PRINCIPLES FOR MONITORING OF ANTIMICROBIAL RESISTANCE AMONG FOOD ANIMALS

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INTRODUCTION

The introduction of antimicrobial agents in human medicine and animal husbandry has been one of the most significant achievements of the 20th century. The first antimicrobial agents were introduced in the 1930's, but shortly after resistance began to emerge and it has now become clear that antimicrobial resistance poses a threat to human and animal health that should be taken seriously. Modern food animal production depends on the use of large amounts of antibiotics for disease control, which provides favourable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans. Food animals and food of animal origin is traded worldwide. Thus, the occurrence of antimicrobial resistance in one country is today a problem for all countries, which emphasise the need for global initiatives and establishment of monitoring systems for determining resistance in all countries.

MONITORING OF ANTIMICROBIAL RESISTANCE

Points	Options to consider
Purpose	General trends, Emerging resistance
Reservoir of interest	Herd, Slaughterhouse, Country/region
Bacterial species	Indicator, Zoonotic, Pathogens
Sampling strategy	faeces, skin, herd, slaughterhouse, retail
Laboratories	Centralised, decentralised
Isolation procedure	Enrichment, CFU, Single bacteria
Susceptibility testing	MIC, Disk, Genes
Recording	Inhibition zones or MIC, Categories
Data handling & availability	Paper reports, Internet Availability of bacteria and results

Monitoring of antimicrobial resistance is a requirement to assess the magnitude of the problem. When establishing a monitoring programme several factors have to be taken into consideration including decision on bacterial species to be included, sampling strategies, isolation procedures, susceptibility testing methods, and data recording, computing and reporting. It is easiest to collect data on resistance in pathogenic bacteria received at laboratories for susceptibility testing. However, results obtained from such isolates will be greatly biased because requisition varies among veterinarians, some infections are more likely to generate isolates and isolates from some infections are more likely to be tested for susceptibility. Furthermore, isolates are in several cases collected after initial treatment and will often include several isolates from the same herds. Reporting of data from these types of collections has to be done with great caution and will be necessary to consider the value of each individual sample. A more optimal method is the collection of isolates from randomly selected animals, based on epidemiological considerations and aimed at covering the target population. Data can be collected from different regional laboratories and compiled centrally. However, differences in susceptibility testing methods might include testing biases. Careful standardisation and inter-calibration between laboratories can make such programmes worthwhile. Data recording, computing and reporting are essential for an appropriate monitoring system. Thus, the database has to include information on the sample population, origin down to specific patient or herd, time of isolation, testing procedure. Furthermore, to enable later studies on the emergence of resistance it is advisable to store the isolates centrally. Thoughtfully designed, longitudinal monitoring networks can provide invaluable insight into where resistance is emerging or increasing and which species pose the main problems for animal and human health. In addition, combing results from several national networks worldwide can provide information on how and where new resistant clones and genes are emerging and spreading.

Purpose of a monitoring system. A monitoring system should always be designed according to its purpose. The data collected during monitoring should always be used otherwise it is just a collection of data for no use or purpose. The purpose of monitoring can be to guide empheric treatment strategies for animals and humans, to study trends in resistance and associations between usage of antimicrobial agents and resistance, to detect emerging resistance, to detect outbreaks with resistant clones, to guide policy and to study the effects of interventions and to provide data for the education of the public, farmers, veterinarians, etc. Since most monitoring systems are based on collected and identified isolates the sensitivity of most systems in detecting emerging resistance is in general low.

Choice of bacteria to include. Most systems are based on: animal pathogens, zoonotic bacteria and indicator bacteria. Animal pathogens are included because it is important to observe trends in pathogenic organisms. Indicator bacteria are included because they can be isolated from healthy individual animals and thus, give a more true value of the occurrence of resistance in the entire animal population than pathogenic isolates. Most programmes use *Escherichia coli* to represent Gram-negative bacteria and *Enterococcus faecalis/faecium* to represent Gram-positive. Zoonotic bacteria are included because they develop resistance in the animal reservoir

and transfer to and cause infections in man. The most commonly included zoonotic bacteria are *Salmonella*, *Campylobacter coli*, *Campylobacter jejuni* and *Yersinia enterocolitica*.

Sampling strategies. Great care should be applied to decide on the sampling sites and strategies. Thus, a decision should be made whether the samples should represent individual herds or entire regions or countries and the number of samples taken adjusted to ensure an appropriate number of isolates to enable epidemiological valid comparisons between reservoirs or over time. If samples are taken to represent entire regions, care should be taken to ensure that the monitoring is not based on several isolates originating from a few animals or farms, but are taken randomly among the sample population. Isolates originating from the same farm might be one single clone, which can greatly bias the outcome of the monitoring.

