance in hospital settings.

Campylobacter and salmonella species typically cause zoonotic infections, i.e. infections which are transmitted from animals to humans. *Pseudomonas aeruginosa* mostly cause infections in hospitals where they rapidly develop resistance against many antibiotics. *Haemophilus influenzae* have shown increased tendency to develop resistance to penicillin during the last 10-20 years and is a common cause of respiratory tract infection. *Helicobacter pylori* is associated with ulcers in the upper gastrointestinal tract, but the prevalence of antibiotic resistance in these bacteria has so far not been evaluated in Denmark. Both *Staphylococcus aureus* and coagulase-negative staphylococci are typically isolated from skin and in blood cultures. Both are significant causes of nosocomial

infections. *Streptococcus pneumoniae* is the most important cause of respiratory tract infection and therefore important to monitor; penicillin-resistance in these bacteria has been rapidly increasing outside Denmark. Enterococci isolated from blood are also included in this group.

Community samples. Another important part of the isolates will consist of bacteria from persons in the community. It is intended to include 850 persons in the investigation. These persons will represent different groups selected on account of their contact with antibiotics, e.g. patients admitted to hospital for treatment of non-infectious diseases, or people either in contact with antibiotics during their work such as nurses or people assumed to be in contact with resistant bacteria during their work. The bacterial species investigated will be coagulase negative staphylococci from skin and *Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli* from rectal swabs. All these bacteria are normally present on the skin or in the gut of most human beings. Also included is *Staphylococcus aureus* from nasal swabs and salmonella species in rectal swabs from abattoir workers. The community material is marked with a "N" in Table 3.1. These results will be presented in a future report.

Indicator bacteria. The third group of bacteria investigated are indicator bacteria. This term designates bacteria which easily become resistant against antibiotics and which are present in or on most humans. Monitoring these bacteria will provide an early indicator of incipient resistance against antibiotics. The three co-operating institutes have chosen the following bacteria in this group: Coagulase negative staphylococci, *Enterococcus faecalis*, *Enterococcus faecium* and *Escherichia coli*.

For comparison among bacterial species it is important to understand, as mentioned in the different tables, that sometimes the bacterial species represent prospectively collected isolates, and at other times clinical isolates collected during a given time span. It is also clear, therefore, that results are not always directly comparable. Unless stated otherwise, the bacterial isolates in this report were collected in 1995.

Table 3.1 shows the number of isolates of each bacterial species that will be included. Furthermore, the origin of the different species are provided. SPF denotes cerebrospinal-fluid. In the column "Strains", "N" denotes that the isolate is part of the normal material. For some of the strains the number of isolates collected during a given time span is shown. The column: "Antibiotic" illustrates which antibiotics the different bacteria are tested against. "Routine" means the antibiotic susceptibility tests which are normally used. "Å+R" denotes that only data from susceptibility testing performed in both Aarhus and Roskilde counties are reported. The rows in Table 3.1 marked in grey show the bacterial species for which data are provided in this report.

Determination of antibiotic resistance

The material in Table 3.1 will be composed of antibiotic resistance data from two key counties (Roskilde and Aarhus counties) as well as data from the whole country. The latter are marked with "X" in the column: "Denmark" in Table 3.1.

It should be noted that the two counties use two different methods for antibiotic susceptibility testing. Roskilde county which is served by the Department of Clinical Microbiology, SSI, use a tablet diffusion method (Rosco), while Aarhus county use a disc pre-diffusion method. All laboratories on the Statens Serum Institut use the tablet diffusion method (Rosco Diagnostica® on SSI resistance agar cat. nr. 784). The breakpoints provided by Rosco have been used.

This means that data are not directly comparable in all cases. Where this is not the case it will be mentioned in the text. Generally the results of susceptibility testing will be reported as resistant or susceptible. Later a sample of bacterial species will be selected for determination of Minimal Inhibitory Concentration (MIC) for the purpose of calibrating procedures between laboratories and for quality assurance.

The two counties have been chosen because they represent two types of Danish counties, Roskilde county as a typical Danish county with non-university hospitals and Aarhus county representing counties with university hospital status. Furthermore, both laboratories use electronic registration of patients samples.

Antibiotic consumption - definitions

ATC-Groups. In this section of the report the registration of antibiotics will follow the international code of ATC-groups (ATC = Anatomical Therapeutic Chemical Classification System). In this code-system all generically similar pharmaceutics will be given the same registration number which also places the pharmaceutics in a therapeutic

group. As an example: Gentamicin has the number J01G B03. "J" shows that it is a pharmaceutical treatment of infectious diseases. "01G", that it is an aminoglycoside. "B03" that it is gentamicin. In the tables the antibiotics are ranked in alphabetical order after ATC-codes. If the antibiotic has not been given an ATC-code it will be placed at the end of the table in alphabetical order, e.g. veterinary therapeutics and growth promoters.

