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OPINION OF THE

SCIENTIFIC STEERING COMMITTEE

ON

ANTIMICROBIAL RESISTANCE

28 May 1999

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Introduction

Resistance to antimicrobials has existed since before they were introduced into human and veterinary medicine. Recent evidence however points to an inexorable increase in the prevalence of drug resistance among bacteria which has paralleled the expansion of their antimicrobial use in all spheres. Particularly difficult management problems are now posed by certain bacterial species which have the ability to acquire resistance to the majority and possibly all available agents. Thus, the increasing prevalence of resistance to antimicrobial agents among pathogenic micro-organisms, and particularly among bacteria, has become an increasingly important problem which has serious implications for the treatment and prevention of infectious diseases in both humans and animals

Mandate

Because of concern over the implications for human and animal health of the rapidly increasing rate of development of antimicrobial resistance the Commission (DGXXIV) asked the Scientific Steering Committee (SSC) to scientifically evaluate the current position regarding the prevalence and development of antimicrobial resistance, examine its implications for human and animal health, particularly with regard to the development and management of infections. The Committee was requested to evaluate factors contributing to the aetiology of the present situation, examine means of influencing or controlling the development of antimicrobial resistance and make recommendations based on scientific evidence. It should also advise on the means of monitoring the outcome of measures, which it might recommend and consider the implications of its advice. In particular the following elements should be considered:

- surveillance and monitoring of the use of antimicrobials,
- use/misuse in human and veterinary medicine (prophylactic and therapeutic), including over-prescription;
- poor compliance of patients with the prescribed treatment (e.g. using lower dosage or interrupting therapy as soon as symptoms disappear);
- poor compliance of the dosage regimen by animal owners;
- nosocomial infections;
- use/misuse as feed additives;
- use/misuse for plant protection purposes;
- use/misuse of antibiotic resistance genes in GMOs;
- prevention of zoonoses - food safety;
- resistant / multi-resistant microbials;
- microbial ecology (changes in normal microbial flora in particular environments e.g. in hospitals due to frequent use of disinfectants);
- identification of the factors involved in the increase in antimicrobial resistance;
- alternative preventive methods in human and veterinary medicine (level of implementation, promotion);

Summary

The SSC's evaluation has revealed that action needs to be taken promptly to reduce the overall use of antimicrobials[1] in a balanced way in all areas: human medicine, veterinary medicine, animal production and plant protection. This should involve improved disease preventive measures, elimination of unnecessary and improper use of antimicrobials, improving the effective use of antimicrobials presently available based on more precise diagnosis of the infectious agent, and on monitoring of antimicrobial resistance and control of antimicrobial usage.

Recommendations

The SSC recommends that there should be EU-wide co-operation and agreement as a matter of urgency, particularly with regard to prioritisation of actions. Those strategies which are most likely to be effective in the control and containment of antimicrobial resistance will be those which can be introduced speedily without undue costs in all countries and which can be monitored and/or enforced across the EU. It may be necessary to support the achievement of these proposals by introducing effective legislation and regulation.

Four important areas of action are proposed:

Prudent Use of Antimicrobials

These strategies relate to controls on the availability and access to antimicrobials within the EU and to the promotion of prudent use *via* education of all prescribers, recipients/clients, manufacturers, and other users. Measures for consideration include:

(1) There should be tighter controls on the sale, supply and distribution of antimicrobials through enforcement of the legal classification mechanisms of individual EU Member States. Member States should review mechanisms in place for the control of sales, supply and distribution of antimicrobials in the light of the recommendations of this report.

(2) The use of antimicrobials in each of the four areas, human medicine, veterinary medicine, animal production and plant protection should be only in accordance with legislative provisions. In particular the use of combinations of antimicrobials should be discouraged.

(3) Action should be taken to eliminate inducements, especially financial, which encourage the inappropriate use of antibiotics.

(4) Guidelines should be drawn up which indicate preferences for use of certain agents in the treatment of human and animal disease and which discourage the practice of prescribing for infections which are likely to be self-limiting and/or non-bacterial in aetiology. The aim should be to establish EU-wide agreements as the bases for local actions, including the development of "best practice" guidelines to support the judicious use of existing and novel agents.

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1 antimicrobials have been defined in Chapter 1.4. of the main report

In this regard, research is needed into:

(a) methods which might improve the prescription use of antimicrobials. including clinical studies which evaluate the important constituents of optimal drug regimens for the treatment of infections.

(b) the motivation of physicians and veterinarians to prescribe, and how prescribing behaviour can best be influenced for the better. The role of audit and participation in the feed-back of data on compliance with guidelines in influencing behaviour needs assessment.

(c) the development of more rapid diagnostic methods for bacterial infections which might allow for better targeting of antimicrobial treatments with minimisation of the unnecessary use of these drugs and limitation of the need for broad spectrum or combination therapy.

(5) Programmes should be developed for education of healthcare professionals (at undergraduate and postgraduate levels), farmers and associated food and feed producers, industries and consumers regarding the existence of this problem and the rationale and importance of interventions proposed. In particular, education should focus on how all these groups may contribute to reducing the unnecessary use of antimicrobials by better understanding of the role of such agents in the management of infectious diseases. These principles should be incorporated into codes of best practice whenever there is a commercial interest involved in the use of antimicrobials.

(6) Regarding the use of antimicrobials as growth promoting agents, the use of agents from classes which are or may be used in human or veterinary medicine (i.e., where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished. Efforts should also be made to replace those antimicrobials promoting growth with no known risk of influencing intestinal bacterial infections by non-antimicrobial alternatives. It is essential that these actions are paralleled by the introduction of changes in animal husbandry practices which will maintain animal health and welfare during the phase-out process. Thus, the phase-out process must be planned and co-ordinated since precipitous actions could have repercussions for animal health. Meanwhile, it should be reiterated to manufacturers and farmers that the continuous feeding of AMGPs to food animals for the purpose of disease prevention is a contravention of EU regulations and represents misuse; more effective enforcement measures should be adopted.

(7) The use of antimicrobials from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) for the purpose of plant protection should be discouraged

(8) While there is no evidence that antibiotic resistance marker genes have transferred from genetically modified plants to pathogenic micro-organisms, and whereas the possibility of such an event has been argued to be remote, it is considered appropriate to recommend that marker genes should be removed from plant cells before commercialisation whenever this is feasible;

failure to remove markers should be justified by the manufacturer. Companies should avoid the use of marker genes which might have the capacity to express and confer resistance against clinically important antibiotics.

(9) Use of genetically modified micro-organisms for commercial purposes either for contained usage or for environmental release was not part of the mandate. However, it is recommended that consideration be given to the potential for development of antimicrobial resistance which might arise from the release of such organisms into the environment.

Prevention of Infection and Containment of Resistant Organisms

These strategies should indirectly contribute to an overall reduction in antimicrobial usage *via* minimising the need for antimicrobial therapy in man, in animals, and in agriculture through the prevention and control of infection and optimal management of infection when it occurs. Measures for consideration include:

(1) There should be agreement and collaboration on the implementation of EU-wide standards of infection control in all types of institutions caring for the unwell and infirm, such as hospitals, nursing homes and day care centres. Policies regarding measures to be taken when transferring patients between units and between institutions should be agreed across the EU.

(2) There should be action to reduce the risk of infection in individuals and in the population as a whole by encouragement of uptake of immunisations, education regarding home hygiene, attention to public health issues, and by the maintenance and/or improvement of housing and social conditions.

(3) There should be a focus on education of veterinarians, farmers, owners of companion animals, food producers and consumers with regard to disease preventive methods in animals and the prevention of zoonotic infections in man and animals.

(4) Efforts should be made to reduce the need for herd treatments by improved husbandry, vaccination, and infectious disease control and eradication. In this regard, herd treatment use of antimicrobials should only be allowed if no other alternative is available and should be regarded as a failure of preventive measures which requires evaluation and investigation.

(5) Similarly, health control programs and other disease preventive methods should be devised and implemented in animal production systems in order to reduce the need and demand for the routine addition of antimicrobials to animal feedstuffs

New Modalities of Prevention and Treatment for Infections

(1) There should be cooperation and coordination between academic departments, the pharmaceutical industry and medical and veterinary research bodies in order to ensure that the necessary appropriate research is conducted which may facilitate the development of truly novel agents and of effective alternatives to antimicrobials as well as preventive therapies.

(2) The identification of novel ways to control and contain resistance may be furthered by investigations into how quickly and to what extent resistance is reversible when antimicrobial use decreases. Other related areas of research include evaluation of the means and likelihood of pathogenic organisms acquiring resistance from normal host flora in vivo, and vice versa, since this may lead to means of interrupting such transfers.

(3) While a connection between the use of antimicrobials in crop protection and resistance adversely affecting humans and animals is less clear, nevertheless the exploration of non-antimicrobials for the prevention and control of plant diseases should be encouraged. In this regard, research is needed to evaluate the potential for the transfer of resistance factors from plant pathogens or environmental micro-organisms to animal and human pathogens.

Monitoring the Effects of Interventions

This report has discussed the fact that there is inadequate evidence to identify with certainty those strategies which may be the most effective in the control and containment of antimicrobial resistance. In particular, it has been mentioned that the data are inadequate to determine which facets of antimicrobial uses and which areas of use are the major contributors to the problem. It has also been pointed out in several chapters that there is a paucity of reliable data regarding the prevalence of resistance across the EU in many pathogenic species, the change in prevalence over time, the incidence of infections due to multiresistant organisms and their clinical outcomes and on antimicrobial consumption within the EU.

While it is recommended above that efforts to control and contain resistance should not await such data since it is felt that the evidence is already compelling that action is needed, nevertheless a baseline should be established regarding resistance and consumption and these issues should then be examined systematically over time. Measures for consideration include:

(1) There should be an EU-wide co-ordination of organism collection and of susceptibility testing methods to monitor resistance patterns over time. Such data are needed to establish the baseline, to determine the effects of interventions, and to allow for meaningful comparisons between countries and regions. This surveillance should involve academic departments, industry (as part of post marketing surveillance) and governments (as part of disease prevention programmes).

(2) Research is needed into methods which might allow for determining and quantifying the impact of antimicrobial resistance on human mortality and morbidity.

(3) There should be EU-wide requirements for monitoring the consumption of antimicrobial agents in humans, animals, plant protection and in the environment; data by prescriber should be available for personal feedback and individual recipient records should be kept where appropriate to species. In particular, it is recommended that all antimicrobials administered on farms should be used only as part of a comprehensive veterinary health programme.

Furthermore, all antimicrobials used on farms, including antimicrobials in AMGPs, should be a matter of record which is kept available for inspection

(4) The effects of all interventions should be kept under constant review. An appropriately constituted EU-wide forum could be assigned the task of monitoring and assessing the outcomes of interventions and of advising on any necessary changes. This body could also serve as a major channel of communication and collaboration with non-EU countries and global bodies including the WHO.

(5) Resistance to antimicrobials is a global problem and interventions in the EU alone might be less effective unless action is also taken in non-EU countries. Therefore, monitoring the efficacy of EU-wide measures must take into account external factors. In this regard, it is possible that regulatory action may need to be considered in order to control access of animals, meat or foods from non-EU countries should there be a significant threat perceived or detected for importation of resistant bacteria.

1. INTRODUCTION

1.1. Background

The introduction of penicillin into clinical practice in the 1940s made a significant contribution to the treatment of a wide range of infectious diseases in humans and animals. The potential for microorganisms to become resistant to antimicrobials was recognised early, for example, by the development of penicillin resistant staphylococci. This problem had been partially addressed by the development of a succession of new effective antimicrobial chemotherapeutic agents. However, in recent years there has been a significant slowing in the rate of development of such agents and at the same time, there has been rapid and extensive development of antimicrobial resistance.

Although there have been important advances in the availability of antiviral and antifungal agents, no truly novel antibacterial drugs have been marketed in more than 10 years. Increasing problems have arisen in finding effective antimicrobial chemotherapy for a number of major bacterial pathogens, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and multiply drug-resistant *Mycobacterium tuberculosis*. This has led to increasing difficulties in the management of a range of human infections.

The rapid and widespread development of resistance is a matter of great concern. It is considered that the extensive use of antimicrobials both in humans and animals is a major contributory factor in the selection of resistant organisms. The precise mechanism for the development and transfer of resistance remains unidentified in some cases and considerable effort needs to be directed towards resolving the scientific basis of this problem. Every administration of an antimicrobial must be considered as an opportunity for the further development of resistance and this attitude needs to be registered by those who use antimicrobials if clinical problems are to be satisfactorily contained.

Although antimicrobial resistance has widespread implications for medical practice and for the treatment of disease in animals, a comprehensive assessment of the implications needs also to take account of the importance and impact of use of antimicrobials in the treatment of animals destined to enter the human food chain, the use of antibiotics as growth promoters in animal production and the possible impact on human health of the use of genetically modified organisms (as food for humans and for animals destined for the human food chain) containing antibiotic resistance (marker) genes, as well as other uses.

The seriousness of the potential consequences of antimicrobial resistance has been considered and debated by numerous academic, professional, industry and Government groups worldwide. Several of these bodies have recently

reported findings and recommendations and a substantial body of information already exists across many scientific disciplines.

1.2. The Proposal

Because of great concern over the implications for human and animal health of the rapidly increasing rate of development of resistance the Commission (DGXXIV) asked the Scientific Steering Committee (SSC) to review the scientific information available on this issue. The SSC created a Working Group with the following mandate:

1.3. Mandate and Terms of Reference

The SSC asked the Working Group to: "Scientifically evaluate the current position regarding the prevalence and development of anti microbial resistance, examine its implications for human and animal health, particularly with regard to the development and management of infections. The group should evaluate factors contributing to the aetiology of the present situation, examine means of influencing or controlling the development of antimicrobial resistance and make recommendations based on scientific evidence. It should also advise on the means of monitoring the outcome of measures, which it might recommend and consider the implications of its advice. In particular the following elements should be considered:

- surveillance and monitoring of the use of antimicrobials,
- use/misuse in human and veterinary medicine (prophylactic and therapeutic), including over-prescription;
- poor compliance of patients with the prescribed treatment (e.g. using lower dosage or interrupting therapy as soon as symptoms disappear);
- poor compliance of the dosage regimen by animal owners;
- nosocomial infections;
- use/misuse as feed additives;
- use/misuse for plant protection purposes;
- use/misuse of antibiotic resistance genes in GMOs;
- prevention of zoonoses - food safety;
- resistant / multi-resistant microbials;
- microbial ecology (changes in normal microbial flora in particular environments e.g. in hospitals due to frequent use of disinfectants);
- identification of the factors involved in the increase in antimicrobial resistance;

- alternative preventive methods in human and veterinary medicine (level of implementation, promotion);

1.4. Scope of Report

To satisfy the overarching requirements of the mandate, and to achieve a balance in the recommendations, which may span all areas of use, the Working Group, considered that the issues should be considered according to four specific areas of application:

i. treatment and prevention of disease in humans

ii. treatment and prevention of disease in animals

iii. improvement of animal production (feed additive use)

iv. plant protection, and the overall effects of antimicrobials in the environment

It is acknowledged that antimicrobial agents may be used for a variety of other purposes (e.g. as food additives, preservatives and for decontamination of surfaces). Two antimicrobials (natamycin and nisin) are authorised as food additives in the EU (Directive 96/85/EC). Natamycin is used on the surface of certain cheeses and dried sausages, and nisin is used in certain puddings and certain cheeses. It is noted that the Scientific Committee for Food recommended that antibiotics used for treatment of infections in humans should not be added to food. Another important area for consideration will be the use of genetically modified micro-organisms for commercial purposes either for contained usage or for environmental release. These applications are not considered further in this report.

There are several reasons to make a distinction between areas (ii) and (iii). Antimicrobial feed additives may confer health benefits but are not administered for the purpose of treating disease. They are used specifically to improve animal production, by affecting the gastro-intestinal flora or the digestibility of feedingstuffs (Council Directive 96/51/EC). They are not administered to individual animals and the number of animals treated is much greater than might be needed for disease control alone. The period of administration, often lifelong, exceeds that needed for the purpose of healing disease and recipient animals enter directly the human food chain thereby providing a greater opportunity for transmission of bacterial resistance directly to humans.

Antimicrobials used as feed additives are found in the three classes of compounds defined by Council Directive 70/524/EEC: 1) antibiotics, 2) coccidiostats and other medicinal substances, and 3) growth promoters (carbodox, olaquindox; but these have been prohibited recently). This report only addresses the substances under category (1). Community authorisation of a feed additive is given only if, at the levels permitted, treatment or prevention of animal disease is excluded. This requirement does not apply to coccidiostats used as feed additives for the prevention of coccidiosis or for other medicinal substances. Typically, antimicrobials are used for life-time

or at least a long period of the animal life and distributed to all the animals in the herd.

The Working Group considered the distinction between antibiotics and chemotherapeutic substances and thought that this was not relevant to the manner in which they are used in practice and select for resistance in bacteria. They have therefore used the term antimicrobial throughout the report for referring to antibacterial and other antimicrobial drugs. The terms antibacterial, antiviral, antifungal are used when referring specifically to one of those drugs. All these are taken to embrace antibiotics, the term most usually used in this context.

The Working Group also considered the relative importance of resistance to antibacterial agents and antimicrobials active against viruses, fungi and protozoa. While it is easy to cite major concerns about resistance in viruses (e.g. *Herpes simplex* and acyclovir), fungi (e.g. *Candida* spp. and fluconazole) and protozoa (e.g. *Malaria falciparum* and chloroquine), antibacterial resistance exists and is perceived to be the urgent problem within the EU. The Report is therefore devoted almost entirely to a consideration of the issues surrounding antibacterial therapy.

The report addresses antimicrobial resistance in a general sense and does not intend to assess in detail any individual products. When needed such an evaluation will need to take place on a case-by-case basis.

1.5. Procedures for Authorising Antimicrobials

The regulatory framework within which antimicrobials are granted marketing authorisation in the EU for human and veterinary use is summarised in Annex 1. Briefly, the therapeutic use of antimicrobials for humans or animals is subject to European-wide and/or national authorisation. Similarly the usage of antimicrobials for phytosanitation may be authorised EU-wide or nationally. In contrast, those agents which are approved for use as feed additives are subject to Council Directive (70/524/EEC), granting community-wide authorisations.