Isolation of the bacteria. Different methodologies for isolation of the bacteria of interest are available. One possibility is to isolate the bacterium from e.g. a faecal sample and then test it for resistance to a panel of antimicrobial agents. This will provide data on several antimicrobial agents at the same time and a randomly well-characterised selected isolate that can be stored for later research purposes. This method has, however, a relatively low sensitivity. Another option is to perform selective enrichment where a larger quantity of e.g. faeces is placed in an enrichment medium supplemented with the antimicrobial agents of interest. The advantage of this method is that it will enable a more sensitive detection of emerging resistance. It will, however, only provide information on a single antimicrobial agent at a time.

Susceptibility testing methods and antimicrobial agents to include. Definition of a bacterial isolate as resistant or susceptible ultimately depends on clinical success or failure of treatment. However, to guide therapy different pheno- and genotypic i*n vitro* methods are used, which both require standardisation and quality control. Antimicrobial agents to be included in a monitoring programme should both fulfil the need for important information and also selected to ensure the highest possible sensitivity in detecting the presence of resistance mechanisms.

Data handling and reporting. Most diagnostic laboratories only report susceptibility to a given antimicrobial agents as susceptible, intermediate resistant or resistant. However, for the purpose of a monitoring programme it would be more optimal if data could be reported as inhibition zones or MIC. This would not only make it possible to compare data over time if breakpoints are changed, but also make it possible to look at the population distributions of the data from different laboratories and if necessary introduce the same or different clinical breakpoints or epidemiological cut-off values. The data obtained from a monitoring programme should always be reported as rapidly as possible to as wide an audience as feasible. Today most monitoring data are published once annually. However, the use of the Internet should make it possible to publish data more rapidly on a web-page in the future.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)

The Danish monitoring for antimicrobial resistance was established in 1995 and is based on the examination of representative bacterial isolates from both healthy and diseased animals. Pathogenic, zoonotic and indicator isolates are sampled. All major food animal sources are covered and the programme is coordinated with a similar sampling of data from bacteria causing infections in humans. In addition, information on the usage of antimicrobial agents is collected. The objectives of DANMAP are to:

- Monitor the usage of antimicrobial agents for food animals and humans
- Monitor the occurrence of resistance in bacteria isolated from food animals, food of animal origin and humans
- Study associations between usage and resistance and identify their trends
- Identify routes of transmission and areas for further research studies

All isolates included in the Danish monitoring system are routinely stored, making it possible to study the occurrence of resistance among historical isolates at a later time and enabling studies into the mechanism of resistance among the different resistant bacteria.

Example of data obtained through the DANMAP programme. Since the ban of avoparcin in 1995 the occurrence of vancomycin resistance has decreased significantly among enterococcal isolates from broilers, whereas no significant change occurred in pigs. It was shown that all VRE isolated from pigs in Denmark belonged to the same clone and that the genes encoding resistance to macrolides (*ermB*) and glycopeptides (*vanA*) were located on the same mobile DNA-element. The consumption of tylosin for growth promotion decreased substantially during 1998. During 1999 and 2000 a significant decrease in the occurrence of VRE among *E. faecium* isolates from pigs have been observed. These findings suggest that the persistence of VRE among the pig population was caused by the continued use of macrolides, mainly tylosin, for growth promotion and therapy. Similarly the occurrence of resistance to macrolides has closely followed the consumption of tylosin for growth promotion and therapy.

Pan-European (ARBAO-II)

The EU is funding ARBAO-II for the period 2003-2005. ARBAO-II is a concerted action involving 19 laboratories in 18 European countries. This concerted action has created a network of national veterinary reference laboratories in Europe and established a surveillance system for monitoring the occurrence and emergence of antibiotic resistance among bacteria from food animals. An external quality control for the capability of laboratories to perform susceptibility testing of bacteria correctly is performed. Different bacterial strains with known susceptibility patterns are sent four times each

year to the different laboratories for testing and the results entered into a central database. Each year the data generated in the individual laboratories are collected centrally and a report on the occurrence of antibiotic resistance among the different bacterial species isolated from food animals generated and published. The data from 2002 are available at: http://www.dfvf.dk/

DISCUSSION

Today national monitoring programmes have been implemented in a number of countries worldwide. Most of these programmes focus on pathogenic bacteria or salmonella, but some of them also report data on resistance in indicator bacteria isolated from healthy animals. However, none of the programmes aim specific at detecting emerging resistance using selective enrichment. In addition, the different programmes differ in their methodology used and antimicrobial agents tested fore. The different programmes are not coordinated and no exchange of data takes place. Furthermore, there is currently no central evaluation of the data.

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