DDD (Defined Daily Dosis) is a technical unit developed by the World Health Organisation (WHO). It represents an assumed average daily dose for a grown-up person receiving the medicine on its main indication. The use of this unit makes it possible to compare consumption of pharmaceutics in different regions. It is important to understand that the figures do not illustrate how many patients have been treated or how many treatments which have been given.

DDD per 100 bed days. The degree of specialisation of the hospitals has great importance, since usually a higher degree of specialization means increased consumption of antibiotics. It is therefore generally assumed that more specialized hospitals such as university hospitals will have a higher antibiotic consumption than other hospitals. For comparison of antibiotic consumption in hospitals it is therefore important to use a common denominator; for this purpose the consumption is given in DDD per 100 bed days. A bed day is defined as each day a bed is occupied by a patient.

DDD per 1000 inhabitants per day. The consumption of antibiotics in the primary health care sector will be calculated as the number of DDD per 1000 inhabitants per day. This should enable comparison between different geographical regions. Table 3.8 gives all the data within the ATC-group J01.

Antibiotic consumption - source of data

Previously the antibiotic consumption in Denmark was collated and published by the pharmaceutical industry in annual publications. However, in the late 80'ies the amount of parallel imported pharmaceuticals which were not included in these publications increased to such a level that the data provided became invalid. The last annual report from the pharmaceutical industry was published in 1991.

Since 1.1.1994 the Danish Medicines Agency has recorded the Danish consumption of pharmaceuticals. These data are remitted monthly to the Agency from all Danish pharmacies. Data concerning antibiotic consumptions in Roskilde county, 1992-95, have been provided by the local authorities. In the collection of data from Roskilde county other codes than the J01 ACT-code have been included, such as A07AA09 (oral antibiotics for treatment of diarrhoea, e.g. vancomycin), G01AF01 (metronidazol suppository) and P01AB01 (metronidazol for amoebic disease).

In the data from the Danish Medicines Agency only pharmaceuticals from ATC group J01 are included.

RESULTS AND DISCUSSION

In the following the results in the different tables will be commented upon by summarising the most interesting data. Differences in the results which are caused by different methods of determining resistance levels will be mentioned.

Antibiotic resistance

Staphylococcus aureus

Figure 3.1 shows the change over time of antibiotic resistance against a number of different antibiotics in *Staphylococcus aureus* isolated from blood cultures in Denmark 1960 to 1995. All *S. aureus* isolated from blood cultures since 1960 have been remitted to the Staphylococcus Laboratory, SSI, for phage typing. All strains, now totalling >22.000, have been kept in the freeze dried or frozen condition.

As illustrated in Figure 3.1 penicillin resistance has increased from 69% in 1960 to 86% in 1995 and has been stable since the late 1970's. In the beginning of the period penicillin resistance was much more prevalent among staphylococci isolated in hospitals than among community-acquired strains. Today the antibiotic resistance pattern is almost identical in strains inside and outside hospitals (data not shown). As illustrated in Figure 3.1, during the end of 1960's and the beginning of 1970's Denmark suffered from an epidemic of multiresistant *S. aureus* including methicillin resistant (MRSA) strains. The reason for the disappearance of the multiresistant strains is unknown, but it was correlated with a marked reduction in the consumption of broad spectrum antibiotics such as tetracycline and streptomycin.

At the same time the clinical microbiology speciality was developed together with an increased awareness of the importance of hospital hygiene. Furthermore, during these years there was an intensive campaign to teach Danish physicians the principles of rational antibiotic treatment.

At present Denmark has a very low prevalence of MRSA i.e. <0,5%, (Table 3.2). This is also be reflected in the low consumption of vancomycin in Denmark. This is an antibiotic which is used to treat infections with multi-resistant gram-positive bacteria such as MRSA. From 1986 to 1995 a total of 249 methicillin-resistant *S. aureus* have been isolated in Denmark. In only 9 of these cases person-to-person spread was identified, including two epidemics involving 7 and 14 secondary cases. More than 50% of all MRSA cases were imported from abroad (data not shown).

Escherichia coli

Table 3.3 shows the prevalence of antibiotic resistance in *E. coli* isolated during 1995 in the two key counties. Generally there were no major differences in the distribution of antibiotic resistance in the two counties. The difference in tetracycline resistance was not statistically significant.

In a Danish study in 1965 of 400 *E. coli* isolated from blood the frequency of resistance against ampicillin was 46%. In two later Danish investigations from the 1970'ies the frequency of ampicillin resistance was 15 and 16%, respectively. Compared to data from Southern Europe these figures are low; in Spain ampicillin resistance in *E. coli* has been reported at around 50-60% in 1995 and for fluoroquinolones at around 10%. In contrast, the frequencies of resistance to fluoroquinolones and gentamicin in the present material is very low (about 1%).

