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SURVEILLANCE OF ANTIMICROBIAL RESISTANCE - REQUIREMENTS FOR MONITORING PROGRAMMES

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Cette étude décrit un programme de surveillance de la résistance aux antibiotiques parmi les bactéries isolées d'animaux destinés à la consommation humaine, et des risques potentiels pour la santé publique. Les bactéries à étudier sont collectées à l'échelon national à partir de poulets, de bovins et de porcs, et comprennent les bactéries pathogènes pour les animaux, les bactéries à l'origine de zoonoses, et des bactéries choisies comme témoins. Les bactéries isolées sont exposées à des disques de diffusion ou à des concentrations minimales inhibitrices d'antibiotiques choisis parmi un large spectre de substances utilisées en thérapie, y compris certains produits utilisés exclusivement chez l'Homme et les antibiotiques utilisés comme facteurs de croissance les plus répandus dans l'Union européenne. Les résistances étaient nombreuses parmi Enterococcus faecium, aux facteurs de croissance et aux agents thérapeutiques de structure chimique voisine. Les différences de résistance constatées entre les porcs et les poulets reflètent les différences de consommation de ces composés dans les deux populations. Si la résistance à certains antibiotiques thérapeutiques était largement répandue, l'efficacité du traitement des infections chez les animaux destinés à la consommation humaine n'est pas en péril, et dans de nombreuses circonstances, les antibiotiques à spectre étroit seront encore efficaces.
Les programmes de surveillance du risque potentiel pour la santé publique des antibio-résistances des bactéries isolées chez l'animal devraient inclure des échantillons prélevés sur une population normale, et l'échantillonnage devrait être conçu de façon à éviter une corrélation intra-classe des résultats (effet élevage). Les bactéries devraient être identifiées au niveau de l'espèce, dans la mesure où la comparaison des niveaux de résistance entre des populations animales n'est souvent valide que si elle concerne la même espèce de bactérie. Les résultats devraient être enregistrés comme des variables continues plutôt que selon la dichotomie résistant ou sensible à l'antibiotique.

INTRODUCTION

Recently, Wiedeman stated that information about the epidemiology of bacterial resistance was fragmentary despite an enormous number of publications and that it remained a field of speculation influenced by fear of the total collapse of antimicrobial therapy by development of resistance (Wiedeman, 1993). This statement, made with reference to human medicine, is even more appropriate when used to describe the effect of therapeutic and growth promoting antimicrobials on occurrence of resistance in animals and the potential public health risk this poses.
This presentation describes a programme to monitor antimicrobial resistance in food animals. The programme is part of an integrated surveillance scheme which also includes the monitoring of resistance in bacteria from foods and from humans. A detailed account of the first results of the joint programme was published recently (Anon, 1997).

DESIGN OF MONITORING PROGRAMME

Collection of isolates.

The bacterial isolates are collected nationwide from broilers, cattle and pigs on a continuos basis and consist of representative samples of animal pathogens from diagnostic submissions, indicator bacteria isolated from samples collected at slaughter, and zoonotic bacteria isolated from either both or the latter source only. Bacteria from diagnostic submissions include *Actinobacillus pleuropneumoniae*, *Escherichia coli*, *Staphylococcus hyicus*, *Staphylococcus aureus* and coagulase negative staphylococci. The zoonotic bacteria include *Campylobacter coli and C. jejuni*, *Salmonella spp.* and *Yersinia enterocolitica* while *E. coli* and *Enterococcus faecium* and *E. faecalis* were chosen as indicator bacteria.
Slaughter samples are collected monthly from cattle (N=24 from 9 plants) and pigs (N=83 from 19 plants) and on a weekly basis from broilers (N=22 from 9 plants). The number of samples from a plant is determined in proportion to the number of animals slaughtered. The samples are collected as a systematic random sample by plant or meat inspection staff, however, formal randomization is not carried out. A quarterly target has been set for each bacterial species. When the target has been met, no further analyses for that species are carried out until next quarter.
For animal pathogens a designated number of isolates are selected each month among bacteria isolated from diagnostic submission to the Danish Veterinary Laboratory, the Cattle Health Laboratory at Ladelund and the Federation of Danish Pig Producers and Slaughterhouses Diagnostic Laboratory at Kjellerup. The selection is done as a systematic random sample without formal randomization.