1.6. Sale and Supply

The control of the supply and sale of medicinal products (i.e. prescription-only or non-prescription availability) rests with the national authorities of the individual EU Member States. In theory, no EU country allows non-prescription supply of systemic antibacterials to humans. It is apparent, however, that such medicines are available through pharmacies in some countries i.e. there is considerable variation in the strictness with which national rules are applied. The ways in which the costs of prescription-only human medicines are partly or wholly subsidised also varies widely because of the very different health care systems which exist and this also impacts on the pattern of use of antimicrobials. Furthermore, whereas many EU Member States restrict supply of antimicrobials approved for veterinary use to prescription-only status, it is not difficult for farmers to access such drugs in some countries for use in food animals. In some European countries veterinarians may acquire a part of their income from the sales of medicines

though the implementation of policies in other countries prevents this practice.

The sale and distribution of feed additives is harmonised within the EU. Antibiotics and coccidiostats are subject to authorisation linked to the person responsible for putting them into circulation. These additives may be supplied only by approved establishments to approved intermediaries or establishments that manufacture premixtures.

2. THE BASIS OF RESISTANCE TO ANTIMICROBIALS

The multiplicity of inherent and acquired mechanisms which may be involved in bacterial resistance to antimicrobial agents, and the different modes of transfer within and between bacterial species of the genes encoding these mechanisms, are important factors in the increasing prevalence of antimicrobial resistance. A consideration of these matters is important for development of the concept that the use of antimicrobial agents exerts a selection pressure in favour of resistant organisms and thus may be considered a crucial factor influencing their prevalence.

2.1. Basis of Antibacterial Action

Therapeutically useful antibacterial drugs must produce a toxic effect on the bacteria but not on the host species by exploiting differences in the composition or metabolism of bacterial and animal cells. For example, some agents interfere with the synthesis of the bacterial cell wall, which is not found in animal cells. Others inhibit bacterial enzymes essential for metabolism or bind to bacterial ribosomes and inhibit protein synthesis but show little or no affinity for corresponding targets in host cells.

2.2. Basis of Resistance and Selection of Resistant Populations

2.2.1. Mechanisms

Antimicrobial resistance may be viewed as the ability of micro-organisms of a certain species to survive or even to grow in the presence of a concentration of an antimicrobial that is usually sufficient to inhibit or kill bacteria of the same species.

Resistance of a bacterium to an antibacterial may be:

inherent - i.e. the species is not normally susceptible to a particular drug. This may be due to an inability of the antibacterial to enter the bacterial cell and reach its target site(s), lack of affinity between the antibacterial and its target (site of action), or absence of the target in the cell. This is also called intrinsic resistance.

acquired - i.e. the species is normally susceptible to a particular drug but certain strains express drug resistance which may be mediated via a number of mechanisms:

i. destroying enzymatically the antimicrobial inside or outside the cell;

ii. lowering the intracellular concentration of an antimicrobial as a result of reduced uptake and/or increased excretion;

iii. altering the target site so that the antimicrobial no longer binds to it;

iv. creating an alternative metabolic pathway that bypasses the target action.

In those strains which have an inherent or an acquired mechanism of resistance, Minimum Inhibitory Concentrations (MICs) of the drug are

deemed to be higher than those which may be achieved for an adequate period at the site of infection and, therefore, a risk of therapeutic failure is predicted. Sometimes two or more mechanisms exist simultaneously in the same organism and combine to produce an even greater degree of resistance.

Finally, it must be noted that a single mechanism of resistance may lead to an ability to withstand the actions of some or all of the drugs of a particular class. Thus, exposure of a bacterial population to a single drug may select for organisms which display resistance to a large number of similar agents.

2.2.2. Selection of Resistant Populations

In the presence of an antimicrobial, organisms with inherent or acquired resistance to the agent will be selected. The bacterial population then comes to consist largely or entirely of resistant bacteria. Three scenarios need to be considered which may lead to clinical and microbiological failure of therapy should the host's immune system not be able to clear the infection without the aid of antibacterial(s):

I If all the organisms at the site of infection are resistant to the agent even before they are exposed to the drug, the infection will persist and may worsen, with potential for complications.

II If the infection is initially caused by a single species within which there is a sub-population of resistant strains, the selection pressure exerted by the antibacterial agent may result in a residual infection caused solely by resistant organisms.

III If the initial infection involves more than one species, at least one of which is not susceptible to the antibacterial agent applied, a change in the predominant pathogen may be detected in parallel with a therapeutic failure.

In all the above cases, the administration of an antibacterial agent will also inevitably exert a selection pressure on the host's normal flora (e.g. in the gut and vagina and on the skin) at sites which may be quite distant from the initial infection. Thus, successful treatment of the presenting infection may be followed by clinically apparent infections in one or more of these sites caused by overgrowth of species which are not susceptible to the antibacterial therapy, such as *Clostridium difficile* enterocolitis and oral or vaginal candidiasis.

2.3. Laboratory Determination of Resistance

For the purposes of therapy, the laboratory reporting of resistance to an antibacterial is based on breakpoints concluded from comparisons of *in vitro* inhibitory concentrations of the agent for target pathogens and its pharmacokinetics, usually in plasma. There is no EU-wide agreement on optimal methods of susceptibility testing, and different methods will give slightly different MICs. Also, plasma profiles of drug concentrations may not be relevant for predicting activity against pathogens at the site of infection. Disk-diffusion methods are most frequently used for determining

susceptibility in clinical laboratories. They are at best only semi-quantitative. Not surprisingly, there is sometimes disagreement on the breakpoints for clinical use appropriate to individual antibiotics and also the need for species-specific breakpoints. Nevertheless, for new antibacterial agents for use as human medicines, breakpoints agreed between the Licensing Authority and the Marketing Authorisation Holder are now stated in the Summary of Product Characteristics (SPC).

Notwithstanding these issues, the finding that an organism is markedly less susceptible *in vitro* to an agent compared with the "normal population" of the species usually correlates with detection of a mechanism(s) which mediates this "resistance" phenomenon. Whether or not an infection with a resistant organism will respond to the agent *in vivo* is not wholly predictable from in-vitro susceptibility test results due to the very many factors which affect the outcome of infection in the living host. These matters are discussed in section 3 of this report.

2.4. Transfer of Resistance

There is a genetic basis for all bacterial resistance to antimicrobial agents. Inherent resistance is determined by the genetic composition of a particular bacterial species. Acquired resistance is brought about either by random mutation of the DNA of the bacterial genome, which is then passed on to offspring, or by the acquisition of DNA containing a gene or genes which code for a mechanism(s) of resistance.

DNA may be transmitted to other bacterial cells by three processes: conjugation, transformation and transduction; though not all species can accept extraneous nucleic acid by all of these methods. Transfer of genetic material encoding a single mechanism may lead to cross-resistance to a large number of closely related drugs. Genes encoding resistance to more than one class of antibacterial agent may also be transferred together and pass on multiple resistance.

In conjugative transfer, DNA passes along a tube which links two bacteria; this may occur between bacteria of the same or similar species. Plasmids bearing genes as transposable elements (transposons) may transfer in entirety or in part between cells; those which carry more than one transposon can encode resistance to many, chemically unrelated, antibacterials. In this way resistances can become linked even though they are brought about by entirely different mechanisms.

Transformation involves the uptake of DNA from the environment; DNA acquired by this process may come from a species unrelated to the recipient, and antibacterial resistance may be acquired even from species not usually responsible for causing disease

Transduction involves the transfer of DNA by a bacteriophage.

2.5. Relationship Between Drug Administration and Prevalence of Resistance

Whatever the mode of spread of resistance determinants, the location of the genes encoding resistance, and the mechanism(s) which mediate the ability of bacterial populations to survive in the presence of an antimicrobial agent, the presence of that agent will favour the survival and multiplication of organisms which are able to withstand the actions of the drug.

Thus, a very plausible hypothesis may be proposed that the more a drug is used, the greater will be the selection pressure in favour of bacterial species and/or sub-populations which are resistant to the actions of that drug. However, it is very difficult to demonstrate a clear relationship between drug use and the prevalence of resistance, not only because of the lack of accurate data on the amounts and modes of consumption (as in section 4) but because the host response to infection is multi-factorial (as in section 3).

Not surprisingly, demonstrations of a strong correlation between in-vitro resistance and therapeutic failure come predominantly from patients who are immunosuppressed, and who have to rely on antibacterial agents to combat infection, and also from those infected with organisms which are situated in cells/tissues to which drugs may not penetrate in high concentrations. It is from hospital units which care for such patients that some of the best evidence available regarding the correlation between drug use and the selection of resistant populations has come.

3. PREVALENCE OF ANTIMICROBIAL RESISTANCE IN PATHOGENS FROM HUMANS, ANIMALS AND PLANTS, AND ITS IMPACT ON HEALTH AND PRODUCTIVITY

Introduction

Reliable information on the prevalence of antimicrobial resistance by drug and by species, together with changes over time, is important for clearly identifying the problem and for establishing a baseline before intervening to contain it. Furthermore, long term surveillance data are needed to monitor the impact of any intervention.

There are few good quality data regarding the prevalence of antimicrobial resistance in important pathogens in man, animals and plants in the EU. Nevertheless, there are sufficient data to show that resistance rates are increasing in many bacterial species, particularly with respect to certain drugs or drug classes, and that there is a considerable variation in the prevalence of resistance across the EU.

The aim of detecting resistance by in-vitro testing is to predict the likely effectiveness of a drug against a pathogen in an infected host. The reporting of resistance in this way is intended to convey the message that a degree of resistance exists in the clinical isolate at which therapy is likely to fail when the infection is treated with the usual dose of an antibiotic (see Chapter 2).

The correlation between *in vitro* susceptibility and clinical and microbiological outcomes is not exact, even when assessed carefully in the setting of a clinical trial. Nonetheless, *in vitro* susceptibility is probably the best available means for predicting successful therapy. This imperfect correlation may be explained by the way breakpoints are determined according to MICs and free plasma concentrations whereas the drug concentration achieved at the site of infection may differ from those observed in the plasma. In addition, the immune system is a vital part of combating infection in patients, many of whom often recovered from bacterial infections before the advent of antimicrobials. Conversely, patients infected with a susceptible pathogen but with impaired immune systems may not recover despite receiving appropriate antibacterial therapy.

For all these reasons, it is difficult precisely to determine the impact of antimicrobial resistance on human and veterinary medicine in terms of outcomes of treatment or prophylaxis. Part of the impact may be a fear of poorer outcomes which encourages the use of newer and often more expensive antimicrobials.

3.1. Resistance in Bacteria from Humans

3.1.1. Prevalence

Many local and national studies of resistance in specific pathogenic species have been performed, but most are point prevalence studies and few have been designed to evaluate change over time. The following summary of information for some of the most important pathogens must be viewed in the light of these deficiencies.

3.1.1.1. Mycobacterium tuberculosis

M. tuberculosis is the commonest cause of death from any single bacterial infectious agent in adults world-wide. The decline in numbers of new infections which occurred during the 20th century ceased or reversed in the mid 1980s due to many factors.

This resurgence was accompanied by an increased rate of multi-resistant isolates defined as those resistant to both isoniazid and rifampicin, with or without other resistances, in which mortality has been documented at 44% and 80-90% in HIV-positive patients. In a survey of cases in England and Wales between 1982 and 1991 (Warburton et al, 1993) 6.1% of 'initial isolates' (ie. first isolates from newly diagnosed patients) were resistant to isoniazid and 0.6% were multi-drug resistant.

3.1.1.2. Streptococcus pneumoniae

Prior to the early 1990s, most pneumococci isolated in the EU were susceptible to penicillin (Appelbaum, 1992). Since then, penicillin resistance has increased significantly (Pradier et al., 1997). The majority of strains with reduced susceptibility are only moderately resistant (inhibited by 0.12 - 1 mg/L penicillin) such that pneumonia and other non-meningitic infections caused by such strains usually respond to high-dose penicillin treatment. Those with high level resistance (MIC > 1 mg/L) are much less prevalent, but all except the most extreme may still respond except in meningitis.

Penicillin resistance is relatively rare in Nordic countries and in the Netherlands (Pradier et al., 1997) but a high prevalence has been reported in Spain (45%) and France (25%); rates between 5 to 10% have been reported in the UK, Germany, Belgium and Italy, and most of these strains are moderately resistant. There have been recent reports of moderately penicillin resistant *S. pneumoniae* with high-level resistance to cefotaxime and ceftriaxone in the United States (Coffey et al., 1995). Many of these Penicillin-insusceptible strains are co-resistant to non-beta-lactam agents. High rates of macrolide resistance have been reported in Spain (18%), France and Belgium (30%) (Pradier et al., 1997, 1994; Campbell et al., 1998).

3.1.1.3. Streptococcus pyogenes

While this species remains exquisitely susceptible to beta-lactam antibiotics, high rates of erythromycin resistance have been reported in Finland (20%), UK (23%), Italy (81%) and Spain (19%) (Borzani et al., 1997; Garcia-Bermejo et al., 1998).

3.1.1.4. Neisseria menigitidis

N. meningitidis strains with decreased susceptibility to penicillin (MIC > 0.16 to 1.28 mg/L) have been described world-wide, but with variable frequency. In Spain, the incidence has increased from 0.4% in 1985 (Saez-Nieto et al., 1988) to 67% in 1996 (Pascual et al., 1996); in the UK, the incidence rose to 11% by 1995 (Kaczmarski, 1995); in Belgium, 6% of the

meningococci showed reduced susceptibility to penicillin in 1998 (Van Looveren et al., 1998). The clinical significance of infection with strains with reduced susceptibility to penicillin is uncertain because treatment with high doses of penicillin has been clinically successful. Also, ceftriaxone remains very active against such organisms (Abadi et al., 1995). Rifampicin resistance is rare and resistance to fluoroquinolones has not yet been reported (Kaczmarski, 1997; Van Looveren et al., 1998).

3.1.1.5. Neisseria Gonorrhoea

The first problem noted was that sulphonamides were almost invariably ineffective against gonorrhoea by 1944 (Campbell, 1944).

Reduction in penicillin susceptibility without B-lactamase production (MICs up to 2mg/l) is associated with marginal clinical resistance but is associated commonly with resistance to tetracycline and erythromycin.

Plasmid-borne B-lactamases were first detected in 1974 in gonococci from the Far East (Ashford et al,1976)and from West Africa (Phillips,1976) and there is now a prevalence of c.50% of Neisseria gonorrhoeae in the developing world, although the prevalence has recently declined in parts of the EU.

Plasmid-mediated tetracycline resistance was reported in 1987 (Hook et al, 1987) but is still uncommon in much of the EU. Ciprofloxacin-resistant strains are still uncommon but their prevalence is increasing.

3.1.1.6. Campylobacter and Salmonella

Campylobacter jejuni, Salmonella and the various enterotoxigenic and enteropathogenic types of *E. coli* are the most frequent causes of bacterial gastroenteritis (DuPont et al., 1993) but are not usually treated with antimicrobial agents. Where treatment is required, the fluoroquinolones are commonly prescribed. However, fluoroquinolone resistance among Campylobacter has been reported at more than 50% by several investigators (Piddock, 1995; Isolates Hoge et al., 1998) and has been correlated with bacteriological and clinical failures (Petruccelli et al., 1992). There are also indications of reduced susceptibility to fluoroquinolones of non-enteric Salmonella serotypes in the UK (Frost et al., 1996) but the prevalence remains very low.

3.1.1.7. E.coli in urinary tract infections

E. coli is responsible for more than 80% of acute uncomplicated cystitis in young women. Increased resistance to several antimicrobials, including ampicillin, trimethoprim-sulphonamide and trimethoprim, has been reported from the UK (Gruneberg, 1994) and a significant increase in the number of *E. coli* resistant to fluoroquinolones has been documented elsewhere (Threlfall, 1997; Garcia-Rodriguez, 1995). Although clinical information is lacking in most of these in-vitro studies, it has been demonstrated by others that these resistant strains are often isolated from patients previously treated with fluoroquinolones (Lehn et al., 1996) or from patients with urinary tract

infections complicated by functional or anatomical disorders of the urinary tract (Ozeki et al., 1997)

Esherichia coli 0157:H7 is an important and common pathogen of the human gastrointestinal tract. Poorly cooked ground beef and unpasteurised milk have been the most frequently implicated vehicles of transmission. In general, antibiotic treatment is not recommended for several reasons. For instance, patients treated with antibiotics tend to have a greater risk of developing hemolytic-uremic syndrome (HUS) in comparison with those who do not receive antibiotics.

3.1.1.8. Vancomycin-resistant enterococci (VRE)

The enterococci are inherently insusceptible to many antibacterials. Therefore serious concern must be expressed at the advent of acquired glycopeptide resistance in *E. faecium* and the recent finding of MRSA resistance to vancomycin.

There are, however, major differences in the epidemiology of vancomycin-resistant enterococci (VRE) between the United States and Europe (for review: see Goossens et al 1998). In the United States, the prevalence of VRE in hospitals has risen considerably in the past 10 years (Martone 1998). Furthermore, there appears to be little genetic variability among these US isolates. In Europe, VRE infections in man are less common and are usually associated with debilitated and immuno-compromised hospitalized patients (Goossens 1998). Moreover, European VRE of human origin are usually highly polyclonal. These human isolates have the same susceptibility profile as VRE isolated from animals (Goossens 1998). The greater prevalence of VRE in US hospitals might be explained by the higher consumption of antibiotics in US hospitals in comparison with European hospitals, particularly glycopeptides.

3.1.1.9. Gram-Negative Bacilli in Intensive Care Units (ICUs)

More than 20% of patients admitted to European intensive care units (ICUs) develop an ICU-acquired infection (Vincent et al., 1995). In a comparison between countries of the incidence of antimicrobial resistance among aerobic Gram-negative bacilli from patients in Belgium, France, Portugal, Spain, and Sweden, the highest rate of resistance was seen in all countries among *P. aeruginosa* (up to 37% resistant to ciprofloxacin in Portugal and 46% resistant to gentamicin in France), *Enterobacter* species, *Acinetobacter* species, and *Stenotrophomonas maltophilia*, and in Portugal and France among *Klebsiella* species (Jarlier et al., 1996; Hanberger et al., 1999). Ciprofloxacin resistance was notable among *Enterobacter* spp from Belgium (31%), France (20%) and Portugal (21%).

In a European study, 26% (Sweden) to 48% (Portugal) of isolates of *Enterobacter cloacae* showed decreased susceptibility to ceftazidime (Archibald et al., 1997). Previous use of third generation cephalosporins has also been associated with the selection of resistance to beta-lactams in blood

isolates of *Enterobacter* spp which is associated with higher mortality (Livermore, 1995).