Enterococci

Table 3.4.1 illustrates the frequency of resistance against a number of antibiotics among enterococci isolated from blood cultures in Roskilde county and Aarhus county. The table also shows the results of a study of enterococci ("Special") submitted to the Streptococcus laboratory, SSI, for further identification in the period from January 1995 to November 1996, inclusive. The latter material can, therefore, not be compared to the two other sets of results in the table.

It should be noted that the degree of antibiotic resistance is very different between different species of enterococci. Table 3.4.2 shows the distribution of different enterococcus species in the different materials. Table 3.4.3 reports the antibiotic resistance for the two common species, *E. faecalis* and *E. faecium*, and illustrates the differences in antibiotic resistance between these two species.

Table 3.4.4 provides data for the number of enterococci isolated from blood cultures in Aarhus county during the last 3 years. It shows a decrease in the number of isolates from blood cultures from this county. This may result from a reduction in the use of broad spectrum antibiotics, as it is known that e.g. overuse of cephalosporins is a predisposing factor for nosocomial infections with enterococci.

Differences in penicillin resistance between counties are probably due to different methods of antibiotic susceptibility testing and reporting from the two laboratories.

Coagulase negative staphylococci

Table 3.5 reports the antibiotic resistance against important antibiotics in coagulase negative staphylococci isolated from blood cultures in Roskilde and Aarhus counties. The major part of these isolates are not associated with infection but can be considered contamination either from the skin of the patients or during handling of the blood cultures. Several investigations have shown, however, that coagulase negative staphylococci are still important indicators of antibiotic resistance due to their occurrence on skin of patients.

The prevalence of antibiotic resistance is much higher in coagulase negative staphylococci than in *Staphylococcus aureus*. Generally, there was little difference between the data from the two counties except for resistance against methicillin, which was significantly higher in Aarhus county than in Roskilde county (48% vs. 14%, $p<0.0005$). This difference could reflect differences in distributions of antibiotic consumption in university hospitals versus non university hospitals. It must be born in mind, however, that determination of methicillin resistance in coagulase negative staphylococci with disc or tablet diffusion is difficult.

Salmonella and Yersinia

Data on antibiotic resistance on salmonella and yersinia are provided in table 3.6 from all 1995 and most of 1996. The material is selected from strains

submitted to SSI either in faecal samples or as strains submitted for sero-typing. As expected the prevalence of antibiotic resistance was very low in *Salmonella* Enteriditis. The occurrence of antibiotic resistance was higher in *Salmonella* Typhimurium, but the occurrence in 1996 was unchanged from 1993, when it was last investigated in detail. In the 1993-study it was shown that most of the multiresistant S. Typhimurium (i.e. resistance to more than 3 antibiotics) were associated with travel in southern European or third world countries. Also provided in this table are data from antibiotic susceptibility testing against antibiotics used only in veterinary medicine or as growth promoters.

Antibiotic consumption

Roskilde county 1992 - 1995.

As shown in table 3.7 the antibiotic consumption in the primary health care sector in Roskilde county constituted 94.5% of the total consumption of the county. This part has increased from 92.6% in 1992. The primary health care consumption in all of Denmark constituted 90.6%.

The pattern of antimicrobial use is very different between the primary health care sector and hospital sector (Figure 3.2).

The primary health care sector used more macrolides, tetracycline, sulphonamides and trimethoprim in contrast to relatively larger consumption of quinolones, cephalosporins and aminoglycosides in hospital sectors. In both sectors the consumption of penicillin has increased in relation to the consumption of ampicillin. The total primary health care consumption varied from 1992 to 1995, first with an increase and since a decrease. The decrease is considered to be due to an audit project performed from 1994 to 1995 involving approximately one third of general practitioners in Roskilde county.

The decrease in the consumption of antibiotics in the hospitals is considered to be partly due to an increased attention to antibiotic use by the department of Clinical Microbiology, SSI, in 1992.

National antibiotic consumption.

Table 3.8 shows data from the Danish Medicines Agency concerning antibiotic consumption in Roskilde and Aarhus counties as well as in the whole country and the distribution between the hospital sector and primary health care sector. It appears that the total consumption in Denmark was reduced by 0.6% in primary health care sector from 1994 to 1995. Overall, there was a decrease in the national antibiotic consumption as well as a decrease in the different counties. Furthermore, this decrease in antibiotic consumption showed beneficial tendencies regarding the development of resistance. There was a reduction in the use of broad spectrum antibiotics such as quinolones, tetracycline and chloramphenicol and an increase in the use of penicillin while there was a reduction in the use of ampicillin.