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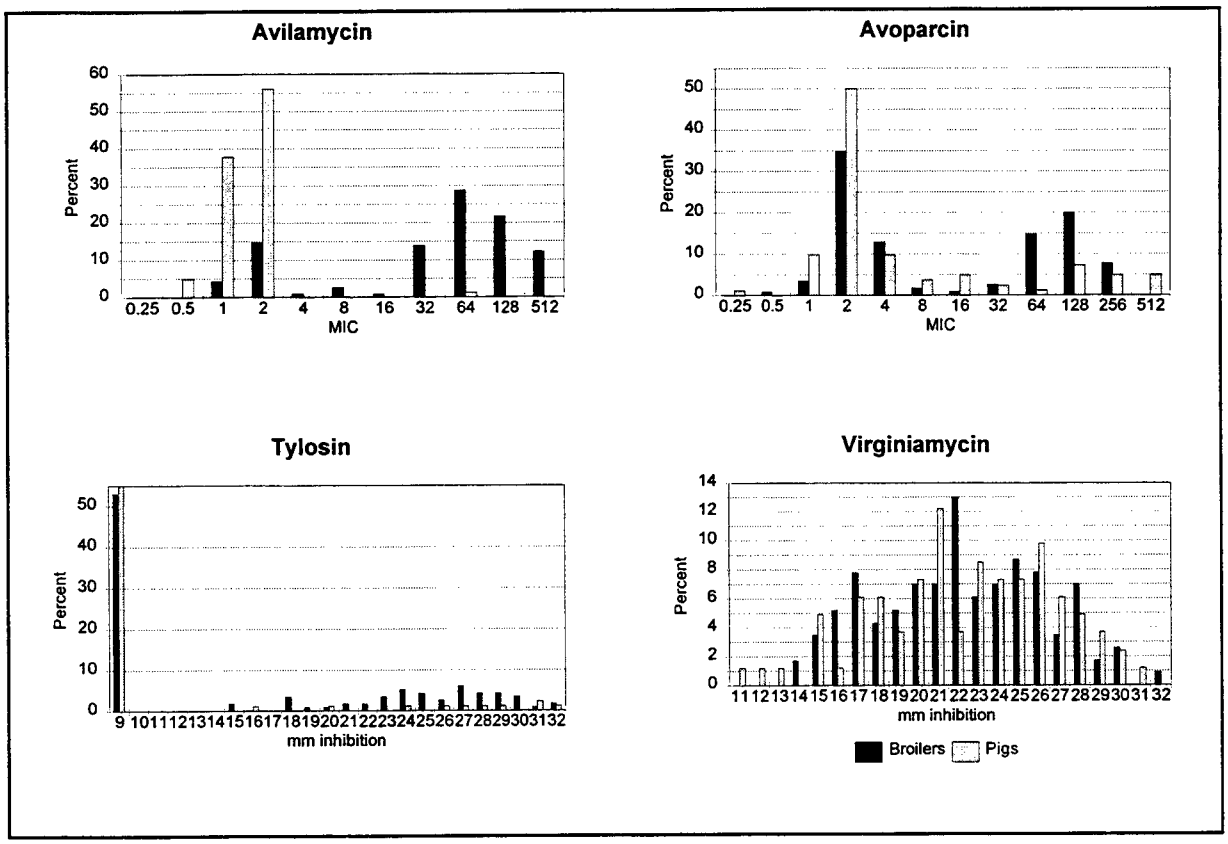
Antimicrobial susceptibility testing.
The isolates are tested for their susceptibility to an extensive panel of antibiotics, including antimicrobial growth promoters. The panel includes all groups of antimicrobials used in animal therapy as well as all antimicrobial growth promoters approved within the EU (including avoparcin and therapeutic agents structurally related to growth promoting agents). Gram positive bacteria are tested against a total of 26 antimicrobials and Gram negative against 15 antimicrobial agents. Susceptibility to therapeutic agents is determined as mm inhibition zone using a tablet diffusion method (Rosco Diagnostica) on Müller Hinton II agar inoculated with approximately 10^5 to 10^6 CFU according to ICS (semi-confluent growth). Susceptibility to antimicrobials used for growth promotion only is determined as minimum inhibitory concentrations (MIC) by the agar dilution method, using spot inoculation of approximately 10^4 CFU. For Campylobacter all determinations are done as MIC.
Evaluation of results. Results (mm inhibition zone or MIC) are entered into a relational data base (Oracle, version 7.14) and processed using SAS (SAS, 1989-1995) and the strains categorized as susceptible or resistant using breakpoints recommended by Rosco. For growth promoters no internationally approved methods exist for susceptibility testing and the breakpoints used must be regarded as tentative.