3.1.1.10. Methicillin-Resistant Staphylococcus aureus (MRSA)

MRSA pose one of the greatest challenges to infection control and have increased in frequency since the 1970s in most countries except in Scandinavia and the Netherlands (Vincent et al., 1995; Voss et al., 1994). They have been problematical in large teaching hospitals, general hospitals and nursing homes and are often resistant to most other anti-staphyloccoccal antimicrobials, except for the glycopeptides. However, treatment failures with vancomycin in patients infected with *S. aureus* described as vancomycin-intermediate *S. aureus* (VISA) have been reported recently in Japan (Hiramatsu et al., 1997) and the US (CDC Update, 1997).

3.1.1.11. Discussion

From the above it can be seen that antimicrobial resistance among pathogens involved in hospital and community-acquired infections is increasing. The possible reasons are probably many, including overcrowding, increased elderly population, population mobility, increased and inappropriate use of antimicrobials, over-the-counter availability of antibiotics, inappropriate prescribing of antimicrobials, lack of compliance, fewer resources for education and infection control, and decreased funding for public health surveillance. Scientific evidence is not available however to confirm the relative contribution of any of these.

Although the hospital and the community might appear to be separate environments, there is considerable potential for the transfer of resistant pathogens in both directions. This might be expected to increase with shorter hospital stays, increased treatment at home of patients with severe and complicated illnesses and increased transfer between acute hospitals and long-term care facilities.

3.1.2. Clinical Impact

3.1.2.1. Morbidity, Mortality and Treatment of Infection

Morbidity and mortality may be increased by delay in administering effective treatment for infections caused by organisms resistant to one or more antimicrobials which would normally be prescribed empirically (Cohen, 1992; Swartz, 1997). Some documented examples are nosocomial infections in hospitalised immunosuppressed patients with infections caused by multi-resistant organisms such as VRE and MRSA. There are special problems with infectious pathogens which are difficult to treat, such as tuberculosis in which the pathogen is increasingly exhibiting resistance to multiple anti-tuberculous agents (Bloom, 1992; Farmer, 1998). It has been debated whether resistant bacterial strains are more or less virulent than their non-resistant counterparts (Levy, 1998; Levy, 1998), but there is no clear evidence to answer this.

Most hospital and community-acquired infections are initially treated empirically and the pathogen involved never confirmed because specimens are not taken for microbiological examination. In addition, failure of first-line therapy is frequently followed by a change of antibiotic not based on laboratory information. When cultures are performed, days can elapse before an alternative drug can be identified on the basis of culture and sensitivity tests. Delays in treatment may not be important to the eventual outcome of less serious infections but the risk of overwhelming sepsis and death, or recovery with sequelae, is increased in many serious infections, such as endocarditis and meningitis (Campbell, 1998; Pallares, 1995; Gold, 1996).

The overall impact of bacterial resistance on morbidity and mortality is impossible to measure precisely, not only because of factors related to the infection, but also those of the patient, which play a major role in the likelihood of a cure. With the exception of those life-threatening infections which are acquired in the community, it is more difficult to conclude that bacterial resistance has a major adverse effect on human health. There is, however, evidence to conclude that nosocomial infections from multi-resistant organisms commonly lead to increased morbidity and mortality (McDonald, 1998; Lucas, 1998; Moeleering, 1998). Notwithstanding the uncertainties expressed, prescribing habits may be changing as a result of practitioners' perceptions of the prevalence of antimicrobial resistance; and the change is towards newer, more expensive and broader-spectrum drugs which might perpetuate the problem.

3.1.2.2. Spread of Infection

A delay in effective treatment due to antimicrobial resistance results in a prolonged risk of spreading an infection both in hospitals and the community (Cohen, 1992; WHO, 1997; WHO, 1998). In hospitals, this leads to an increased risk of outbreaks of nosocomial infection since large numbers of highly susceptible patients are cared for in close proximity with considerable intra-institution movement of both staff and patients. In the community, such delays are especially significant when prevention depends to a large extent on the treatment of cases and contacts (e.g. tuberculosis, sexually-transmitted diseases).

The increase in international travel, within and outside of the EU, has facilitated the spread of resistant pathogens between countries, with importation of resistant organisms occurring not only into the community but also directly into hospitals (Shanahan, 1994). Examples of the introduction of multi-resistant pathogens from outside the EU include nosocomial outbreaks which have stemmed from the direct transfer of seriously ill patients infected with such organisms into EU hospitals, and also the spread of multi-resistant community-acquired infections such as gonorrhoea.

3.1.2.3. Adverse Effects on Complex Treatments

The emergence of antibiotic resistance can also adversely affect treatments and diagnostic procedures that have been made possible by the existence of effective antimicrobial agents. These include antineoplastic therapy and

organ transplantation (Cohen, 1997). This could potentially annul important progress in medicine over the last 25 years.

3.1.2.4. Costs

It is impossible to attribute increases in the costs of health care specifically to the rise in resistance to antimicrobials as there is insufficient data of clarity from the community to establish such a relationship. In hospitals also there are many confounding variables which make it difficult to determine the specific impact of resistance. The economic implications of resistance include prolonged hospitalisation and the need for additional diagnostic and therapeutic procedures as well as the obligatory use of alternative drugs which are generally more expensive and may need to be given parenterally rather than orally. Additional factors include the psychological trauma to the patient of single room isolation, denial of full hospital facilities, prolonged separation from family, and reduction in income. (Cohen, 1992; Cohen and Tartasky, 1997; Williams and Heymann, 1998; Weekly epid. Record 12, 333-340, 1997).

3.2. Resistance in Bacteria from Animals

Antimicrobial resistance varies according to species, degree and nature of the disease(s) present and antimicrobial use.

Intensive breeding systems with large numbers of young animals housed in limited areas create favourable conditions for the emergence and spread of infectious agents. Although antimicrobials are indispensable in the control of these infections, they exert a selection pressure for the development of resistant pathogenic and commensal bacteria. It is difficult to assess the impact on veterinary practice and animal health of resistance in pathogens which cause specific infections in animals. With regard to the potential effects of resistance in animal bacteria on human health, the direct transfer of pathogens to man and the possibility of spread of resistance determinants between animal and human pathogens must both be considered.

3.2.1. Prevalence

Few countries have initiated continuous surveillance of antimicrobial resistance in bacteria isolated from farm animals, and resistance in bacteria from domestic pets is hardly monitored at all on a systematic basis. Data collected over time is especially valuable since it allows changes in the prevalence of resistance to be studied. Denmark has gathered important evidence in a comprehensive surveillance programme covering pathogenic, zoonotic and indicator bacteria from all farm animals and from food sources. (DANMAP, 1997). In France, a veterinary surveillance network has monitored pathogenic bacteria, including salmonellae, isolated from cattle since 1980 (Martel, 1995). In Germany, strains isolated from clinical specimens are regularly assessed for resistance and the results are published on a regular basis (Trolldenier, 1995). A detailed review of the impact of the development of antibiotic resistance in all stages of the food chain has recently been published in the UK. (MAFF, 1998).

3.2.1.1. Escherichia coli

E. coli is one of the most important enteric pathogens in food producing animals. Quantitative data of varying quality on resistance among porcine *E. coli* are available from many countries and high resistance rates are reported all over Europe (Baquero, 1996; DANMAP, 1997; Mc Kinnon, 1993; Morvan, 1994; Pohl, 1991; Wray, 1993). Resistance has been reported to tetracyclines, sulphonamides and streptomycin (≥50%), to ampicillin (20 to 50%) and to trimethoprim-sulphonamide (18 to 46%). Among non-pathogenic *E. coli* strains isolated from healthy pigs at slaughter, the resistance rates were lower than those observed in sick animals for tetracycline (37%) and ampicillin (10%) (DANMAP, 1997).

In Sweden, the prevalence of antimicrobial resistance among porcine *E. coli* has not changed in ten years despite a reduction of antimicrobial usage, particularly of tetracycline (Björnerot, 1996; Odensvik, 1997). However, the situation is still favourable in comparison with other countries (Melin, 1996). In 1994, 38% of clinical isolates were resistant to tetracycline, 41% to streptomycin, 10% to trimethoprim-sulphonamide and 8% to ampicillin, whereas all 1994 Swedish strains and 2% Danish isolates were inhibited by < 0.5 mg/L enrofloxacin (DANMAP, 1997; Melin, 1996). In Finland the resistance situation in 1996 was 30, 21, 24, 9 and 7% for tetracycline, sulpha compounds, streptomycin, ampicillin and sulpha trimethoprim respectively (Tast, 1997).

Very high rates of resistance to tetracyclines, ampicillin, trimethoprim-sulphonamide, neomycin, and gentamicin in clinical isolates from calves has been reported from Denmark, France and the UK (DANMAP, 1997; Martel, 1995; Wray, 1993). In the Netherlands, where fluoroquinolones are extensively used in cattle, 40% of a large number of calf herds investigated harboured fluoroquinolone-resistant *E. coli* to a varying extent (MAFF, 1998). Furthermore, in the UK plasmid encoded beta-lactamases resistant to inhibition by clavulanic acid are reported to occur among coliform bacteria in cattle, probably due to a wide usage of the ampicillin/clavulanic acid combination (Hunter, 1993). As in porcine *E. coli*, the resistance rate was markedly lower in isolates from healthy calves than from diseased animals (DANMAP, 1997).

In Spain, high rates of resistance in poultry isolates (67 to 94%) was seen for streptomycin, tetracycline, sulphonamides, and trimethoprim-sulphamethoxazole while 13 to 24 % were resistant to fluoroquinolones (Blanco, 1996). The numbers in Finland in 1996 were 4 and 15% for streptomycin and tetracycline respectively (Tast 1997).

The limited information available on the prevalence of resistance among bovine *E. coli* O157 strains, or other verotoxin-producing strains, shows that resistance rates are low (CDC/FDA/USDA/UK, 1996).

As far as domestic pets are concerned, antimicrobial resistant *E. coli* have been isolated from a wide variety of sites in dogs with the pathogens often resistant to more than one antimicrobial. In some veterinary practices

multiresistant *E.coli* are present in many of the dog infections and includes fluoroquinolone and co-amoxyclav resistance.

3.2.1.2. Salmonella typhimurium

Salmonellosis is a zoonotic infection in man and some serovars cause clinical problems in animals, mostly in calves and pigs. Both in animal and human specimens antimicrobial resistance is most widespread in the *S. typhimurium* serovar and there are many reports of high rates of resistance including multiple resistance, especially among bovine strains (Heisig, 1993; Martel, 1995; Morvan, 1994; Threlfall, 1997; Wray, 1993). The epidemic spread of phage type DT 104 in the 1990s has been particularly worrying. Some strains are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. In 1995 90% of UK isolates belonging to this particular phage type had this resistance pattern. Resistance to fluoroquinolones (13%) and trimethoprim has also emerged in the UK (Wray, 1997).

In contrast, resistance of *S. typhimurium* to fluoroquinolones, trimethoprim, neomycin and gentamicin is almost non-existent in Scandinavia and resistance to ampicillin, chloramphenicol and tetracycline is uncommon (DANMAP, 1997; Franklin, 1994) Resistance rates for *S. typhimurium* have also decreased considerably in Denmark since the early 1980s when 80% were resistant to one or more antimicrobials (Jorgensen, 1986). In the Netherlands few of 394 animal isolates recently investigated were drug-resistant. (van den Pelt, 1998), although fluoroquinolone resistance was 8% among broiler isolates (Jacobs-Reitsma, 1994).

Salmonella isolates from dogs and cats reflect the isolates from food and farms. In horses *S. dublin* are usually sensitive to all antimicrobials whereas 50% *S.typhimurium* show evidence of resistance. In a study in a Dutch veterinary teaching hospital 28 of 69 isolates were resistant to three or more antibiotics Van Duijkeren et al (1994). Reptiles are common sources of salmonellae with most infections derived from holding tanks or farms of origin. Gentimicin is widely used in turtle farms to produce salmonella free eggs yet D'Aoust (1990) found 21% of turtle eggs still contained salmonella of which 81% were gentimicin resistant.

3.2.1.3. Serpulina hyodysenteriae

This anaerobic spirochete is the causative agent of swine dysentery. Various antimicrobial classes are used to control it. Tylosin (a macrolide) and lincomycin (a lincosamide) have been extensively used for a long time to treat this infection and resistance is common in clinical isolates (Buller, 1996) (Gunnarsson, 1991) (Kitai, 1979). The feed additives carbadox and olaquindox, used until recently as growth promoters in swine production in Europe, have probably exerted an inhibitory effect on *S. hyodysenteriae* in fattening pigs. MICs of these agents for *S. hyodysenteriae* are generally low and clinical disease may have been prevented due to their use.

Tiamulin, a pleuromutilin, has been used more recently and resistance is so far uncommon. (Gunnarsson, 1991; Ronne, 1990) (Buller, 1996), although

the emergence of resistant strains has been reported (Buller, 1996) (Gresham, 1998). In view of the limited selection of antimicrobials available it seems to be of utmost importance closely to monitor the spread of resistance, especially that of tiamulin in *S. hyodysenteriae.*

3.2.1.4. Pasteurella spp and Actinobacillus spp.

These bacterial species are inherently susceptible to beta-lactam antimicrobials. In many countries, however, a high percentage of pasteurellae and actinobacilli are now resistant to penicillin, often caused by beta-lactamase production (Burrows, 1993; Martel, 1995; Rossmanith, 1991; Schwartz, 1989). Resistance to tetracyclines is also common in bovine isolates of *P. haemolytica* and *P. multocida* (Hörmansdorfer, 1996; Watts, 1994). High rates of resistance to ampicillin (61%), chloramphenicol (35%), tetracycline (71%), and trimethoprim-sulphonamide (39%) have been reported for bovine *P. haemolytica* strains in France (Martel, 1995). Beta-lactamase producing porcine *A. pleuropneumoniae* or bovine *Pasteurella* spp. strains have not so far been detected in Scandinavia and other antimicrobial resistance rates are also reported to be low in these countries (Franklin, 1988) (DANMAP, 1997)

3.2.1.5. Staphylococcus aureus

S. aureus is the most important pathogen in bovine mastitis. Although treatment and prevention regimens differ between countries, antimicrobials are widely used for both. Resistance rates are difficult to assess since few studies are published. The frequency of penicillin-resistant strains varies between 5 and 90% (Aarestrup, 1998; IDF, 1991). In some countries (UK and Switzerland) a decline has been observed lately from very high levels (70% and 50%, respectively). In Denmark, penicillin resistance in bovine *S. aureus* has been increasing despite restrictive antimicrobial policy (20%). Fairly constant and low levels through the 1960s to the 1990s were reported from Norway and Sweden (5 to 15%) and from Germany (30 to 40%) (Aarestrup, 1998). To date the occurrence of methicillin resistance in bovine *S. aureus* strains has not been confirmed.

Staphylococci are commonly present on the skin of pet animals with pyoderma and in cases of otitis externa and in the nares. MRSA in a veterinary hospital has been described by Tomlin et al (1998)

3.2.1.6. Streptococcus and Enterococcus spp.

Penicillin resistance has not been reported in *S. agalactiae, S. dysgalactiae* and *S. uberis*, important causative agents of bovine mastitis.

Enterococci only occasionally cause disease in farm animals. Since enterococci in farm animals often carry transferable resistance genes mediating resistance to antimicrobials used in human medicine, the potential impact of this resistance gene pool on human health has caused much concern lately. The use of glycopeptides (avoparcin), macrolides (tylosin and spiramycin) and streptogramins (virginiamycin) as feed additives in animal production has been especially questioned (WHO, Berlin, 1997).

However SCAN have concluded that the use of virginiamycin as a growth promoter did not constitute an immediate risk to public health in Denmark (SCAN, July 1998) and that there was no demonstration of time-dependent increase in resistance in tylosin (SCAN, February 1998)

A high prevalence of high-level glycopeptide resistance *(VanA*-mediated*)* among animal isolates has been associated with the use of avoparcin as a growth promoter (Aarestrup 1995, Bates et al, 1994, Devriese et al, 1996; DANMAP 1997). In contrast, in Sweden where glycopeptides have not been used in animals since 1985, no vancomycin -resistant enterococci have been detected in animals so far (SOU 1997; Van den Bogaard et al, 1998).

Until recently, quantitative data on macrolide and particularly streptogramin resistance in enterococci have been scarce. The assessment of the available data has also been confounded by the use of different methods of testing and unclear definitions of resistance. Data recently produced have clearly shown that the use of macrolides and streptogramins in broiler and pig production is associated with emergence of macrolide and streptogramin resistance in enterococci (Aarestrup et al, 1998; DANMAP 1997.

3.2.1.7. Aeromonas salmonicida

More than 80 bacterial pathogens have been identified among fish farmed in Europe with only a few having significant impact on aquaculture. Plasmid mediated resistance to antimicrobials have, however, been identified in a number of these bacterial fish pathogens (Aoki 1988, DeGrandis and Stevenson 1985) with transferable R-plasmids found in *Aeromonas. salmonicida* encoding resistance to chloramphenicol, sulphonamide and streptomycin in Japan, to combinations of sulphonamide, streptomycin, spectinomycin, trimethoprim and/or tetracycline in Ireland (Aoki 1997) and in 11 out of 40 isolates in Scotland to combinations of oxytetracycline, streptomycin, sulphamethoxine and/or trimethoprim (Inglis et al. 1993) .Beta-lactamases occur naturally and widely among aeromonads including fish pathogens (Rossolini et al 1996). A useful review of bacteria associated with fish disease and human disease was produced by Smith et al.(1994).

3.2.1.8. Discussion

The use of antimicrobials for therapy and preventive measures in animal production has led to an increase in antimicrobial resistance both in animal pathogens and in commensal bacteria. The emergence and spread of antimicrobial resistance in animal farms parallels that in hospitals and the same antimicrobial classes are used in both environments. As a consequence, bacterial infections in farm animals may be untreatable or have to be treated with the most recently developed antimicrobials such as fluoroquinolones and third generation cephalosporins. For antibiotics such as trimethoprim and the fluoroquinolones there is epidemiological evidence of a correlation between licensing and the emergence of resistance. The situation varies between countries, which may reflect different climate, breeding and management systems in animal production as well as

differences in antibiotic policies. In general, the prevalence of resistance is lower in Scandinavian countries.

3.2.2. Impact on Veterinary Medicine and Effects on Human Health

3.2.2.1. Morbidity, Mortality and Treatment of Infections

The primary impact of resistance to antibacterials is failure of empirical therapy of bacterial infection, which causes an increase in morbidity and mortality and hence prolonged suffering of infected animals. Untreatable infections do occur but are very rare and are not yet a major problem in veterinary medicine.

The management of diseases in animals raises issues, including economic ones, which do not apply in human medicine. For example, infections in food animals raised in flocks or large groups often run an acute or subacute course in young animals and the entire group may have to be treated empirically by the addition of therapeutic antibiotics to feed or water. Resistance in such situations may cause therapy failure with increased morbidity and mortality and require substitution of unique alternative antimicrobial formulations suitable for herd administration. These are frequently not generally available, and if so, are more expensive.