The total consumption of antibiotics of 13.8 DDD/1000 inhabitants/day is somewhat lower than the other Nordic countries and much lower than countries in southern Europe (i.e. > 20 DDD/1000 inhabitants/day), according to published data. Surprisingly, the antibiotic consumption at the university hospital in Aarhus county was lower than in the non-university hospital in Roskilde county. Similarly there was a lower overall consumption in Aarhus county than in Roskilde county. However, the trend in antibiotic consumption was similar in the two counties. The reason for the differences in the antibiotic consumption will be a subject for further detailed investigations. Several explanations are possible; a simple explanation could be differences in the registration of bed days or it could be due to demographical differences in populations between the two counties. So far the differences in antibiotic consumption are not reflected in the prevalence of antibiotic resistance in the strains investigated in this report.

Table 3.1. Bacterial strains from humans included in DANMAP
Oversigt over humane stammer der indgår i overvågningsprojektet

Bacterial species Bakterier	Source Kilde	Type of data indsamling: Århus Roskilde counties/ Amter	Nationwide Danmark	Res. data	Isolates Stammer	Antimicrobial Antibiotikum	Approx. no. of isolates Ca. antal stammer	Comments Bemærk-ning
E. coli	Blood Blod	x	x	x		Amp, Cef, Cip, Gen, Sul, Tet, Tri, Mec	1500	
	Faeces Fæces	x			N		850	
	Other Andre	x		x			100	
E. cloacae	Blood Blod	x		x		Å+R	1000	
	Other Andre	x		x				
K. pneumoniae	Blood Blod	x		x		Å+R	1000	
	Other Andre	x		x				
Campylobacter	Faeces Fæces		x	x		Routine Rutine	300	
Salmonella	Faeces Fæces		x	x	N	Routine Rutine	2500	
Yersinia	Faeces Fæces		x			Routine Rutine	100	
P. aeroginosa	All Alle	x		x		Å+R	200	
H. influenzae	All Alle	x		x		Ampi,Chl,Cefu	500	
H. pylori	Biopsi	Ros		x		Routine Rutine		1
N. meningitidis	Blood, CSF Blod, SPV	x	x	x		Routine Rutine	100	
S. aureus	Blood Blod		x		x	Pen, Tet, Mec, Ery, Fec, Gen, Cipr	1200	
	Other Andre		x				25000	
	Nasal swab Næsepodn.	x			N		850	
CNS	Blood Blod	x			N + 100/6m	As S. aureus	1250	2
S. pneumoniae	Blood, CSF Blod, SPV	x	x	x	x Red. sensitivity	Pen + Ery	1000	3
	All Alle	x		x			1000	
S. haemolyticus (Gr.A)	All Alle			x	100 per 3 years/år	Pen + Ery	200	4
Enterococci	Blood Blod	x		x		Tet, Amp, Cef, Cip, Pen, Mec, Sulf, Ery	100	5
	Faeces Fæces	x			N	Pen, Tet, Ery, Met, van, Cip, Str, Gen	850	
	Other Andre	x			x	Same	100	

1) *Helicobacter pylori* are only collected in Roskilde County.
Helicobacter pylori kun indsamlet i Roskilde Amt.
2) 100 consecutive isolates are collected twice a year in the two key-counties.
100 på hinanden følgende isolater indsamlet to gange årligt i de to nøgle-amter.
3) All resistant isolates from all Denmark are sent to SSI for futher investigations.
Alle resistente isolater fra hele Danmark er sendt til SSI til videre undersøgelse.
4) 100 random isolates every third year are tested for resistance against penicillin in each of the key-counties.
Hvert tredje år testes i hvert nøgle-amt 100 tilfældige isolater for resistens overfor penicillin
5) 100 isolates from the two key-counties are tested annually by SSI.
Der testes årligt 100 isolater fra de to nøgle-amter ved SSI.

Please see text for detailed discussion of this table.
Der henvises til teksten for uddybende kommentarer.

Table 3.2 Prevalence (%) of methicillinresistant *Staphylococcus aureus* in selected countries (1990-1995).
Prevalens (%) af methicillin-resistente Staphylococcus aureus i udvalgte lande (1990-1995)

Land	% Methicillinresistens 1)
Denmark	0.2 - 0.7%
The Netherlands	3%
Germany	6%
United Kingdom	8%
USA	10%
France	30%
Greece	40%
Japan	60%

1) Average from several reports.
Gennemsnit fra adskillige rapporter.

Table 3.3 Resistance against antibiotics in % among *E. coli* isolated from blood cultures in 1995.
Resistens mod forskellige antibiotika hos E. coli isoleret fra blod i % af undersøgte stammer i 1995.