RESULTS

There was widespread resistance among *E. faecium* from broilers and pigs to growth promoting antibiotics and to structurally related therapeutic agents. Sixty-nine and 2 percent were resistant to avilamycin, 59 and 29 percent to avoparcin, 59 and 90 percent to tylosin and 43 and 47 percent to virginiamycin, respectively. Figure 1 shows the distribution of MIC-values and mm inhibition zones for these antibiotics. Staphylococci from cattle and pigs were almost fully susceptible to growth promoters with the exception of S. hyicus from pigs which had a high occurrence of resistance to macrolides (35-40 percent).

Figure 1
***E. faecium* from broilers (dark columns) and pigs (light columns). Distribution of MIC and mm inhibition zones to selected growth promoting agents**



Among bacteria isolated as pathogens resistance to certain therapeutic antimicrobials was widespread, for example resistance to ampicillin, sulphonamides and trimethoprim among E. coli and resistance among coagulase negative staphylococci to penicillin, sulphonamides and trimethoprim. Nevertheless, the efficaceous treatment of infections in food animals is not at risk and use of narrow-spectrum antimicrobials will usually be effective.

Table I shows resistance among zoonotic bacteria. Among Campylobacters, the occurrence of resistance was higher among isolates from pigs than broilers or cattle. However, a direct comparison is not possible because the bacterial species differ. *C. coli* predominates among pigs while it is rare among broilers. Salmonella from pigs was also more often resistant than were Salmonella from broilers or cattle. More than 75% of isolates from broilers and pigs were *Salmonella* Typhimurium and therefore comparable while most of cattle isolates were *Salmonella* Dublin. Direct comparison in this case is therefore difficult.
Among *E. coli* isolated from diagnostic submissions from cattle all were resistant to at least 1 antimicrobial compared with only 14% of *E. coli* from slaughter samples, and in pigs 99% compared with 73%.

Table I
Percent resistance to selected therapeutic agents among zoonotic bacteria

Antibiotic	Broilers		Cattle			Pigs	
	C. jejuni n=55	Salmonella n=40	C. jejuni n=29	Salmonella n=58	C. coli n=99	Salmonella n=150	Yersinia n=73
Ampicillin	7	18	3	0	17	9	97
Chloramphenicol	5	0	0	2	12	20	1
Gentamicin	0	0	0	0	0	1	1
Nalidixic acid	2	0	14	5	27	3	5
Streptomycin	2	13	7	5	44	21	5
Sulphonamide	0	65	28	45	8	50	3
Tetracycline	4	0	0	3	1	22	0
Trimethoprim	ND	0	ND	0	ND	6	45

DISCUSSION

Results of antimicrobial resistance monitoring programmes are often most conveniently summarized as percent isolates among a particular bacterial genus, for example *E. coli* or Salmonella, that are resistant or susceptible to the antimicrobials tested. This practice reflects the use of results to provide a basis for decisions about adequate antibiotherapy in animals or in humans. It is, however, an inadequate basis for comparison of results obtained in different countries or using different testing methods. It is well known that differences in commercial preparations of media may have a considerable effect on the results and Wiedeman (1993) demonstrated that use of different breakpoints in USA, Germany, the Netherlands and Sweden effectively makes it impossible to compare results for resistance of *E. coli* to ampicillin. Courvalin (1992) stressed that identification of bacteria to species level is necessary for results to be interpretable and Martel and Cloudert (1993) reported that even within a bacterial species such as E. coli the occurrence of resistance may vary with serotype. Furthermore, as indicated by the present results, the sampling frame used may have a considerable effect on the outcome.
In programmes to monitor the potential public health risk of antimicrobial resistance in bacteria from animals, sampling should include samples from the normal population of animals and be designed to avoid intra-class correlation of results (i.e. herd effects). Bacteria should be identified to species level as comparisons of resistance levels between animal populations often are valid only if based on the same bacterial species. Results should be recorded as continuous variables rather than as resistant or susceptible.
When these requirements are met differences in the occurrence of antimicrobial resistance may be used as a basis for inference about selective pressure or clonal spread of resistant strains. The differences in occurrence of resistance among *E. faecium* from broilers and pigs as shown in Figure 1 most likely reflect differences in the consumption of the compounds in the two animal populations.

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