The prevalence of drug resistance has encouraged a move away from the prescription of narrow spectrum antimicrobials. In many European countries penicillin can no longer be used in the treatment of mastitis since beta-lactamase producing *S. aureus* strains are common. In regions with a low (5 %) prevalence of resistance, the cheaper narrow spectrum benzylpenicillin is still used as first choice for staphylococcal mastitis in cattle, while in regions where the prevalence of resistance is up to 90 % more expensive and broader spectrum agents are the drugs of choice. (Aarestrup, 1999).

3.2.2.2. Spread of Infection

There is evidence of a spread of bacteria bearing determinants for antimicrobial resistance from animals to man – the zoonoses are the best example. This spread can be partly prevented but not completely interrupted by good hygiene. For this reason preharvest pathogen control e.g. of salmonella is important. Other measures are supportive.

Drug resistance can prolong infectivity so increasing the risk of transmission of the infection and of resistant bacteria to other animals within a herd and to the environment, as well as to humans by direct transfer or via the food chain. In this regard, it should be noted that antimicrobial treatment for Salmonella infections in animals may result in prolonged carriage, as happens in man (Wierup 1994). Transportation of live animals to other regions and countries also provides a potential for the spread of infection with resistant organisms to animals reared at geographically distant sites. The OIE regional commission for Europe has recently focused on this aspect and made specific recommendations (OIE, 1998).

Antibiotic resistant bacteria may be present in the intestinal tract of a large proportion of food-producing animals (Nijsten, 1996; van den Bogaard, 1996; van den Bogaard, 1998). As it is difficult to prevent carcass contamination during slaughter and subsequent steps in food preparation, antibiotic resistant bacteria derived from the intestinal tract of food producing animals may be transmitted to humans via food (Hinton, 1988; Gustafson, 1997) This facilitates the spread of antibiotic resistance genes (Nesbakken, 1996; Borch, 1996; Berends, 1997) and aspects of farm hygiene, transport, slaughter and food processing hygiene as well as personnel training are important in this regard.

3.2.2.3. Reduced Colonisation Resistance

Antimicrobial administration disrupts the normal intestinal flora and may increase susceptibility to colonisation with organisms such as *Salmonella* due to a reduction in the colonisation-resistance of the gut. This is well documented for avoparcin which, in chickens, leads to a decrease in the infective dose of salmonella while such an effect was not found for other antimicrobials studied (Smith, 1975; Barrow, 1984). Avilamycin and bacitracin do not do this in the dosages used for growth promotion (Humbert, 1991; Matthes, 1982; Nurmi, 1974; Smith, 1975; Smith, 1978) and flavomycin has been shown to give a some protection against Salmonella infections (Ford, 1981).

Treatment with several antimicrobials can result in severe, even fatal infection, by *Clostridium difficile* in humans (Mc Farland, 1995) and horses, (Baverud, 1997; Gustafasson et al 1997), and by *Clostridium perfringens* in horses following use of tetracycline (Andersson, 1971).

3.2.2.4. Effects on Human Health

Veterinary antimicrobial use may constitute a threat to human health but the impact of resistance among zoonotic bacteria and the risk of transfer of resistance determinants between animal and human pathogens is still an unquantifiable hazard . In relation to the use of AMGP a review of the literature did not produce evidence to support a proposal that avilamycin, bambermycin (flavomycin), monensin or salinomycin induce or co-select bacterial strains with cross-resistance to antibiotics used in human medicine. However, van den Bogaard and Stobberingh (1996) have identified that cross-resistance exists between avilamycin and the everninomycin Ziracin, a new antibiotic being studied for use in humans. The medical impact of the use of antimicrobials as feed additives in farm animals has been extensively addressed (WHO, 1997) as has the potential risk for human health associated with the use of fluoroquinolones in farm animals (WHO, 1998).

At a first WHO meeting in Berlin in October, 1997, it was concluded that, although the magnitude of the medical and public health impact of antimicrobial use in food animal production is not known, there is enough evidence to cause concern and take action. At a second WHO meeting in Geneva in June, 1998, it was concluded that the use of fluoroquinolones in food animals has led to the emergence of fluoroquinolone-resistant Campylobacter and Salmonella. However, it was also concluded that there

has been little documented impact of this resistance on human health and further research and data gathering are essential to quantify this potential. Finally, it was acknowledged that fluoroquinolones should remain at the disposal of veterinarians to treat sick animals but that these antibiotics should be used in accordance with principles of prudent use.

3.2.3. Impact on Animal Production

The use of antimicrobials has greatly facilitated the present methods of animal production but the emergence of resistance has limited the number of effective antimicrobials available, increased the cost of their use, and has sometimes lead to the adoption of drastic measures to combat the problem. For example, the dramatic ongoing spread in several member states of multiply resistant *Salmonella typhimurium* DT 104 has prompted an attempt to stop its introduction into animal production in Denmark by means of a total eradication policy of infected swine herds (Baggesen and Bager, 1998). This is the first time such a draconian strategy, usually applied to epizootic diseases, has been used against a bacterial pathogen due to the presence of resistance.

Early studies with AMGPs showed clear growth promoting benefits. This lead to their widespread use to improve animal production. With time, however, the growth promoting effects of AMGPs have been diminishing. It has been observed that AMGPs are most effective on farms with poorer animal health and hygiene records and that their mode of action as growth promoter is not always clear (Stahly, 1994). Whether the AMGPs were preventing intercurrent low grade infection or promoting growth by inducing microbial intestinal changes was unclear. The results of the ban on specific drugs by individual member states (Denmark, The Netherlands and Germany), and the 1998 EU ban on four agents, have also highlighted the need for a justification for the continued use of AMGPs. Any use at higher concentrations of AMGPs for the purpose of disease prevention is a violation of Directive 70/524/EEC which states that AMGPs should have no clinical effect. If the real role of these antimicrobials is to control intestinal infections, then a case could be constructed for them to be regulated as veterinary medicines and used only at therapeutic doses for a limited period instead of continuous administration in feed.

It is noteworthy that when all AMGP were withdrawn from use in Sweden in 1986 significant clinical problems due to post weaning diarrhoea were seen in pigs which also showed decreased growth (Robertsson and Lundeheim, 1994). However, no adverse clinical or other effects were observed in Denmark (Jorsal et al 1998) or Sweden when the AMGPs were withdrawn from fattening pigs (Wierup, 1998). The growth rate of these animals (25 kg at slaughter) achieved on conventional farms in the latter country with controlled production efficiency was on average greater than 850 g per day without any antimicrobials in the feed, a growth rate no less than in countries using AMGPs (Wierup, 1998).

The impact of resistance on aquaculture has been to significantly improve husbandry practice as a necessary response and has also greatly encouraged the development of alternatives such as vaccines.

3.3. Resistance Amongst Bacteria in Plant Protection

Antimicrobials used in plant protection are considered to provide an advantage over conventional pesticide chemicals (Misato et al. 1977) in that they may reduce conventional pesticide use by one tenth or one hundred fold. Furthermore antimicrobials may degrade faster in the soil and thus accumulate less in the environment.

3.3.1. Prevalence

Resistance to kasugamycin, an aminoglycoside, was found very early in plant pathogens. Tabei and Mukoo (1955) found streptomycin-resistant plant pathogenic bacteria associated with potato ring rot and soft rot (Misato *et al.* 1977). Wakimoto and Mukoo (1963) isolated 16 streptomycin-resistant *Xanthomonas oryzae* strains (leaf blight pathogen) from infected rice plants (Misato *et al.* 1977); these strains showed cross-resistance to chloramphenicol and cellocidin. Streptomycin and oxytetracycline resistance have been documented in orchards in US during the past years. In the EU, no information on resistance has been available.

3.3.2. Impact on health and productivity

3.3.2.1. Risks to humans and animals

The risk of disease due to drug-resistant plant pathogens in man is negligible since these organisms are not known to be pathogenic to humans or to animals. Nevertheless, species of the same genus can cause disease in both plants and mammals (*e.g. Pseudomonas*). Therefore, while it may be reasonable to presume that there is little or no direct risk for production of human or animal disease by plant pathogens, a risk of acquisition of genes encoding drug resistance in plant pathogens by human or animal pathogens might exist through exposure to antimicrobials of possible mammalian commensals or pathogens present in the environment, and then horizontal gene transfer. Ingestion of water, soil and vegetables containing resistant bacteria could be a possible source of resistance genes for humans and animals. There is also a potential that transmission of bacteria on some vegetable crops from animals to man, which is well documented, may lead to direct infection of consumers with animal pathogens, such as *E. coli* O157:H7, if crops are fertilised with animal manure. These pathogens may develop resistance to the antimicrobials used in plant protection. It has been estimated that in an average salad meal of tomato, lettuce and cucumber about 10^9 bacteria would be ingested (Levy 1984) and it is clear that vegetables and fruits can be a source of antimicrobial resistant bacteria (Khachatourians 1998). Levels of antimicrobial resistance as high as 70-90% have been recorded in microorganisms associated with fruits and vegetables (Levy 1984) and resistant bacteria could be isolated from most fruits and vegetables tested.

Thus an undefined risk exists that resistance genes from organisms in the environment could be transferred to mammalian pathogens. The risk increases if resistance develops against an antimicrobial which is related to (*e.g.* kasugamycin) or is the same as (*e.g.* streptomycin) that used in medicine.

3.3.2.2. Risks to plants

The greatest impact of resistance in plant pathogens will be on plant protection practices. It is inevitable that resistant plant pathogens will emerge. The fact that antimicrobial resistance is now common suggests that plant disease control with antimicrobials may not be possible in the long term. Studies have shown that resistant pathogens adapt quickly to newly introduced antimicrobials implying that cyclic changes between several antimicrobials or other methods will not solve the resistance problem. Effects of resistance on productivity will be particularly marked where pest control relies heavily on antimicrobials rather than on other types of pesticides. The application of streptomycin in fire blight control in apple and pear orchards (Misato *et al.* 1977, Chiou and Jones 1991, 1993) may be significantly compromised by resistance. For example, the frequent occurrence of streptomycin resistance reduces the effectiveness of chemical control of *E. amylovora* in many areas, such as in California on pear trees.

3.4. Resistant bacteria in the environment

3.4.1. Prevalence

Antimicrobial resistance is widespread in the environment. Resistant bacteria can currently be isolated from continental and coastal waters and from soil and sewage treatment plants. Many authors have reported over the past 20 years that 10 to 46 % of the heterotrophic bacteria from waters and sediments contain resistance genes and this may be as high as 76% in bacteria from sewage waters. Since the percentage of soil bacteria that can be cultured is low (1-10%), an accurate estimate of the percentage of natural antimicrobial resistance in soil microorganisms is not possible. The bacterial genera found differ depending on the source of the isolate. For example, soil and manure samples contain *Pseudomonas spp, Alcaligenes faecalis* and *Xanthomonas* spp while in sewage samples enterobacteria dominate (Pukal et al. 1996, Gotz et al. 1997).

Kanamycin/neomycin-resistant bacteria are ubiquitous in nature, the prevalence being dependent upon the source of the bacterial isolates, the highest level (39%) being found in pig manure. Most of the resistance genes are currently restricted to Gram negative bacteria, which often have plasmid-borne genes located on transposons encoding for aminoglycoside modifying enzymes (Courvalin and Carlier 1981). Similarly, genes encoding streptomycin resistance can be found at high frequency in natural populations of bacteria (Shaw et al. 1993). Ampicillin-resistance is seen in up to 10% of environmental bacteria (Parveen et al. 1997).

Antibiotic medication in food producing animals is controlled in the EU to ensure that antibiotic residues are not present in food. However, during use,

antibiotics may enter the environment having been excreted in the faeces and / or urine of treated animals. Antibiotic run off from plant applications is also possible. Animals have also been shown to acquire antibiotic tissue residues from contact with an environment in which other animals have been treated with sulphadimidine and furazolidone. Similarly, oxilinic acid has been detected in crabs and mussels in the vicinity of fish farms for up to 13 days after treatment (Coyne et al (1997) and antibiotic resistant bacteria have also been recovered from sediment from fish farms (Kerry et al 1994, Depaola et al 1995).

3.4.2. Impact on health

Bacterial gene transfer is now thought to occur not only in the human and animal intestine but throughout the biosphere, especially in nutrient-rich sites such as aquatic systems, sediments, soils, in the vicinity of plant roots, and in the sludge of the biological sewage treatment systems. Resistant bacteria can be isolated from all of these sites. Resistance may also be spread from bacteria borne onplants and vegetables treated with antimicrobials or fertilised with wastes containing animal or human faecal residues or derived from fish farms. Resistance should therefore be taken as a phenomenon of global genetic ecology.

A number of reservoirs and habitats may be sites for the emergence and maintenance of antimicrobial resistant microorganisms. These include hospitals, farms, aquaculture, human or animal commensal bacteria, and habitats where faeces and urine from humans and animals are found. Sewage from humans and livestock given antimicrobials is a mechanism of spreading resistance genes. Antimicrobials excreted by humans and animals are found in sewage water and may degrade slowly and exert a continuous selection pressure. In a similar way, antimicrobials used in plant protection are washed into the soil and ground water where they may select resistant bacteria, so favouring the dissemination of resistance genes. The US EPA recognises that there are deficiencies in the present knowledge of the environmental fate and ecological effect of streptomycin (EPA Pesticide Fact Sheet, 1988, updated). No information is currently available on its breakdown in soil and water. Also the Agency is unable to assess the potential for oxytetracycline to contaminate groundwater because the environmental fate of oytetracycline has not been characterised, and neither is it able to assess the ecological effects of oxytetracycline on terrestrial or aquatic wildlife, again because no data are available.

Common factors between the four ecological compartments (humans, animals, plants and soil-water) are the antimicrobials, the bacteria and the genes that code for resistance. The genes move between the bacteria in each compartment, and the bacteria may move between the compartments.

3.5. Antibiotic resistance marker genes in genetically modified plants

Genetic modification of plants usually involves two steps where selectable markers are being used: 1) engineering of the gene construct which is used to transform the plant; this will normally be done in *E. coli;* 2) transformation of the plant and selection of transformants. Markers are used for selecting the

desired transformants among the non-transformed individuals. Some of the antibiotic resistance marker genes encode resistance to: ampicillin, chloramphenicol, kanamycin, streptomycin, amikacin, tetracycline, hygromycin, gentamicin and phleomycin resistance markers.

3.5.1. The fate of plant DNA in the gastro-intestinal tract

Consumed as a component of e.g. fresh fruit or vegetable, antibiotic resistance marker genes are treated in the gastro-intestinal (GI) tract in a similar way to any other gene present in food of plant or animal origin. During movement in the GI tract, plant DNA is rapidly degraded into small fragments. According to an estimate, 1 g of maize tissue will yield no more than 100 µg of genomic DNA in the gut, and estimated 50 pg intact marker gene in transgenic maize. About 1-2% of orally ingested M13 DNA persists transiently as fragments between 1 and 7 h after feeding in the gut and faeces of mice (Schubber *et al.*, 1994). The bulk of these fragments are 100-400 bp in size (the size of the gene which codes for TEM-1 beta-lactamase is <900 bp). The small intestine contains about 2.2-0.7% (1-8 h after feeding), the cecum 2.4-1.1% (2-18 h), the large intestine 0.2-1.7% (2-8 h) of the DNA orally administered. A few percent (<5%) of the ingested DNA (up to 1700 bp) may be excreted as fragments in the faeces (Schubbert et al., 1994, 1997).

3.5.2. The potential for integration of DNA from food into intestinal microorganisms

Transformation of intestinal bacteria with food-derived plant DNA has never been demonstrated and attempts to transform competent *E. coli* bacteria with plant DNA *in vitro* have been unsuccessful. In nature, the processes of integration, heterologous transcription and translation, and not DNA flux, are likely to be the limiting factors in functional gene exchange. Recombination is probably the most serious barrier to functional inter-specific gene transfer. Because of this, gene transfer events mediated by natural transformation are most likely to occur between members of the same or closely related species. It is important to note that most transgenic plants have pUC 18 plasmid, which does not have homology to most bacterial genomes, and no transfer functions. Thus it seems unlikely that pUC18 DNA could sucessfully transform bacteria pathogenic to man.

3.5.3. The probability of expression of an integrated gene in intestinal microorganisms

The antibiotic resistance marker genes used for selection of plant transformants have regulatory sequences that may not function in gut microorganisms; in those cases, recombination would have to occur to restore functionality. Complicated rearrangements, especially under selective pressure, may bring a prokaryotic promoter in front of the marker gene, leading to its expression. The expression of antibiotic resistance marker genes which serve to facilitate the selection of transformants is under the control of regulatory sequences. In principle, these regulatory sequences might allow the marker genes to be expressed at least in some types of intestinal bacteria. However, promoters of genes from one phylogenetic

group of bacteria usually do not work in a member of another phylogenetic group (Salyers 1997). A 'silent' resistance can become activated by insertion of an insertion sequence in the promoter region or mutations in the promoter region that cause it to become active. Only if a selective pressure is present, which gives an advantage to the recipient of the gene, will the gene transfer event have any concrete consequences.

3.5.4. The protein encoded by antibiotic resistance gene in plant as a potential safety issue

A special concern with respect to antibiotic resistance genes is the theoretical possibility that clinical therapy could be compromised due to inactivation of an oral dose of antibiotic as a result of consumption of food derived from the transgenic plant. Any such risk arising as a result of the protein should correlate with the amount of enzyme remaining functionally active in the GI tract. This, on the other hand, depends on a) the estimated daily intake (EDI) and thus the level of the active protein in food, and b) stability of the protein in the GI tract. FDA calculated that the EDI of APH(3')II (kanamycin resistance gene) was 480 µg/person/day (FDA 1994). Proteins present in food and entering the GI tract are broken down to smaller peptides and amino acid constituents by digestive enzymes. The purified APH(3')-II protein was degraded in 10 seconds in *in vitro* assays developed to simulate the human GI environment. Tomato extract and non-fat milk, added to determine whether the presence of additional food-source proteins might slow the proteolytic degradation of the enzyme, did not prevent the effective degradation of the protein.

It has been calculated that, under conditions where the maximum amount of kanamycin could be inactivated by the marker protein in ingested tomatoes, the loss of antibiotic efficacy would be 1.5% of a 1 g dose of neomycin (Redenbaugh *et al.* 1993, 1994), and only after oral administration.