ATC code ATC kode	Antimicrobial agent Antibiotikum	Roskilde county Roskilde Amt		Aarhus county Århus Amt	
		%	n 1)	%	n
J01 AA 07	Tetracycline	9 2)	32	23 2)	443
J01 CA 01	Ampicillin	35	98	32	443
J01 CA 11	Mecillinam	4	87	-	-
J01 DA 06	Cefuroxime	1	97	5	443
J01 EA 01	Trimethoprim	18	84	15	443
J01 EB.02	Sulfamethizol	-	1	34	443
J01 GB 03	Gentamicin	1	96	1	443
J01MA 02	Ciprofloxacin	1	90	< 1	442

1) n=number of isolates tested.
n=antallet af resistensbestemte isolater
2) This difference is not significant.
Denne forskel er ikke signifikant.

Table 3.4.1. Resistance against antibiotics in % among enterococci isolated from blood cultures in 1995.
Resistens mod antibiotika hos enterokokker isoleret fra blod i % af isolerede stammer i 1995.

ATC code ATC kode	Antimicrobial agent Antibiotikum	Roskilde county Roskilde Amt		Aarhus county Århus Amt		Special 1)	
		%	n 2)	%	n	%	n
J01 AA 07	Tetracycline	-	3 3)	60	57	35	65
J01 CA 01	Ampicillin	-	3	14	57	26	65
J01 CA 11	Mecillinam	-	3	-	-	-	-
J01 CE 02	Penicillin	-	3	18	57	34	65
J01 CF 01	Dicloxacillin	-	-	-	-	98	65
J01 EA 01	Trimethoprim	-	-	12	57	18	65
J01 EB 02	Sulfamethizol	-	1	96	57	-	65
J01 FA 01	Erythromycin	-	3	42	57	29	65
J01 GB 03	Gentamicin	-	3	21	57	9	65
J01 MA 02	Ciprofloxacin	-	-	68	56	-	65
J01 XA 02	Teicoplanin	-	-	-	-	1,5	65
J01 XA 01	Vancomycin	- 4)	2	0	57	1,5	65
J01 XC 01	Fucidin	-	-	-	-	86	65

1) Special material. See text.
Specialindsamling. Se tekst.
2) n=number of strains tested.
n=antallet af resistensbestemte isolater.
3) In Roskilde County only three enterococci were isolated in 1995.
I Roskilde Amt er der kun isoleret tre enterokokker fra blod i 1995.
4) None of the strains investigated were resistant towards vancomycin.
Af de to undersøgte stammer var ingen resistent overfor vancomycin.

Tabel 3.4.2. Distribution of enterococcal species
Fordeling af enterococcer

Enterococcal species Enterokok species	Roskilde county Roskilde Amt	Aarhus county Århus Amt	"Special"
	n	n	n
Enterococcus faecalis	2	44	25
Enterococcus faecium	1	4	29
Enterococcus; others/andre	0	9	11
Total/I alt	3	57	65

Tabel 3.4.3. Number of enterococci in Århus County, different years.
Antal enterokokker i Århus Amt, forskellige år

Year År	Number of isolates Antal isolater	Number of patients Antal patienter
1994	71	41
1995	57	34
1996 1)	27	15

1) Data from 11$^1/_2$ month of 1996
Data fra 11$^1/_2$ måned af 1996

Table 3.4.4. Resistance among *E. faecium* and *E. faecalis* in "special" study
Resistens hos E. faecium og E. faecalis i specialindsamling

ATC code ATC kode	Antimicrobial agent Antibiotikum	E. faecium n = 29 %	E. faecalis n = 25 %
J01 AA 07	Tetracycline	34	36
J01 CA 01	Ampicillin	59	0
J01 CE 02	Penicillin	69	8
J01 CF 01	Dicloxacillin	100	96
J01 EA 01	Trimethoprim	19	9
J01 FA 01	Erythromycin	41	28
J01 GB 03	Gentamicin	14 1)	8 1)
J01 XA 02	Teicoplanin	3	0
J01 XA 01	Vancomycin	3	0
J01 XC 01	Fucidin	76	100

1) High-level resistance MIC>500ug/ml as determined by MIC
Høj resistens MIC>500ug/ml ved MIC-bestemmelse

Table 3.5 Antibiotic resistance in % among coagulase negative staphylococci isolated from blood cultures in 1995.
Resistens mod antibiotika hos koagulasenegative stafylokokker isoleret fra blod i % af antallet af undersøgte stammer i 1995.