3.5.5. Impact on human health, on animal health and animal production

In general, it can be concluded that:

(1) Most, if not all, of the antibiotic resistance genes ingested (in the form of plants) will be degraded in the gastrointestinal tract before reaching the critical areas where potential transformation of microorganisms take place.

(2) Even if intact DNA is present, the probability that competent microorganisms will be naturally transformed by this exogenous DNA in humans, animals or environment is very low.

The probability that gene transfer between plant and microorganisms occurs and creates health problems seems to be extremely low and needs to be considered only in special cases where the antibiotic is administered by the oral route and there is also heavy selection pressure. The nature of the gene and its expression product as well as the conditions in the GI tract will determine whether or not a food safety problem exists. Aspects to consider are the importance of the substrate antibiotics in human and animal therapy

and whether there are alternatives (*e. g.* vancomycin, other glycopeptides, fluoroquinolones, tetracycline, gentamicin, newer derivatives of beta-lactam antibiotics), frequency of use (probability of selection pressure) and route of administration. The substrate profile of enzymes should be carefully analysed to see whether there is any chance that they may catalyse the inactivation of an important antibiotic used in human therapy. Use of the antibiotic in the environment may cause additional selection pressure; e.g. streptomycin and oxytetracycline are used as pesticides.

Hence, although the risk of gene transfer is extremely small, each plant containing antibiotic resistance genes should be evaluated on a case-by-case basis. The main emphasis should be on the evaluation of the selection acting on the bacterial recipients after possible horizontal gene transfer. Assessment of potential for transfer of antibiotic resistance marker genes from plant into the bacterial community should be examined more closely.

4. AMOUNTS OF ANTIMICROBIALS USED

Introduction

If the assumption that increased antimicrobial use correlates with an increased prevalence of antimicrobial resistance holds true, then information on the consumption of antimicrobials in human and veterinary medicine, as feed additives, or for phytosanitation purposes should provide insight into the relative contribution of the four areas to the overall problem. Data by country might even point to a correlation between consumption of individual antibiotics and the variations in prevalence of resistance to each across the EU.

The amount of accurate information available, however, is limited and it is not known whether the key factor is the total tonnage of active substance consumed or the way in which antibiotics are administered (e.g. dose regimens, duration of courses, in hospitals or in the community).

Consistent sources of information across the community have been difficult to find. As a broad generalisation, FEDESA (1997) has provided data which shows that of 10493 tons of active ingredient antibiotic consumed during 1997, approximately 52% was used in human medicine, 33% in veterinary medicine and 15% in animal production. (see Annex, Table 2).

4.1. Human medicine

Of the approximately 50% of total antimicrobial consumption (in terms of tonnage) which is used in human medicine approximately 80% is used by generalists and 20% by specialists in hospitals (Harrison, 1998). There may be variations in these proportions between countries but evidence submitted in the UK confirms that by far the greatest use occurs in the community (House of Lords, 1998).

There is no consensus on the unit of measurement to compare the consumption of antibiotics between hospitals and the community. Most of the commercially prepared data gives information based on costs, with limited or no information on the way antimicrobials are prescribed by indication, dose, dosing regimen, duration of treatment, compliance or treatment outcome. Some information prepared by pharmaceutical companies gives approximate quantities prescribed, whereas that released by governmental authorities, is sometimes transformed into indices such as defined daily doses (DDD) which accommodate average daily dose and duration of treatment. Most of the data from these different sources is not comparable so that a precise survey of use is not possible.

The ATC/DDD (ATC – Anatomical and Therapeutical Classification) methodology has been used by several investigators to compare the use of drugs in hospitals. The DDD (Defined Daily Dose) is given for each drug by the World Health Organisation and in a very few studies DDD per 100 beddays has been examined. However, drugs may be used at different dosages in different countries. In one of very few comparative studies on antibiotic consumption in the EU, Janknegt et al concluded, based on DDD

per 100 beddays, that antibiotic consumption in Belgium was higher than in the Netherlands and Germany. However, higher DDD per 100 beddays were found in Belgium because in the latter more coamoxyclav is used, for which the authors had used a parenteral DDD of 1g (whereas this drug is prescribed in Belgium at a dosage of 3 to 6 g daily). Thus, the conclusion was biased by a single antibiotic. For this reason, DDA (Defined Daily Administration) which is based on the prescribed daily administration, is used to compare antibiotic consumption between hospitals in Belgium (Anonymous, Rijksinstituut voor Ziekte en Invaliditeitsverzekering, Brussels, Belgium, 1996). Also, Janknegt et al provided no information on the hospitals selected in the three countries (number of intensive care, haematology or oncology beds, teaching hospitals, etc). Similarly, there is no consensus on the methodology for comparison of antibiotic consumption in the community (DDD per 1,000 inhabitants? DDD per 10,000 inhabitants? etc.)

The data do reveal that human consumption of antimicrobials is on the increase. A recent French study found a mean annual increase of 3.7% in antimicrobial use between 1981-82 and 1992 (Guillemot, 1998). In the UK, the number of prescriptions showed an annual increase of 5% from 1989 to 1991(Davey, 1996), although the trend levelled off in 1993 (Prescription Pricing Authority, 1996). Respiratory tract infections (RTIs) are the most frequent reason for prescribing antimicrobials in the community, despite the likely non bacterial aetiology of respiratory tract infections (RTIs) (Gonzales, 1997) (Guillemot, 1998).

There are further differences between countries within Europe. A recent report from France (report to Observatoire National des Prescriptions et Consommation des Medicaments) compared prescribing for RTIs in France, UK and Germany. The annual number of consultations per 1000 inhabitants for pharyngitis and rhinopharyngitis is greater in France than in the other two countries and proportion of patients receiving an antimicrobial for presumed viral RTIs is greater in France and UK than in Germany. The types of drugs used also differ. Oral cephalosporins are more often used in France and penicillin derivatives more frequently used in the UK. In England and Wales sufficient oral antibacterials were prescribed annually in the community to treat every member of the population for 5 days a year (Prescription Pricing Authority, 1996).

4.2. Veterinary medicine and animal husbandry

Governments have not so far requested that data on antimicrobial consumption by animals be supplied by industry, suppliers or pharmacists. The most comprehensive regularly available data comes from Sweden (SOU, 1977). Some other EU countries also publish data, but the quality does not allow reliable comparison between countries and between the usage in different animal species where antimicrobial dose regimens often vary greatly. The European Federation of Animal Health industries (FEDESA, 1997) provided data on the animal health product market (as monetary values per animal) for Europe in 1995 using manufacturers' prices. Total sales were 3.3 billion Euro of which therapeutic pharmaceutical products comprised 48%, feed additives 37%, and biologicals 15%.

The World Federation of Animal Health Companies (COMISA) have stated that nutritional feed additives comprise the largest individual sector in sales values accounting for 24% of antibacterials, other antimicrobials 16%, and medical feed additives 12% (FEDESA, 1997). Of the world market, "Western Europe" accounted for 25% and Central Europe 9% of total consumption. The figures for individual countries were: France 6%; Germany 5%; UK, Spain and Italy each 3%. Further information is given Annex 2.

Antimicrobials are also used in fish farming and this has given cause for environmental concerns. (Husevag, 1995; Lunestad, 1993; Yndestad, 1993). Accurate records of the amounts used are not available but there is evidence to show that it has decreased considerably of late. This has resulted from development of good husbandry with consequent reduction of disease transmission, together with the development and use of effective vaccines. In the Norwegian Atlantic salmon industry, antibiotic usage has fallen by 90% from 70 tonnes per year in 1987 despite a doubling in the tonnage of salmon produced.

4.3. Human vs. animal consumption

It is difficult to compare with any degree of precision the relative consumption of antimicrobials between human and animals in the EU. Such comparisons should accommodate the relative sizes of human and animal populations and some data are given in Annex 2. In Finland in 1997 the amount of therapeutic antimicrobials registered for animal use was 20 tonnes of active substance which is about 47% of the amount used annually for humans (Mannerkorpi, 1996).

In France the only reliable data were considered to be sales values but, as emphasised by Van den Bogaard (1997), expenditure rather than volume is an unreliable guide to usage. A more detailed comparison of the animal and human usage was presented from Sweden 1980 (Wierup, 1984). The national average annual human usage was calculated to be 415mg of active substance/kg metabolic body weight (see glossary for conversion of metabolic weight). The corresponding data for therapeutic usage in food animals was 59mg/kg, and was 89mg/kg when AMGPs were included. The intensity of the use was thus approximately 7.0 or 4.6 times larger in humans compared to animals. However, in the Netherlands Van den Bogaard (Van den Bogaard, 1997) found the usage of any active antibiotic substance per kg body weight/year in 1990 was 100 mg for humans, 430 mg for poultry and 125 mg for pigs.

4.4. Plant Protection

World-wide, a variety of antimicrobials have been allowed for use on plants, including chloramphenicol, griseofulvin, nystatin, oxolinic acid, streptomycin and (oxy)tetracycline. In the US, the annual use of antimicrobials is 23 million kg, of which over 8 million kg is used in the agrifood industry (Khachatourians 1998). Fire blight (*Erwinia amylovora*) is one of the major plant diseases for which antibiotics are used. For fruit trees (22,000 kg) mainly oxytetracycline (ca. 9,600 kg; on pears and peaches; EPA

Pesticide Fact Sheet 1988, last modified 03/17/1998) and streptomycin (ca. 10000 kg; on apples and pears; ENVI/471) are used. Streptomycin is also used on vegetables and non-food crops such as tobacco and greenhouse ornamentals, though there is no accurate record of the amounts used.

Only limited information on the amounts of antimicrobials used for plant protection within EU is available. A recent enquiry by DGVI showed the use of kasugamycin in Spain as 1,994 kg (1997). The areas treated represented 3% of horticultural cultivation, 7% of bean cultivation and 0.1% of seed fruit or citrus fruit orchards. Usage of streptomycin in Austria was 12 kg (1998; apples), Belgium 755 kg (1997; apples, pears), and the Netherlands 170 kg (1997; apples, pears).

4.5. Conclusions

The quality of information available on antibiotic use is poor and is inadequate as a basis from which to draw useful scientific conclusions regarding the relation between antimicrobial resistance and the amounts of antimicrobials used. Improving the quality and quantity of this information in the EU will be important for the evaluation of any proposed intervention.

Good quality data collected according to standardised methodology will be essential to establish a baseline for the relative consumption (human, veterinary, animal feedstuffs and plants) and would make possible the detection of changes over time in response to voluntary or statutory curbs on usage.

5. RELATIONSHIP OF THE USE OF ANTIMICROBIALS TO RESISTANCE AND ITS TRANSFER BETWEEN ECOSYSTEMS

Introduction

The emergence of antibiotic resistance and its spread or transfer between different ecosystems is complex and several important steps occur between the emergence of antibiotic-resistant bacteria in animals and the occurrence of treatment failures which may have serious consequences in humans and animals. These involve the **emergence** of antibiotic resistance, **selection** as a result of antibiotic use, the **spread** of antibiotic resistance from one ecological compartment to another and the **clinical impact** in humans and animals of infection with resistant bacteria.

The **Emergence** of antibiotic-resistant bacteria may be due to mutational changes and/or transfer of resistance genes among identical or different bacterial species and the genetic events involved have been reviewed in Chapter 2.

The **Selection** of antibiotic resistant bacteria is driven by exposure to antibiotics and several studies have demonstrated this link. The transmission of resistant bacteria between different ecological compartments may also be facilitated if the number of resistant organisms increases as this increases the probability of transfer of genetic resistant determinants.

The **Spread** of antibiotic-resistant bacteria from one ecological compartment to another (e.g. from animals to humans) can either be direct or indirect (e.g. via the food chain). However, bacteria may be pathogens to some hosts but natural inhabitants in others, and bacteria present in one host (as commensal or pathogen) may not be able to colonise another. Thus, for zoonotic bacteria (*Salmonella spp, Campylobacter spp*), it may be relatively easy to study a link between antibiotic use in animals and the development of infection with resistant organisms in humans. But for those bacteria that are generally considered commensals in humans (enterococci, E. coli), such a link is more difficult to assess. Firstly, all bacteria should be considered as potential pathogens, depending on factors such as the presence of specific virulence determinants, site of infection and efficacy of the host immune system. Secondly, even for bacteria derived from animals that are not able to colonise humans, studies have shown that antibiotic resistance genes can be transferred to other human commensal and pathogenic bacteria. However, such data are very limited and focus on specific antibiotics and bacterial species. Indeed, it is clear that all genes are not equally successful in their transfer. Yet, the probability of such transfer will increase if the resistant bacterial population is enriched following the use of antibiotics in humans.

Finally, the **Clinical impact** of infection with resistant bacteria in humans is substantial but it is difficult to study and has not been investigated in scientifically designed, prospective experimental studies. Conclusions in this respect must therefore be based mostly on observational studies. Moreover, patients who die of infection with resistant bacteria and / or superinfecting pathogens are frequently immunocompromised.

5.1. Human Medicine

Although many important individual case studies have been reported, precise quantitative evidence from scientific prospective studies are generally lacking in the area of human medicine. The issue has therefore been examined from the perspective of patterns of use and access to antibiotics.

5.1.1. Use and availability of antibacterials

Countries with the highest rates of antibacterial resistance amongst human pathogens also have high per capita consumption of antimicrobials. The USA and Japan account for about 10% of the world population but over 60% of the world market in antimicrobials and both have very high rates of resistance amongst common human pathogens. A parallel increase in antimicrobial prescriptions and the development of resistance has been demonstrated for *S. pneumoniae* and beta-lactam use (Baquero, 1996). Equally important, a clearly demonstrable fall in the rate of macrolide resistance in *S. pyogenes* followed a decreased use of erythromycin in Finland (Seppala, 1997). However, this was paralleled by an increase in the prevalence of erythromycin resistant *S. pneumoniae*. Further evidence comes from examples where the introduction and policing of hospital policies which effectively control antimicrobial use have led to a significant reduction in the prevalence of some resistant pathogens (Betts, 1984; Pear, 1994; Shlaes, 1997; King, 1992).

Using genetic methods and epidemiological observations, Austin et al. (1999) reported an analysis of the influence of the selective pressure imposed by the volume of drug use on temporal changes in resistance. Analytical expressions were derived to delineate key relationships between resistance and drug composition. The authors showed that there is a critical level of drug consumption required to trigger the emergence of resistance to significant levels. Their analysis also indicates that the time scale for emergence of resistance under a constant selective pressure is typically much shorter than the decay time after cessation or decline in the volume of drug use and that significant reductions in resistance required equally significant reductions in drug consumption. Besides continuing the relation between resistance and use, these results also highlight the need for early intervention once resistance is detected.

In all EU member states systemic antibacterials are licensed for use as prescription-only medicines. In some countries, however, they can be purchased from pharmacies without prescription and such ease of access is considered a contributory factor to the high rate of resistance amongst common pathogens in some countries. However, severe problems with resistance may occur even in those countries (e.g. Hungary) where prescription only status is strictly enforced. Outside the EU, in Africa, Latin America and SE Asia antimicrobials are readily available for purchase. High prevalences of resistance have been reported from at least some of these areas. As in the EU, it is difficult to obtain a clear picture of trends over time.

5.1.2. Type of antibacterial

Some antimicrobial agents select for resistance more readily than others. For example, rifampicin resistance is commonly mediated by a single mutation and resistant sub-populations are readily selected when the drug is administered alone. In addition previous administration of third-generation cephalosporins has been demonstrated to be more strongly associated with the selection of multi-resistant *Enterobacter spp* compared with other drug types (Chow, 1981).

5.1.3. Method of use of antibacterials

Antibiotic use selects for resistant microorganisms, but little is known about the relative contributions of dose, dose interval and duration of therapy to selection pressure. It would seem reasonable to suppose that the longer the duration of therapy, the more likely it would be that the normal flora will be deranged and colonisation and superinfection with micro-organisms resistant to the antibacterial being used would occur. The dose administered may also be an important factor. Conversely, short courses may encourage the survival of the least susceptible members of the population of bacteria. Indeed, the emergence of penicillin-resistant gonococci has been attributed to extensive use of single dose therapy. A recent study has shown a relationship between the amount of parenteral fluoroquinolone usage and risk of nosocomial infection due to *Acinetobacter alcaligenes* (Villers, 1998), especially when low doses are used. The route of treatment may also play a part in selecting for resistance. While oral therapy is more convenient and cheaper than parenteral, antibacterials which are poorly absorbed may put a considerable selection pressure on bacteria in the lower small bowel and colon. Systemic or topical therapy may encourage the overgrowth of drug-resistant species of the normal flora, on the skin and in the gut, and the dissemination of these resistant strains to contacts (Miller, 1996).

5.1.4. Hospital vs. community use of antimicrobials

The environment in which antimicrobials are used is important because of differences in the intensity of the selection pressure and chances for the spread of resistant organisms. The intensity of the selection pressure is greater in hospitals where patients receiving antibacterial therapy are concentrated. Although the total selection pressure may be greater in the community because total consumption is greater, it is less likely that several members of any group would be taking antimicrobials at the same time. However, it is not known whether the total selection pressure or the intensity of the selection pressure is the more important contributor to the overall prevalence of resistant micro-organisms.

Resistant organisms are spread in the same way as all other bacteria. Spread may occur more readily in hospitals, where it is most commonly by staff-patient contacts and may be exacerbated by overcrowding, insufficient attention to hygiene measures and movement of patients within and between hospitals. Spread in the community occurs by many different routes; oral

inoculation of gastro-intestinal pathogens and person to person spread of respiratory pathogens are among the most important.

5.2. Veterinary medicine and animal production

Several retrospective and prospective studies have been performed to evaluate the correlation between antimicrobial usage in animals and the development of resistance in bacteria from animals. Most have shown that the introduction of specific antimicrobials into veterinary practice is followed by detection of resistance to those antimicrobials.

Approximately 90% of antimicrobials used in veterinary medicine and all AMPGs are given orally to food animals either in the feed or in drinking water or milk. As a result of EU legislation and the effects of the Swann report (Joint Committee, 1969), most of the antimicrobials used for clinical purposes in humans and/or animals are not used as AMGPs. Drugs of the same class, to which cross-resistance can occur, are used, however, and this may be an important issue. Some specific examples include:

5.2.1. Fluoroquinolone use in Poultry

Fluoroquinolone are used for treatment of animals but are not used as AMGPs in the EUQuinolones do not eradicate *Campylobacter jejuni* from chickens; it has been shown that birds with previously quinolone-susceptible strains harbour resistant strains within a few days of treatment (Jacobs-Reitsma, 1994). In Great Britain enrofloxacin was registered for veterinary used in 1993, at which time 14% of *C. jejuni* isolated from poultry carcasses imported from the Netherlands (where it was introduced in 1987) were fluoroquinolone-resistant, whereas in locally raised broilers it was only 1% (Gaunt, 1996). By 1997 the percentage fluoroquinolone resistant *C. jejuni* from English broilers (10%) had approached that on the Continent. The comparatively low prevalence of fluoroquinolone resistance among isolates of *C. jejuni* from Swedish broilers may be a reflection of the fact that these agents have been used only occasionally in broiler production in Sweden (Berndtson, 1995).