ATC code ATC kode	Antimicrobial agent Antibiotikum	Roskilde county Roskilde Amt		Aarhus county Århus Amt	
		%	n 1) N=121 2)	%	N=732
J01 AA07	Tetracyclin	18	34	17	730
J01 CA 01	Ampicillin	69	105	52	731
J01 CE 02	Penicillin	73	116	78	732
J01 CF 03	Methicillin	14	112	48	296
J01 EA 01	Trimethoprim	15	26	30	730
J01 EB 02	Sulfamethizol	-	-	36	731
J01 FA 01	Erythromycin	26	110	26	731
J01 GB 03	Gentamicin	8	110	24	731
J01 MA 02	Ciprofloxacin	-	-	-	-
J01 XA 01	Vancomycin	0	107	0	296

1) n=number of isolates tested.
n=antallet af resistensbestemte isolater.
2) *N=total number of isolates.*
N=antal isolater i alt.

Table 3.6 Antibiotic resistance in % among salmonella and *Y. enterocolitica* collected between 01.01.1995 and 01.12.1996.
Antibiotikaresistens i % blandt salmonella og Y. enterocolitica indsamlet i perioden 01.01.1995 til 01.12.1996.

ATC code ATC kode	Antimicrobial agent Antibiotikum	S. Typhimurium		S. Enteritidis		*Y. enterocolitica*	
		%	n	%	n	%	n
G04 AB 01	Nalidixic acid	<1	353	<1	167	0	110
J01 AA 07	Tetracyclin	20	353	<1	167	0	110
J01 BA 01	Chloramphenicol	7	353	0	167	1	110
J01 CA 01	Ampicillin	10	353	3	167	98	110
J01 CA 11	Mecillinam	0	353	0	167	0	110
J01 CR 02	Amoxicillin + Clavulan acid	0	353	0	167	1	110
J01 DA 13	Ceftriaxon	0	353	0	167	0	110
J01 EA 01	Trimethoprim	3	353	0	167	0	110
J01 EB 02	Sulfamethizol	18	353	3	167	<1	110
J01 GB 03	Gentamicin	0	353	1	167	0	110
J01 MA 02	Ciprofloxacin	0	353	0	167	0	110
J01 XB 01	Colistin	0	353	0	167	0	110
	Apramycin	0	353	0	167	0	110
	Enrofloxacin	0	353	0	167	0	110
	Furazolidon	1	353	1	167	60	110
	Spectinomycin	7	353	2	167	<1	110
	Streptomycin	20	353	3	167	2	110

Table 3.7 Annual use of antibiotics in the county of Roskilde since 1992 in primary health care and in hospitals 1)
Antibiotikaforbrug i Roskilde Amt (1). Antal "Definerede Døgn Doser" fordelt på år og sted 1)

Year År	Primary health care Primærsektor		Hospitals Sygehuse		Total Ialt	Consumption in hospitals in % of total Sygehusforbrug i % af total
	DDD (2,3)	DDD/1000 pop./day indb./dag	DDD	DDD/100 bed days/year sengedage/år	DDD	
1992	1,126,561	14.0	89,464	43.1	1,216,025	7.4
1993	1,261,931	15.6	97,218	45.6	1,359,149	7.2
1994	1,319,708	16.2	87,965	40.8	1,407,673	6.2
1995	1,290,655	15.8	75,618	36.7	1,366,273	5.5

1) Data from the county of Roskilde provided by the Health Department, Roskilde county administration and the hospital pharmacy.
Data fra Roskilde Amt, Sundhedsafdelingen og sygehusapoteket.
2) DDD = Defined Daily Doses. See text.
DDD = Definerede Døgn Doser. Se tekst.
3) In Roskilde are used the following ATC-codes (Anatomical Therapeutic Chemical Classification System): J01 + A07AA09 + G01AF01) + P01AB01. See text.
ATC = I Roskilde-opgørelsen er anvendt følgende ATC-koder: J01 (antibiotika til systemisk brug) + A07AA09 (tarmaseptika: po vancomycin) + G01AF01 (antibiotika og antiseptika til gynækologisk brug: metronidazol) + P01AB01 (amøbemidler: metronidazol, se tekst).

Table 3.8. Use of antibiotics in DDD (Defined Daily Doses) in Denmark and in selected counties.
Antibiotikaforbrug i Definerede Døgn Doser i Danmark og udvalgte amter.