5.2.2. Glycopeptides

Avoparcin, a glycopeptide not used in man but until recently commonly used as an AMGP in most EU states, selects for VRE in the intestinal flora of animals fed avoparcin (Bates, 1994). VRE are found in the faecal flora of healthy humans and animal pets in countries where avoparcin is used as a AMGP on farms (Endtz, 1991; van Belkom, 1996; van den Bogaard, 1997; van den Bogaard, 1996).

An association between avoparcin and a high prevalence of glycopeptide resistance (VanA) among animal isolates was reported by Aarestrup, 1998; Bates, 1994; Bager, 1997; Devriese, 1993; DANMAP, 1997. In Denmark, the isolation of VRE from faecal samples of pigs and poultry was three times higher in animals fed avoparcin than from other animals (relative odds ratios were 2.9 (1.4-5.9) for poultry and 3.3 (0.9-12.3) for pigs. Mevius (1999; Health Council of the Netherlands, 1988; Hanekamp JC et al. 1999)

observed a higher percentage VRE per gram faeces (10%-100%) in veal calves from farms using avoparcin as an AMGP than in faecal samples of calves fed bacitracin (1%-10%). The prevalence of VRE in turkey flocks fed avoparcin was 60% in contrast to 8% in flocks not exposed to avoparcin (van den Bogaard, 1996); the relative odds ratio was 7.5.

In contrast, in Sweden, where glycopeptides have not been used in animals since 1985, no vancomycin-resistant enterococci have been detected in animals so far (SOU, 1997; van den Bogaard, 1998) and in the USA, where avoparcin has never been used, no high- level VRE has been found in faecal samples of food animals or healthy humans outside hospitals (van den Bogaard, 1997; Coque, 1996). Following the suspension of the use of avoparcin in Denmark, the prevalence of vancomycin- resistant *Enterococcus faecium* in broilers has decreased from 52% in 1995 to 12% in 1997 (Aarestrup, 1998;DANMAP, 1997).

5.2.3. Macrolides and streptogramins used as AMGPs

Resistance to macrolide-lincosamide-streptogramin-type (MLS)-antimicrobials like erythromycin and pristinamycins is common in enterococci from animals fed tylosin (a macrolide) or virginiamycin (a streptogramin) (van den Bogaard, 1997) as AMGPs. In a 1995 Danish study (DANMAP, 1997), the prevalences of resistance among enterococci from pigs and poultry to erythromycin were 91% and 59%, and were 53% and 37% to the streptogramin mixture dalfopristin – quinupristin, which is being developed for use in man. In Finland, however, where tylosin is only used to treat swine dysentery and not used as an AMGP, the prevalence of erythromycin resistance in enterococci is significantly lower at 18% in pigs and 9% in poultry (Tast, 1997). Linton (Linton, 1985) found a significant increase in the prevalence of resistance to tylosin in faecal enterococci of pigs and poultry but virginiamycin use was not associated with an increase in resistance. The data show that the use of macrolides and streptogramins in broiler and pig production is associated with the emergence of macrolide and streptogramin resistance in enterococci (Aarestrup, 1998) (DANMAP, 1997) (van den Bogaard, 1998).

5.2.4. Tetracyclines

In the Netherlands the prevalence of tetracycline resistance in human and animal salmonella isolates increased until the ban of tetracycline as an AMGP (Manten, 1971), after which it declined gradually (Voogd, 1977). In Great Britain, after the ban of tetracycline as an AMGP, tetracycline-resistant *S. typhimurium* isolates from calves fell from 60% in 1970 to 8% in 1977 (Cherubin, 1984). There is a view, however, that natural phenomena and the spontaneous ending of epidemics by virulent tetracycline resistant *S. typhimurium* clones might have contributed to this decrease (National Academy of Sciences, 1980). Despite stopping use of tetracycline in pigs in 1971 in the UK there was no reduction in resistance among *E. coli* between 1970 and 1975 (Smith 1975).

5.2.5. Olaquindox and carbadox

After the introduction of olaquindox in 1982, the prevalence of resistance in faecal *E. coli* of pigs increased in three years from 0.004% to 6% in farms using olaquindox as an AMGP. In farms not using olaquindox the prevalence of resistance increased as well, but to a significantly lesser degree (Linton, 1988). Ohmae (Ohmae, 1983) noted an increase of resistance to carbadox in faecal *E. coli* isolates of pigs after its introduction; resistant isolates from six farms that fed carbadox continuously to pigs either as AMGP or for prevention of swine dysentery, carried the same transferable plasmid conferring carbadox resistance. However, carbadox is not used in poultry and no resistance was found in *E. coli* from poultry in the same region. Mills and Kelly (Mills, 1986) found an increase in resistance to carbadox among *E. coli* isolates from pigs from 37% to 61% during a period when carbadox was used not only used as an AMGP, but also for prevention of swine dysentery and therapy of salmonellosis.

5.3. Plant protection

The relationship between antibiotic use in plant protection and resistance can be considered at two different levels: resistance development in human and plant pathogens.

No significant human dietary exposure is anticipated from antimicrobials (streptomycin, oxytetracycline) used as pesticides in fruit and vegetables. A survey conducted in Finland by the National Food Administration in October 1998 revealed no antimicrobial residues in imported vegetables. According to the EPA Pesticide Fact Sheet (1988), streptomycin residues are non-detectable (<0.5 ppm) in or on crops. Potential daily exposure to streptomycin as a pesticide is <0.01% of the daily clinical dosage.

However, antimicrobials used in plant protection are washed into the soil and ground water where they may select for resistant bacteria, favouring the dissemination of resistance genes. The US EPA recognises that there are deficiencies in the present knowledge of the environmental fate and ecological effect of streptomycin and oxytetracycline (EPA Pesticide Fact Sheet, 1988, updated).

Resistance development in plant pathogenic bacteria has been observed after introduction of antimicrobials as plant protection products. For example, there is a correlation between streptomycin use and resistance development in plant pathogenic bacteria associated with apple and pear trees treated with streptomycin (Burr *et al.* 1988; Chiou and Jones 1991, 1993). The resistance genes were completely homologous to those found in the well-known clinically isolated streptomycin/sulphonamide resistance plasmid RSF1010; the transposon harbouring the resistance genes was shown to be present in a variety of streptomycin-resistant bacterial isolates suggesting its involvement in spread of streptomycin resistance under selection in the environment.

5.4. Resistance transfer between components of the ecosystem

Four ecological compartments may be considered as important for the transfer of resistance to antimicrobials; humans, animals, plants and soil-water. The common factors between the four ecological compartments are the antimicrobials, the bacteria, and the genes that code for resistance. Some resistance genes have been shown to move between bacteria in each compartment and it is possible for bacteria to move between the compartments.

Bacterial gene transfer is now thought to occur not only in the human and animal intestine but throughout the biosphere, especially in nutrient-rich sites such as aquatic systems, sediments, soils, in the vicinity of plant roots, and in the sludge of the biological sewage treatment systems. Antimicrobial resistant bacteria have been isolated from all of these sites (Dahlberg, 1998; Hill et al., 1998; Ashelford et al. 1997; Wellington and van Elsas, 1992; Fry and Day, 1992). Resistance may also be spread from plants and vegetables treated with antimicrobials or fertilised with wastes containing animal or human faecal residues. Thus resistance should be considered as a phenomenon of global genetic ecology.

The crucial questions are whether resistance genes are transferable between environmental microorganisms and mammalian pathogens, and whether there are cascades of exchanges between related species or genera. The chain of resistance transfer is probably much more complicated and longer from plant pathogens to mammals than from animals to man. At present, no definitive antimicrobial resistance rates and predictive models are available.

5.5. Transfer of resistant bacteria and resistance genes from animals to man

There is considerable evidence to support the view that antimicrobial use in animals, both in the therapy of infections and as AMGPs, is associated with an increasing prevalence of bacteria exhibiting resistance to the agents used. Many drugs used in animals can select for bacteria which are resistant to antimicrobials used in man. An important question is to what extent the increasing prevalence of antimicrobial resistance in animals contributes to the increasing prevalence of resistance among human pathogens.

Transmission to man of zoonotic agents such as *Salmonella* spp. and *Campylobacter* spp. is of particular importance in assessing this relationship. Antimicrobial treatment of salmonella infections in animals generally results in prolonged carriage, as found in man (Wierup, 1994). Resistant bacteria and salmonella are generally present in the intestinal tract of a large proportion of food-producing animals (Wierup, 1994; ECOSOC, 1998) and it is impossible to prevent carcass contamination by the intestinal microflora during slaughtering and subsequent steps in the food chain (Bôgel, 1991). Thus humans will acquire both pathogenic and non-pathogenic antibiotic resistant organisms from animals. This can be partly controlled but not entirely prevented by good food hygiene. Zoonotic bacteria like salmonella therefore have to be controlled primarily during food production, according to the concept of pre-harvest pathogen control (WHO, 1983; Wierup, 1995).

5.5.1. Zoonotic bacteria

Most investigations on the transfer of resistant bacteria from animals to humans concern food-borne infections caused by *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp.

5.5.1.1. Salmonella

Before the introduction of antimicrobials salmonellae were fully susceptible to most agents (Datta, 1983). Evidence for asymptomatic passage of salmonellae from animal products to man came from a study of serotypes from isolated asymptomatic carriers in a meat packing plant which were shown to correspond with those isolated from raw meat (Deleener, 1980). In most EU states *S. enteritidis* is the most common serotype implicated in human infections, probably due to its extensive dissemination among poultry since 1980. Because this serotype does not usually cause clinical symptoms in affected birds, they are not treated with antimicrobials. The selection pressure is therefore low and most isolates are still susceptible to most antimicrobials.

Sporadically, however, epidemics due to clones with enhanced pathogenicity for animals occur, such as *S. typhimurium* phage type 29 from 1963 till 1969, definitive type (DT) 204 in 1977 and DT 204 and DT 193 in 1980 (Bezanson, 1983; Holmberg, 1984; Spika, 1987;Cherubin, 1984). Because these strains cause serious disease, animals are treated with antimicrobials and, as a result of the selection pressure, multi-resistance emerges. During all these epidemics the same phage type with identical resistance profiles was isolated from animal and human infections. Calves are the primary reservoir of *S. typhimurium*, but sheep, goats, pigs, poultry and horses can also become infected. *S. typhimurium* DT 104 has caused an epidemic in animals since 1994. From its outset this strain was resistant to most of the antimicrobials normally used to treat enteric infections in animals, but it has acquired in addition resistance against trimethoprim and fluoroquinolones (Wray, 1997), most likely because animals could only be treated with these antimicrobials.

5.5.1.2. Campylobacter and Yersinia

Poultry form the most important reservoir for human campylobacter infections. Endtz et al. (Endtz, 1991) observed that the emergence of fluoroquinolone-resistant *C. jejuni* infections in humans in the Netherlands coincided with the introduction of enrofloxacin (a fluoroquinolone) for poultry therapy in early 1987. However, ciprofloxacin was introduced for human use in the Netherlands in 1988 so that the 1989 finding that 14% of poultry and 11% of human isolates of *C. jejuni* were resistant is a little difficult to interpret. The *C. jejuni* strains from chickens in Sweden were considerably more susceptible to fluoroquinolones than the human domestic strains (33%) isolated the same year indicating that Swedish chickens were not the primary source of human infections with *C. jejuni* (Berndtson, 1995) (Sjögren, 1993). Campylobacter from dogs and cats have been found to be resistant to neomycin, tetracycline, tylosin, erythromycin and metronidazole

Transfer of chloramphenicol resistant *Yersinia enterocolitica* strains from animals to humans has been described (Perez-Trallero, 1988).

5.5.2. Commensal bacteria

Exposure to antimicrobials is associated with an increased prevalence of resistance among bacteria of the normal flora of both humans and animals which may be . considered a good indicator for the selection pressure exerted by antimicrobial use (Murray, 1992) and for the resistance problems to be expected in pathogens (Lester, 1990). Resistant bacteria from the intestinal flora of food animals may spread to farm workers as a result of direct contact and poor hygiene; bacteria also contaminate carcasses of slaughtered animals and reach humans via the food chain. Investigation of the prevalence of resistance of certain indicator bacteria like *E. coli* and enterococci in the intestinal tract of different populations of animals and humans makes it feasible to detect a possible transfer of resistant bacteria from animals to humans and vice versa.

5.5.2.1. E. coli

Corpet showed that the prevalence and degree of resistance in faecal *E. coli* flora of humans who ate only sterilised food decreased significantly (Corpet, 1988). Nijsten found significantly more resistant *E. coli* in the faecal flora of pig farmers compared with urban residents (Nijsten, 1996) but the personal antimicrobial usage of the farmers was much higher than that of urban residents. A Netherlands study which compared the prevalence of ciprofloxacin-resistant *E. coli* in faecal samples of turkeys and turkey farmers (enrofloxacin used) with pigs and pig farmers (no enrofloxacin) suggested that transfer occurred from turkeys to turkey farmers in that strains were indistinguishable on typing (van den Bogaard, 1997). The infection risk seemed much lower for workers in processing plants despite the fact that - ciprofloxacin-resistant *E. coli* have been isolated from the turkey carcasses after slaughtering (van den Bogaard, 1996). In contrast, there was no difference between the prevalence of furazolidone-resistant *E. coli* between the pig and turkey populations in the same study, or between the two groups of farmers, which likely reflects the fact that furazolidone was used extensively in both species.

5.5.2.2. Enterococci

In the same Netherlands study, VRE were isolated from a turkey farmer and from his turkeys, which were not only identical on typing but had also a *VanA*-gene with a unique mutation (van den Bogaard, 1997). This may be an indication of transfer of resistant strains from animals to humans. Also in the Netherlands, no VRE were isolated from faecal samples of a group of vegetarians, but VRE were frequently found in meat-consuming humans (Schouten, 1997). In Sweden, no VRE were found in the faecal flora of healthy humans and animals. This is in concordance with the results Quednau et al., who were able to isolate VRE from Danish, but not from Swedish meat (products) (Quednau, 1998). No VRE could be detected in stool samples of healthy Swedish volunteers after taking a course of vancomycin orally (Edlund, 1997). In contrast a similar experiment in

Belgium showed that volunteers, in which no VRE were found in their stool samples before the study, all became positive after oral vancomycin. (van der Auwera, 1996).

5.5.3. Transfer of resistance genes from the animal flora to human pathogenic and commensal bacteria

In 1976, Levy demonstrated that tetracycline resistance genes were transferred between chicken, and from chicken to human, *E. coli* (Levy, 1976). Hummel et al. (Hummel, 1986) examined the consequences of the 1982 DDR introduction of nourseotricin as an AMGP for pigs. This is a streptotricin class antimicrobial, none of which have been used in man and which do not show cross-resistance with other classes. Within a year, resistance to nourseotricin was common in faecal *E. coli* from pigs fed this antimicrobial and the responsible gene was located on a transposon (Tn 1825) which, within two years, was found not only in faecal isolates from pig farmers and their families, but also in urban residents and amongst *E. coli* isolated from urinary tract infections in humans. A few years later it was also found in other pathogenic bacteria, not only zoonotic bacteria like *Salmonella* spp. but also *Shigella* spp., which only affect humans and do not have an animal reservoir. Outside the DDR nourseotricin resistance has never been found.

Despite the fact that apramycin and hygromycin are only used in animals, genes encoding resistance to these two, which are co-transferred, have not only been found in animal isolates and zoonotic bacteria isolated from humans but also from Enterobacteriaceae in the environment, the intestinal flora of farmers and hospital isolates (Hunter, 1993; Chaslus-Dancla, 1986; Chaslus-Dancla, 1989; Chaslus-Dancla, 1991).

5.5.4. Discussion and Conclusions

Current knowledge is too ambiguous to identify all of the factors that are important in the selection and spread of antimicrobial resistance. It is known, however, that heavy use of antimicrobials is one important factor, and restriction in the use of antimicrobials should lead to a containment or possibly a reduction in the extent of the problem.

It is likely that the ways in which antimicrobials are administered (e.g. dose regimens and compliance in therapeutic use, chronic administration of agents as AMGPs, crop spraying) are also important for selection and that, dissemination of resistant micro-organisms results from the breakdown in common hygiene practices in the home, the community, in hospitals, on farms, and in further food processing.

As the relative contributions of the many factors likely to be involved in the development of antimicrobial resistance are still poorly understood, it is not possible to predict the rates of emergence or disappearance of resistance based on amounts or patterns of antimicrobial use. Consequentlyfurther and future restrictions on antimicrobial use should be instituted only after careful

analysis of each antimicrobial, its mode of use, and the prevalence of resistance

The prevalence of resistance in each ecological compartment appears to be related to the characteristics of the resistance gene(s), the composition of the bacterial population, and the antimicrobial selection pressure. However, there are links between the compartments, and from a microbiological point of view, the resistance gene pool may be considered to be common to all bacteria. Evers et al (1996) and Davies (1994) concluded that antibiotic resistance gene clusters were derived from a large and diverse environmental gene pool. The way in which resistance genes may move via gene cassettes, integrons, transposons and plasmids is well known (Hall and Collis, 1995).

There is evidence to support a continuous flow of resistant bacteria and of resistance genes between pathogenic and commensal bacteria, as well as between the different compartments. Consequently, the use of antimicrobials and the occurrence of resistance in one compartment should be expected to influence the occurrence of resistance in others.

An example of such a gene flow between different compartments is vancomycin resistance in enterococci, caused by a gene cluster of nine genes. *VanA* is the commonest gene encoding glycopeptide resistance in *E. faecium*. The origin of this gene cluster is considered to be exogenous (environmental) but not exactly known (Evers et al. 1996). This gene cluster has entered bacterial populations of different animal species and has been amplified through the selective pressure exerted by use of Avoparcin (Bagen et al, 1997) and has spread between animals and man (Haaheim et all 1997; Jensen et al 1998). The most likely route of transmission is via the food chain (Bates et al 1995A; Wegener et al 1997).

5.6. Secondary Ecological Implications of Antimicrobial Resistance

Resistance is an important ecological regulation phenomenon known as allelopathy. This natural phenomenon also includes natural antimicrobial resistance. Despite this fact, there are hypothetical threads which may be very unlikely, but should be scientifically addressed.