Antibiotika ATC-kode	Roskilde Amt						Århus Amt						Danmark					
	Hospitals/Hospitaler			Primary health care Primærsektor			Hospitals/Hospitaler			Primary health care Primærsektor			Hospitals/Hospitaler			Primary health care Primærsektor		
	1994 1)	1995 1)	+/- % 2)	1994 3)	1995 3)	+/-%	1994 4)	1995 3)	+/-%	1994 3)	1995 3)	+/-%	1994 4)	1995 3)	+/-%	1994 3)	1995 3)	+/-%
Tetracycliner J01A	735	543 (545)	-26	126392	121994	-5	-	2093	-	333975	340774	2	-	41657	-	3263169	3016850	-8
Amfenikoler J02B	19	0	-100	8	17	113	-	76	-	167	17	-90	-	532	-	1110	223	-79
Penicilliner J01C	56058	44604 (48135)	-20	709724	663794	-6	-	135186	-	1373313	1434147	4	-	1409518	-	14575223	14808080	2
Cefalosporiner J01D	6138	5483 (5904)	-15	1023	874	-15	-	18606	-	2820	1974	-30	-	202732	-	32760	32544	-1
Sulfonamider/ Trimethoprim J01E	4428	4162 (1757)	-6	66646	67887	2	-	6808	-	152975	154961	1	-	83577	-	1621922	1575500	-3
Makrolider/ Lincosamider J01F	6613	7950 (10587)	20	217442	211318	-3	-	15141	-	384169	367240	-4	-	261973	-	3928952	4025490	2
Aminoglykosider J01G	5533	4682 (5353)	-15	3	1	-67	-	5980	-	53	34	-36	-	74472	-	3751	3416	-9
Quinoloner J01M	1745	1959 (2414)	12	20114	18784	-7	-	6303	-	75973	53073	-30	-	86571	-	687318	587586	-15
Other/andre J01X	3173	2932 (863)	-8	3872	3228	-17	-	8604	-	6708	6073	-9	-	54723	-	77518	75760	-2
Total DDD I alt DDD	84442	72315 (75558)	-14	1147228	1087901	-5	-	198799	-	2330157	2358298	1	-	2217753	-	24191728	24125472	-0.3
Total DDD/1000 pop./day I alt DDD/1000 indb./dag	-	-	-	14.1	13.3	-6	-	-	-	10.4	10.4	0.4	-	-	-	12.8	12.7	-0.6
I alt DDD/100 bed days sengedage	39.2	35.1	-10.5	-	-	-	-	28.7	-	-	-	-	-	-	-	-	-	-

1) Data from: The hospital pharmacy, Roskilde county. Data in () are from Danish Medicines Agency.
Data fra sygehusapoteket i Roskilde Amt. Data () er fra Lægemiddelstyrelsen.

2) Changes calculated from consumption in Roskilde county.
Ændringer beregnet på grundlag af forbruget i Roskilde Amt.

3) Data from: The Danish Medicines Agency.
Data fra Lægemiddelstyrelsen.

4) Data not valid. See text.
Data ikke valide. Se tekst.

Figure 3.1 Resistance in *Staphylococcus aureus* since 1960. 23036 isolates from blood cultures in total.
Resistensudvikling for Staphylococcus aureus siden 1960. Baseret på 23036 stammer isoleret fra blod.