There is consensus that the introduction of antimicrobial resistance genes by various pathways into aquatic and terrestrial systems changes microbial ecology by changing the genetic resources. This fact remains true, even considering that resistant microorganisms have generally no competitory benefit. Consequences for soil and water microbiology, however, are unclear. There is no scientific information whether there is an impact of antimicrobial resistance as a secondary ecological effect in soils and waters on higher organisms.

In order to improve the knowledge base systematically to address these questions, information would be needed on:

- The long term fate of resistance genes in soils and waters, the time for resistance to be lost (reversibility),

- Investigate conditions and duration of reversibility of resistance and the consequences for soil and water microbial ecology,

- Establishment of NOECs for resistance development,

- Elaborate strategies, methods and data to justify the assumption, that apart from microbial ecology there are no harmful effects on the environment from resistant organisms.

6. OPTIONS FOR THE CONTROL AND CONTAINMENT OF RESISTANCE

Introduction

The preceding chapters have indicated that many factors are involved in the selection and spread of antimicrobial-resistant bacteria. Most important amongst these appears to be the extent of usage since the relationship between the amount of antibacterials used and the prevalence of resistance is broadly quantitative. Thus, the control and containment of resistance is likely to be successful only if the measures employed include a reduction in the use of antimicrobials in all spheres of current application. The measures which need to be considered for control and containment arediscussed in this chapter in terms of Improving Prescription Use, Improving Non Prescription Use, Reducing the Need for Antimicrobials, Providing New Antimicrobials and Educating Prescribers and Users..

6.1. Improving prescription use

The prescribing of an antibiotic presumes that an indication for its use exists and that a presumptive or definite diagnosis has been made. It is important that any intervention aimed at reducing the use of antimicrobials should not restrict antimicrobial use in cases of genuine human and animal need Even then, it is difficult clearly to define appropriate and inappropriate use because of deficiencies in present knowledge regarding the impact of antimicrobials on clinical and microbiological outcomes, duration of morbidity, and risk of sequelae..

There is, however, a lack of precise information regarding the optimal dose, dose interval and duration of therapy which will achieve a resolution of the infectious process with minimal risk of selecting for resistant organisms among target pathogens and with least disruption of the normal flora of the host. Nor are there good data regarding the relative risks and benefits of different agents in these respects. Some of the reasons for these deficiencies lie in the fact that the clinical trials performed to support the marketing authorisation of new antibacterial agents are usually designed to demonstrate equivalence between a regimen of the new agent and approved comparators. Due to the many host factors involved in the response to infection, and to frequent uncertainty regarding the identity of the bacterial pathogen(s), if any, in patients enrolled into trials, there is commonly no correlation between in-vitro susceptibility and clinical and microbiological outcomes in these trials.

A combination of these factors makes it difficult not only to define what constitutes "prudent use" of antimicrobials, but also to write guidelines for practitioners which might promote prudent use. Ideally, prudent use would encompass the use of antimicrobials in a way which minimises the risk of developing resistance while optimising therapeutic effect.

6.1.1. Human medicine

6.1.1.1. Guidelines for antimicrobial use

Many guidelines regarding the use of antimicrobials have been proposed by international and national organisations and professional associations, by governments, and by local bodies such as formulary committees. Some of these cover only specific uses (*e.*g. the treatment and/or prevention of certain types of infection), some offer wide-ranging advice and provide guidance on both the drug, route of administration and regimen to be used, while others mention only the drug(s). The basis of these guidelines is often not presented to their audience; it is clear that some attempt to provide advice derived from available "evidence-based medicine" while others may be prompted more by local needs to contain costs of drug use. It is also not clear to what extent guidance reflects knowledge of patterns of antimicrobial resistance, nor how often they are considered for revision according to changes in the prevalence of resistance and the launch of new drugs/formulations.

The existence of multiple sources of guidance, providing what is often quite different advice, is confusing to practitioners. In addition, while several studies have looked at compliance with guidelines, there are few studies which have compared the clinical outcomes among patients treated for the same infection but according to different guidelines.

Thus, there is a lack of data which might be used to convince doctors that changing their prescribing habits is both worthwhile and not likely to be detrimental to patients. Not surprisingly, simply making guidelines available does not necessarily have a measurable effect on patterns of antimicrobial use. Indeed, except in local situations where monitoring of use is possible, or in countries where reimbursement schemes allow for the determination of usage patterns, audit of compliance with guidance of any sort is not routinely performed.

Despite these potential problems and the frequent absence of reliable information on which to define "best practice" and "prudent use" with regard to the treatment of infections, the production and implementation of guidance is potentially a means of reducing the unnecessary prescription of antimicrobials and, where data are available, could be used to promote rational use of these drugs.

Ideally, guidelines should aim to:

– diminish or stop unnecessary prescribing, while preserving quality of care;

– give advice on choice of the drug and regimen which might optimise outcomes while minimising emergence of resistance;

– avoid the use of unnecessarily broad-spectrum antimicrobials.

The effectiveness of guidelines, and compliance with them, has to be evaluated on a routine basis to ensure that they are being implemented

correctly and so that the need for revisions can be identified promptly. In practice, this requires investment in skilled manpower, such as physicians expert in the management of infection, pharmacists, and electronic prescribing linked to clinical records. Means of auditing need to be devised both in hospital and community practice. These principles are equally applicable to the practice of veterinary medicine.

6.1.1.2. Computer-assisted prescribing

This mode of providing guidance offers the possibility of influencing prescribing decisions while taking into account locally-derived resistance and other factors such as drug availability. In this way, broad national or even international policies could be adapted to the local situation. Such systems would be most useful when computerised clinical records already exist but these do not yet exist in many, or perhaps most, EU hospitals and in only rudimentary form in general practice, so that huge investment in appropriate hardware, software and training would be needed.

6.1.1.3. Rapid identification of pathogens and their susceptibilities

Much antimicrobial prescribing is empirical, and many doctors do not utilise diagnostic laboratories unless first or even second-line treatment has failed. More rapid identification of the pathogen involved would not only aid the choice of therapy but also could prevent the use of an antimicrobial in some cases. For example, many common infections, particularly those producing signs and symptoms related to the respiratory tract, are not due to bacteria, so that an antibacterial would not be expected to influence the course of the disease except in patients who have considerable risk of bacterial superinfection. In addition, where a wide range of species may cause the same symptomatic presentation, as in urinary infections, broad spectrum agents may be prescribed when a narrow-spectrum agent might be curative if knowledge of the pathogen and its susceptibilities were to be speedily available.

In the USA there is evidence that physicians are more likely to change therapy if they have susceptibility data before there is clinical response to the empirical prescription (Trenholme et al 1989). Currently, there are few good tests of this kind available so that the manufacturers of diagnostics and scientific researchers need encouragement to seek out more rapid means of identifying pathogens and determining their susceptibilities. Attempts to assess the likely cost-benefit of employing such tests *vs.* prescribing unnecessarily or inappropriately would be needed, and would have to be repeated at intervals.

6.1.2. Veterinary medicine

Access to effective antimicrobials, a necessity for animal production, is increasingly threatened not only by antimicrobial resistance itself but also by the real and potential threat to public health from the use of antimicrobials in animals. In order to preserve the ability to treat bacterial infections in animals, action must be taken to reduce the overall use through the implementation of preventive measures and by prudent use; the

implementation of such measures should parallel efforts to change patterns of use in clinical medicine.

6.1.2.1. Modes of Prescribing in Veterinary Medicine

In veterinary medicine antimicrobials may be prescribed for prophylactic or therapeutic purposes.

In addition, a special situation exists when some members of a group or a flock of animals are infected. In those situations it is often necessary to administer antimicrobials to the whole group or flock even though all animals do not yet demonstrate clinical signs of infection at the time of administration, but it is likely that most of them will get the disease in the next days. Such use of antimicrobials is often referred to as metaphylactic use, but this is not an internationally-accepted term.

The veterinary surgeon prescribing antimicrobials should have knowledge of the disease history not only of the current case but also of the current and previous health situation of the whole farm, including disease preventive and other measures undertaken. This is of particular importance when evaluating the need for prophylactic use of antimicrobials. It should be considered whether professional advice on measures which might prevent new infections in the future such as changes in management, improving hygiene, housing or vaccination are indicated as well.

6.1.2.2. Prescribing guidelines

To achieve an optimal and prudent use of antimicrobials, guidelines and policies for the use of antimicrobials may be established as a help to the veterinary practitioner. Such guidelines would also support the practitioner against demands for antimicrobials by farmers. Farmers, like veterinary practitioners, receive a great deal of confusing and sometimes biased information from the pharmaceutical industry which tend to encourage the use of new antimicrobials when older drugs may be as effective. These principles are equally applicable to the practice of human These principles are equally applicable to the practice of veterinary medicine. medicine.

The strategic aims of policies expressed in guidelines should be to achieve three goals; optimal therapeutic effect and/or protection of animals at risk; control of antimicrobial resistance; and provision of practical, affordable treatment that avoid risks of residues in or damage to animal products for human consumption.

Application of these principles should result in a preferred and limited list of antimicrobials for more than 90% of the conditions commonly presenting in practice and provide a rational treatment choice based on scientific data, results of disease surveillance and practical experience. Guidance should include data on appropriate dosage, range and duration of application, and residue problems (withdrawal times). If several antimicrobials can be used, guidelines must differentiate between first, second and third choice antimicrobials. Consultation with a specialist is an effective means of

controlling usage of third choice antimicrobials while at the same time assuring that sick animals will be treated effectively and appropriately.

Audit, peer consensus and review could apply to veterinary practice, and without them, there is unlikely to be professional acceptance and compliance.

Veterinary surgeons should be regularly informed about their own prescribing and the average amounts and types of antimicrobials used per numbers of animals by species, both locally and nationally. This would enable them to compare their prescribing habits with those of colleagues, bringing differences to their attention

6.1.2.3. Surveillance of resistance

Emergence and spread of resistance is the most serious unwanted effect of antimicrobial use in animals, so facilities to monitor and analyse regularly the prevalence and patterns of resistance should be developed as a priority. This could be funded as part of post-marketing surveillance by the pharmaceutical industry and by government as part of disease prevention programmes. Antimicrobial surveillance programmes should be running continuously and cover as large a territory in each country or region as possible. Antimicrobial susceptibility data should be quantitative and produced under strict quality assurance. Samples must be representative and sampling strategies uniform. Priority bacterial species should be selected and all antimicrobial classes used in human and veterinary medicine and in agriculture should be represented. Interpretation and reporting of results from different laboratories must be harmonised.

More detailed knowledge about the usage of antimicrobials and the impact on epidemiology and prevalence of antimicrobial resistance in different environments will assist a greater understanding about the forces behind the development and dissemination of antimicrobial resistance. The information obtained may be useful in the development of useful mathematical models. Reliable antimicrobial surveillance data will form the basis for antimicrobial policies and interventions when needed. Data on antimicrobial resistance should be targeted not only to doctors and veterinarians, but also to the general public and to farmers.

6.1.2.4. Recording of antimicrobial usage

Recording, qualitatively and quantitatively, of the antimicrobials used in veterinary medicine is of paramount importance, not only to monitor the impact of the antimicrobial policy, but also to look for a possible correlation between the usage of a certain antimicrobials and the prevalence of resistance. It is difficult to obtain data on the amounts of antimicrobials used in veterinary medicine as most pharmaceutical companies are reluctant to make these figures available. Registration of the amounts of veterinary medicines sold by each company should be made compulsory as well as the recording of indications and use of veterinary drugs and pre-medicated feeds by means of a logbook on each farm and in each veterinary practice. Such a recording system of health status and use of medicines will make it not only

feasible to monitor the usage of antimicrobials on a farm and by prescription, but also the indications for antimicrobial usage and efficacy of antimicrobial therapy under practice conditions. In fact, reports of lack of efficacy are at least as important for an antimicrobial policy at those recording adverse reactions or resistance spread. Because such recording and the correlations which could be made from such data are highly labour intensive, some form of computerised system, ideally with on-line transmission of data, requires detailed consideration.

All antimicrobials administered on farms should be used only as part of a comprehensive veterinary health programme. Furthermore, all antimicrobials used on farms, including AMGPs, should be a matter of record which is kept available for inspection (see Chapter.7). This would not only control the extent of use; but also provide opportunity for advice on alternative methods of control and preventing future outbreaks of infection.

6.1.2.5. Conclusions

Effective use of resources, such as antimicrobials, is a professional duty of the veterinary surgeon. Antimicrobial formularies and guidelines for domestic animal species could be used to assist veterinary surgeons in providing optimal therapy and preventing bacterial infections with minimal risk of selection and dissemination of resistance. It is of paramount importance that the introduction of formularies, which includes advice on optimal veterinary use of antimicrobials, is backed by facilities for monitoring usage of and resistance against antimicrobials. This is necessary to monitor the compliance with the formularies, to be able to adapt timely the advice given in the formularies to changing circumstances and to take, if necessary, intervention measures. Farms should consider introducing comprehensive veterinary health programmes which are a matter of record available for inspection, which identify all antimicrobial use, including use of antimicrobials as AMGPs.

6.2. Controlling non-prescription use

6.2.1. Animal production

As was summarised in chapter 3.2.2.4, special attention has been focused on the medical impact of the use of antimicrobials as feed additives for growth promoting purposes (AMGP) and the scientific grounds for a continued use of AMGP have been questioned.

For an evaluation of this non prescription use of antimicrobials and a possible future use of AMGP, those antimicrobials used as growth promoters can be considered in three groups:

1. Antimicrobials also used for the treatment of disease

   There seems to be a general agreement that, in accordance with the recommendations of the Swann Committee (1969), antimicrobials used for

therapy should not be used as AMGP and this is why antimicrobials like penicillin and tetracycline are not licensed for use as AMGP.

2. Antimicrobials which can select for cross-resistance to antimicrobials used for therapy

   Antimicrobials used as AMGP can result in antibacterial resistance against related antimicrobials used for therapy and jeopardise treatment in humans and animals. WHO recommends that any use of antimicrobials for growth promotion in animals should be terminated if it is used in human therapeutics or known to select for cross-resistance to antimicrobials used in human medicine(WHO 1997).

3. Antimicrobials not used for therapy and which do not give rise to cross resistance against such drugs

   Theoretically, use of this group of antimicrobials as AMGP should not affect antimicrobial therapy in humans or animals. Nevertheless, there is a possibility that:

   – In its search for new antibacterials, the pharmaceutical industry may focus on development of molecules related to those now used as AMGP, as is the case for molecules related to avilamycin and virginiamycin.

   – Any use at higher concentrations of AMGPs for the purpose of disease prevention is a violation of Directive 70/524/EEC.

   – Strategies to replace the use of AMGPs can be successful in pig as well as in poultry production, as was shown for Sweden where the total use of antimicrobials in the entire animal sector decreased by 50 % during a 10- year period following the ban on AMGP in 1986. However, a withdrawal of AMGP from the EU may increase costs in certain types of production and breakthrough of infections previously controlled by the AMGP may need to be treated. Thus, during a transition period an increase in costs might be expected in certain types of animal production especially in sites where a lack of knowledge or ambitions and incitements to implement disease prevention exist, or where dated production facilities prevent an optimal implementation of such methods.

In summary, antimicrobials used for therapy or those which can cause cross resistance to such antimicrobials should not be used as AMGP. The use of other antimicrobials as AMGP does not seem to be necessary for animal production as they can, to a certain extent, be replaced by non-antimicrobial feed additives and, when needed, by disease preventive management methods supplemented by therapeutic use of antimicrobials. A continued use of AMGP may lead to negative consequences to human and animal health in relation to specific antimicrobials. In contrast, a withdrawal of the use of AMGPs could be expected to lead to a considerable overall decrease in the use of antimicrobials and thereby the risk for exacerbating antimicrobial resistance. It is possible that the removal of antimicrobials from AMGPs

could result in a transient increase in the veterinary use of antimicrobials for the treatment of clinical disease, but this would be expected to be small by comparison with the amounts of antimicrobial no longer used in AMGPs. With appropriate changes in agriculture and animal production practices, even this use of antimicrobials would be expected to diminish.

6.3. Reducing the need for antimicrobials

Apart from reducing the amount of antimicrobials used in treating and preventing infections, reducing the number of infections would in itself lessen the need for antimicrobial use, both prescription and non-prescription.

6.3.1. Human medicine

6.3.1.1. Infection control

Fast recognition and early containment of transmissible infection in institutions and in the community are key factors for the reduction of cases of infection which require antibacterial therapy. These strategies require professional input to infection control so as to raise environmental standards, such as intensified cleaning schedules (the current state of cleanliness of clinical areas being sometimes poor) and the provision of more single room accommodation to isolate patients. The development of more practicable and affordable laboratory methodologies for speedy identification of particular species in patient samples and for the detection of specific mechanisms of resistance would also aid infection control by reducing the time during which the patient reservoir goes unrecognised.

Financial constraints in some countries mean that the availability of beds and nursing staff and the standards of cleanliness have been reduced to levels which are frequently below what is acceptable. Patients can be moved from ward to ward many times, sometimes occupying a bed only recently vacated. Nurses are too busy to observe basic hygiene measures and may be inadequately trained in infection control practises. The physical design of some hospitals is inimical to good infection control; modification of existing buildings and thoughtful new hospital construction would assist institutional infection control.

Apart from hospitals, transmission of infection is a particular problem in day-care centres, long-stay nursing homes and any residential institutions. In the community, a reduced incidence of bacterial infection might come about largely through general improvements in health, especially with regard to respiratory infection, and by public education on infection avoidance. Raising the standards of housing conditions and improving the public health has long been known to reduce the incidence of infections and the potential for spread of infection between household members. The quality of food and potable water is an important issue. For example, hitherto unimportant deficits in the clearing of cryptosporidium from drinking water has now become an issue following the recognition that such infections are common, and may be chronic and debilitating in HIV-infected individuals. While *cryptosparidia* have undoubtedly been causing self-limiting infections in some healthy persons for many years, the symptoms have been ascribed to

other causes. All these issues impinge on health policies and the price a society is able and willing to pay for its own health.

6.3.1.2. Non-antimicrobial treatments for the management of infection

For certain conditions, well-conducted research may be able to define the true contribution of antimicrobials to clinical recovery in comparison with other treatment modalities such as physiotherapy and inhalational therapies in respiratory tract infections. The latter may enhance pathogen elimination, so reducing the potential for spread and for selection of resistance, or even replace antimicrobial use altogether.