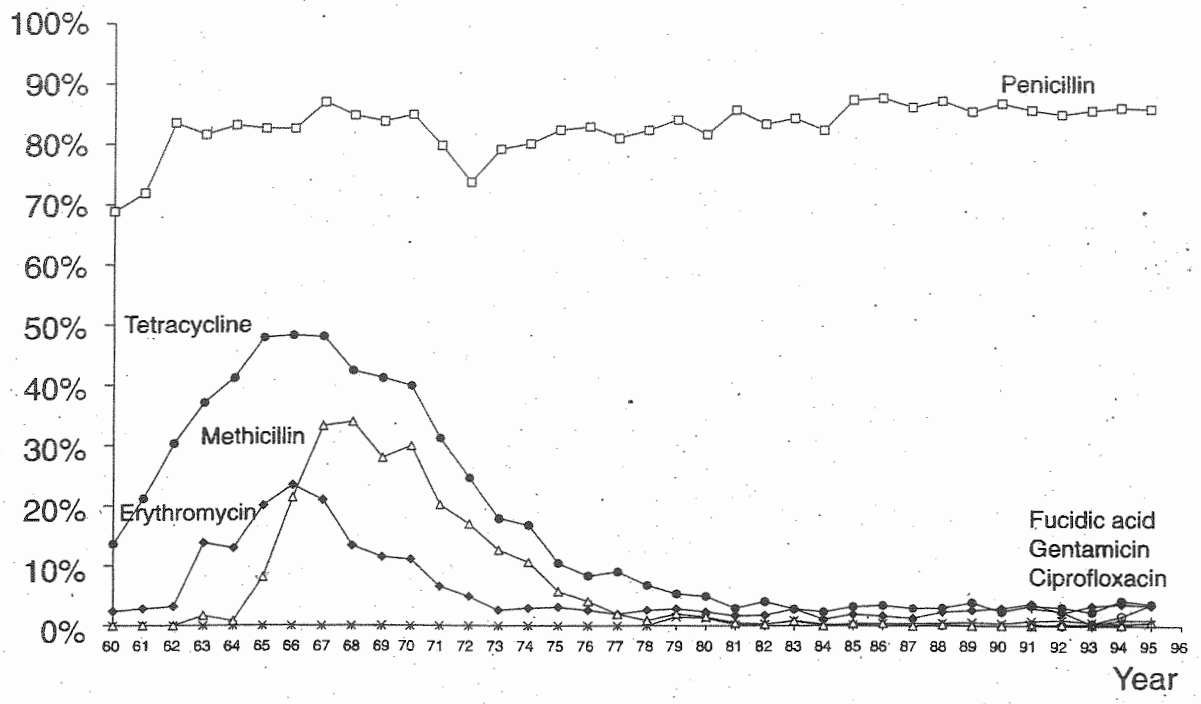
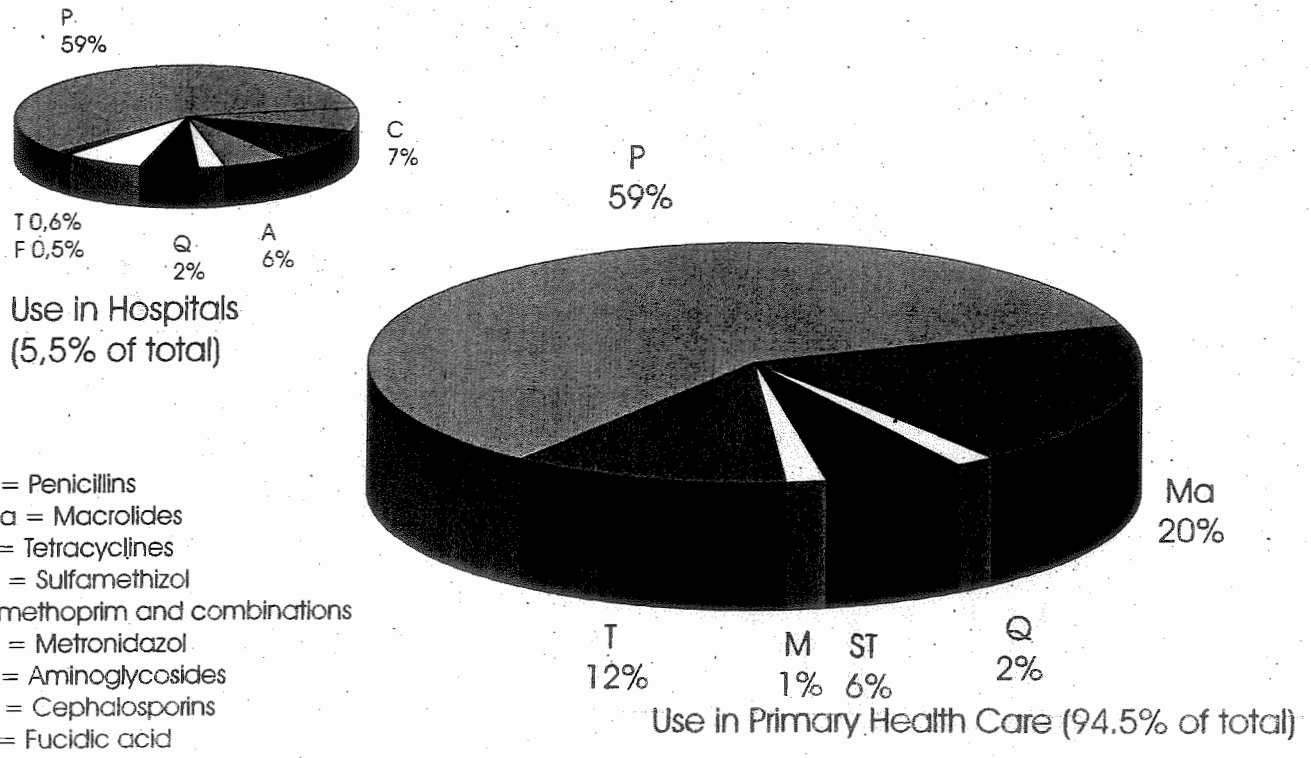


Figure 3.2 The use of different antibiotics in the county of Roskilde 1995
Forbruget af antibiotika i Roskilde Amt i1995



P = Penicillins
Ma = Macrolides
T = Tetracyclines
ST = Sulfamethizol Trimethoprim and combinations
M = Metronidazol
A = Aminoglycosides
C = Cephalosporins
F = Fucidic acid
Q = Quinolones

Figure 3.3 Development in the use of antibiotics in the county of Roskilde 1992-1995
Udviklingen i forbruget af antibiotika i Roskilde Amt.