6.3.1.3. Vaccines

Vaccines have played a major role in reducing the incidence of many viral and some bacterial diseases. However, vaccines which might prevent the commonest bacterial infections which are treated with antimicrobials are not available and would need to cover a wide range of species to have an impact. Nevertheless, the 23-valent pneumococcal vaccines and the recently developed heptavalent conjugates need evaluation for the possible cost-effectiveness of widespread vaccination programmes.

The potential importance of vaccines in controlling life threatening infection must not be underestimated and research should be encouraged since vaccines may become increasingly important in preventing infections due to multi-resistant pathogens.

6.3.1.4. Immunostimulation

It may be possible to identify substances which have a beneficial effect on the protective immune system without increasing the risk of autoimmune disease. While the effects would be non-specific (unlike vaccines), a lower risk of acquisition of infection reduces the importance of antimicrobial resistance as a threat to human health and, for any remaining circulating strains, the chance of exposure to selection pressures is reduced.

6.3.1.5. Providing Colonisation: Resistance in man

Probiotics are live microorganisms which can establish themselves in the microbial population of the lower gut and enhance the colonisation resistance effect. Particularly in the field of antibiotic-associated diarrhoea due to selection of drug-resistant species (mainly *clostridia*), double-blind and placebo-controlled clinical trials have shown a benefit of administering *Saccharomyces boulardii*. Other probiotics, such as *Lactobacillus acidophilus* and *Bifidobacterium species* have been effective in various clinical conditions related to the intestinal tract. However, a great deal of information has accumulated from anecdotal reports rather than from well designed clinical trials.

Prebiotics, which are chemical supplements (often oligosaccharides) may favour the proliferation of "healthpromoting" bacteria at the expense of pathogenic species. However, these have yet to be studied in clinical trials.

6.3.2. Veterinary medicine and animal husbandry

6.3.2.1. Reduction of use by disease preventive measures

In contrast to the situation in human medicine more drastic and effective management and other disease preventive methods can be undertaken in animal production.

Batch-wise, age-segregated production using all-in/all-out systems with biosecurity routines together with optimal nutrition, environment (including housing and ventilation) and management routines can greatly improve animal health and decrease the need for antimicrobials. For example, following the withdrawal of AMGPs in Sweden from slaughter chicken production, the introduction of a wide variety of disease control methods prevented the expected outbreaks of necrotising enteritis. Those outbreaks that occurred could be treated by penicillin in the drinking water for 2 days, and thus the total usage of antimicrobial decreased. The previous annual usage of up to 1 tonne virginiamycin as growth promoter was replaced during the first two years after the ban by a general usage in therapeutic doses of up to 2 tonnes, but during the following year this was replaced by about 150 kg of penicillin, and that later decreased further to a negligible level (Wierup, 1998).

In piglet production, outbreaks of infectious diseases can often be controlled by age- segregated batch production, and the use of antimicrobials against post weaning diarrhoea has been found to be 3-4 times greater for piglets weaned in conventional pens compared to pens with deep straw bedding (Holmgren, 1994). It is not possible to undertake some of these measures in old facilities, but the design of new buildings should be such that optimal husbandry is facilitated. Following any restriction and/or withdrawal of AMGPs, any outbreaks of infection would need to be treated and may lead, as in Sweden, during the first two years after withdrawal of AMGPs to a temporary increase in use of antimicrobials until such time that husbandry practises are optimised (Björnerot, 1996).

Thus, some methods of animal rearing may have to be modified or even abandoned, as in veal calf production where animals are fed a diet free from iron in order to obtain light coloured meat. This results in anaemic animals with increased susceptibility to infections, which largely contributes to a heavy use of antimicrobials (Franken, 1990; Bosch, 1994).

6.3.2.2. Vaccines

Vaccines can be especially effective in preventing bacterial infection in animals. Vaccination against coliform infection in piglets has virtually eliminated piglet diarrhoea which was previously a major indication for the use of antimicrobials. Similarly, the introduction of vaccines against furunculosis in fish farming in Norway has eliminated an alarmingly large use of antimicrobials (Markestad, 1997).

6.3.2.3. Alternatives to antimicrobial growth promoters in animal husbandry

It is important to examine possible alternatives to AMGPs which may assist in maintaining optimal animal production (Vanbelle, 1989). Organic acids, probiotics and prebiotics, and enzymes will be considered. Some of the non-AMGPs discussed will not be available for many years, and others are speculative

Organic acids

Various organic acids have been used for decades to improve feed hygiene, sometimes mixed with mineral acids, and to benefit feeding (Kirchgeszner, 1988). More recent work (Roth and Kirchgeszner, 1995) has shown organic acids are acting by exerting an antimicrobial effect in the feed itself, enhancing acidity in the stomach and ileum, increasing food digestibility, acting as an antimicrobial in the intestine (by lowering pH, reducing the acid-binding capacity and by acting against moulds and mycotoxins and also by the anionic form of organic acids acting in the small intestine especially against the accompanying flora), and having an intrinsic energy content.

Probiotics and prebiotics

The principles of use are as discussed in 6.3.1.5. in man.

Probiotics, such as appropriate strains of viable lactic acid bacteria, are able to improve performance in calves, pigs and domestic birds (Vanbelle, 1990;Teller, 1991). Most studies in animals have been done in conjunction with administration of fructo-oligosaccharide (inulin) and oligofructose, which seem to promote not only the growth of Bifidobacteria but also inhibit pathogens such as clostridia and E. coli.

Recent studies showed that living yeasts enhance defence mechanisms through harmless immunogenic stimuli in both animals and humans, especially in infectious diarrhoeal disease (Bertin, 1997). Living yeast is also used in ruminant feeds to improve productivity (Wallace, 1993;Chaucheyras, 1995).

Enzymes

Enzymes for animal feed have been developed over the last 20 years, particularly in Scandinavia (Cowan, 1995) where the addition of β-glucanases to barley for poultry feed was first used. Within the EU 40 % of the broiler and piglet feeds contain a cocktail of enzymes, while only 10 % of the feed for laying hens and growing pigs is estimated to be supplemented with enzymes. The nutritional yields of soya, peas and rape seed meal, sunflower, copra, rice bran, and sorghum are also improved by addition of specific enzymes. Enzymes appear to be particularly beneficial to monogastric animals. Enzymes are not really alternatives to growth promoters but do fit the literal definition of digestive enhancement in that they enable more efficient use of feed materials.

6.3.3. Plant protection

6.3.3.1. Cultural practices

Good cultural practices (choice of proper planting site, prevention, sanitation, fertilization, integrated pest management strategies, pruning) may help to minimize the problems caused by plant pathogens without an excessive use of antimicrobials.

6.3.3.2. Biocontrol (competitive exclusion)

Biocontrol agents have shown promising results in the prevention of bacterial diseases. It should be recognized, however, that antibiosis (the ability of the microbes to produce and excrete antimicrobials) plays a role in the efficacy of bacteria in biological control.

6.3.4. The environment

All the methods by which antimicrobial usage may be reduced would reduce contamination of environment by wastes containing resistant bacteria and antimicrobials from humans, animals, aquaculture and agriculture.

6.4. Providing new antimicrobials

6.4.1. Developing new antimicrobials

No antibacterials with novel modes of action have been introduced in the last decade (Shlaes, 1993) though there are several currently in clinical development – e.g. oxazolidinones and everninomycins. Firstly, it is difficult to find or create truly novel agents which are patentable. Secondly, there may be a finite number of appropriate targets in bacteria. Thirdly, it is commercially unattractive to invest in research having little chance of producing a return. The cost of research, development and testing of a novel antimicrobial is now probably in excess of US$350 million (Gold, 1996), and the time required for effective marketing is at least 6-7 years (Cohen, 1992; Billstein, 1994). As a result, the number of companies investing in antibacterial research declined even before the recent trend towards company mergers occurred. Moreover,there is the risk that a costly new antimicrobial drug may well become obsolete within a few years, reducing the economic returns that can be expected to a level that is insufficient to justify the investment (Goldmann, 1996).

However, the rapidly expanding phenomenon of antimicrobial resistance has begun to stimulate industry interest. In particular, the wealth of information derived from bacterial genetics has opened new avenues for the development of antimicrobials with novel mechanisms of action which may also be less susceptible to known mechanisms of antimicrobial resistance. Collaboration between the academic institutions, WHO, national public health bodies and the drug industry has already been useful in the war against bacterial resistance (Heymann, 1996). Encouragement should be given for the development of truly novel antibacterials and also for clinical trials to establish optimal treatment regimens for defined clinical conditions.

Inevitably, bacteria will eventually develop resistance to new agents, although this may take a long time. Therefore, efforts are needed to protect the utility of novel agents by establishing more carefully the principles of prudent use.

6.4.2. Novel approaches to antimicrobial chemotherapy

While standard antimicrobials have the capacity to inhibit pathogen replication and achieve killing of organisms, with or without the aid of the immune response of the host, new types of drugs are being sought by several workers and companies. These include searches for drugs which might interfere with virulence factors or mechanisms of resistance, or which seek to modify the molecular biology of multiple resistant pathogens.

6.5. Educating prescribers and users of antimicrobials, and the public

6.5.1. Prescribers

Although the undergraduate curricula for students of human and veterinary medicine is crowded, some courses still lack a definitive slot on antimicrobial use which covers technicalities and clinical perspectives as well as sociological and world implications of their use.

Aspects of antimicrobial therapy are also poorly addressed in the sphere of post-graduate education. Thus, there is scope for expanding education of health professionals both in terms of good prescribing and how to minimise antimicrobial use by preventing infection occurring. For medical practitioners this includes all aspects of hygiene in clinical areas. For veterinary practitioners this might include updating on organised health control programmes mentioned above.

Audit of antimicrobial use and its clinical outcomes might be an important means to monitor the impact of educational efforts.

6.5.2. Patients and Clients

Education of potential users appears to give encouraging results (Belongia, 1998). Much of the pressure on professionals to prescribe antibacterials comes from their patients or clients, which implies that public education is needed on the social consequences of overuse of antibacterials. In the case of veterinary usage, education of farmers, including discussion of enforcement policies designed to ensure compliance with the law and regulations covering sale, prescription, and usage could be expanded. Consumption data of antimicrobials by users would be vital so that feedback is available on trends and changes in usage. Such data could be supplemented with data as patterns of resistance derived from systematic surveillance.

The other major aspect of education is hygiene, i.e. measures for avoiding infection in all spheres of life. Examples in man are improved awareness of how to avoid food-poisoning by correct food preparation and maintenance of kitchen hygiene and how to reduce the transmission of sexually transmitted

diseases by use of barrier methods of contraception. Optimally, such education is needed from school age onwards.

7. AREAS FOR FURTHER RESEARCH

7.1. Data collection

Chapters 3, 4 and 5 have pointed out that knowledge about the ways in which the use of antimicrobials may be related to resistance has been severely hampered by a lack of good quality information on prevalences of antimicrobial resistance, amounts of antimicrobials used, the applied modes of use, and the outcomes of use. The first priority is therefore to establish what information is currently available on all these matters in individual member states and to make suggestions on how it can be improved.

In particular, EU-wide information of resistance should be acquired as a matter of priority. This will involve both research and consensus on the best methods to ensure comprehensive, reliable and cost-effective surveillance systems.

7.2. Selection pressure and transfer of resistance

More research into factors which influence the selection of resistant microorganisms is needed, particularly at the *in vivo* and epidemiological levels. Examples are: investigate how quickly and to what extent resistance may be reversible when antimicrobial use decreases; investigate the mechanisms through which pathogenic bacteria can acquire resistance from the host flora *in vivo*, and vice versa.

Research on the factors (biological, chemical and physical) that stimulate or prevent the genetic transfer of resistant genes among bacteria could result in the definition of novel strategies and medical products aimed at reducing antimicrobial resistance and improving the efficiency of antibiotics.

Once the factors and substances that influence the genetic transfer among bacteria are identified, new avenues for the control and prevention of antimicrobial resistance will be opened.

The genetic basis of antimicrobial resistance should be studied in more detail to find ways to circumvent the resistance mechanisms of bacteria. For example, by combining antimicrobials with compounds that inhibit the resistance mechanisms of bacteria at the molecular level, the effectiveness of an antimicrobial *in vivo* may be preserved. In this way, the lifespan of classic antimicrobials may be considerably prolonged.

7.3. Define impact

Find methods to measure the impact of antimicrobial resistance on human mortality and morbidity, including the application of mathematical models; determine the impact of social factors on antimicrobial efficacy and on the development of resistance. These aims may well require the use of interventional or case-control studies, both in the community and in hospitals.

More research and epidemiological data are needed to clarify the issue of inter-species transfer of antimicrobial resistance. In the case of agricultural uses of antimicrobials, more studies are needed on the potential for plant pathogens or environmental micro-organisms to transfer resistance factors to animal and human pathogens. Antimicrobial resistance surveys should include resistance factors which are linked to antimicrobials used solely for plant protection (*e.g.* kasugamycin). This model approach could also be valid with other 'single-purpose' antimicrobials (e.g. feed additive flavophospholipol) in exploring other routes of resistance transfer and the possible impact of the various routes in the apparent 'total' resistance.

7.4. Define prudent use

Research is needed into ways that might improve the prescription use of antimicrobials. Clinical trials of efficacy are presently almost entirely funded by the pharmaceutical industry and have objectives largely confined to satisfying regulatory authorities. Thus trials which seek to optimise the dose, dose interval and duration of treatment are rarely done.

7.5. Prescribing practices

Applied research is needed into the motivation of physicians and veterinarians to prescribe as at present, and how prescribing behaviour can best be influenced for the better. The role of audit and participation in the feed-back of data on compliance with guidelines in influencing behaviour should be assessed.

7.6. Infection control

Focus is needed on methods of reducing the need for antimicrobials by lowering the prevalence of infection. Widespread implementation and enforcement of methods for reducing transmission of pathogens is needed, not only in hospitals but also in sites of high population density in the community, such as day-care centres.

7.7. Supplementary measures

More studies are needed on the impact of changes of conditions of animal husbandry on animal health. Research into alternative approaches for phytosanitary purposes should be encouraged.

7.8. Rapid diagnosis

The development of more rapid diagnostic methods for bacterial infections should be encouraged. Thereby antimicrobial treatments will be aimed directly at the infectious agent and the need for broadspectrum or combined antimicrobial usage will be more limited

7.9. Steering Committee for EU

In establishing priorities for European-wide research, the involvement of experts representing appropriate scientific and professional bodies is recommended.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1. Conclusions

Resistance to antimicrobials existed before these drugs were introduced into human and veterinary medicine. All available evidence points to an inexorable increase in the prevalence of drug resistance among bacteria which has paralleled the expansion of their antimicrobial use in all spheres. Particularly difficult management problems are now posed by certain bacterial species which have the ability to acquire resistance to the majority (possibly all) available agents. Thus, the increasing prevalence of resistance to antimicrobial agents among pathogenic micro-organisms, and particularly among bacteria, is now an important problem which has serious implications for the treatment and prevention of infectious diseases in both humans and animals This requires urgent attention by Governments and by all users of antimicrobials.

Although much scientific information is available, not all aspects of the development of antimicrobial resistance are well understood. It is, however, known that the selection of resistant bacteria is promoted, and their dissemination is enhanced, by the use of antimicrobial agents. To obtain robust evidence which might confirm and quantify the relationship between modes and extent of antimicrobial usage and the prevalence of resistance would probably take several years. Furthermore, it could not be expected that studies would be able to identify and quantify the contribution of all possible factors. It is considered that to wait for incontrovertible evidence before taking action would be to miss an important opportunity for intervention. Indeed, the best opportunity to acquire causal evidence may be during the monitoring of interventions to control resistance.

While intervention may not achieve a reversal of the problem of antimicrobial resistance, at the very least some containment of current trends should be an expected outcome of successful strategies. Thus, the aim of interventions commenced now is to prevent antimicrobial resistance from becoming an even greater problem and to preserve the utility of the antimicrobials currently available. The core strategy - to make every effort to reduce the amounts used - should apply to each of the four component areas discussed in this report. Certain other strategies may apply to specific areas of use. The outcomes of any actions taken need careful monitoring to identify the most and least successful strategies so that there may be modifications of interventions over time.

In summary, actions should be taken promptly to reduce the overall use of antimicrobials[2] in a balanced way in all areas: human medicine, veterinary medicine, animal production and plant protection. This should involve improved disease preventative measures, elimination of unnecessary and improper use of antimicrobials, improving the effective use of antimicrobials

[2] antimicrobials have been defined in Chapter 1.4. of the main report

presently available based on more precise diagnosis of the infectious agent, and on monitoring of antimicrobial resistance and control of antimicrobial usage. It is proposed that the following recommendations are relevant to these conclusions:

8.2. Recommendations

The SSC recommends that there should be EU-wide co-operation and agreement as a matter of urgency, particularly with regard to prioritisation of actions. Those strategies which are most likely to be effective in the control and containment of antimicrobial resistance will be those which can be introduced speedily without undue costs in all countries and which can be monitored and/or enforced across the EU. It may be necessary to support the achievement of these proposals by introducing effective legislation and regulation.

Four important areas of action are proposed:

8.2.1. Prudent Use of Antimicrobials

These strategies relate to controls on the availability and access to antimicrobials within the EU and to the promotion of prudent use *via* education of all prescribers, recipients/clients, manufacturers, and other users. Measures for consideration include:

8.2.1.1. There should be tighter controls on the sale, supply and distribution of antimicrobials through enforcement of the legal classification mechanisms of individual EU Member States. Member States should review mechanisms in place for the control of sales, supply and distribution of antimicrobials in the light of the recommendations of this report.

8.2.1.2. The use of antimicrobials in each of the four areas, human medicine, veterinary medicine, animal production and plant protection should be only in accordance with legislative provisions. In particular the use of combinations of antimicrobials should be discouraged.

8.2.1.3. Action should be taken to eliminate inducements, especially financial, which encourage the inappropriate use of antibiotics.

8.2.1.4. Guidelines should be drawn up which indicate preferences for use of certain agents in the treatment of human and animal disease and which discourage the practice of prescribing for infections which are likely to be self-limiting and/or non-bacterial in aetiology. The aim should be to establish EU-wide agreements as the bases for local actions, including the development of "best practice" guidelines to support the judicious use of existing and novel agents.

In this regard, research is needed into:

(a) methods which might improve the prescription use of antimicrobials. including clinical studies which evaluate the important constituents of optimal drug regimens for the treatment of infections.

(b) the motivation of physicians and veterinarians to prescribe, and how prescribing behaviour can best be influenced for the better. The role of audit and participation in the feed-back of data on compliance with guidelines in influencing behaviour needs assessment.

(c) the development of more rapid diagnostic methods for bacterial infections which might allow for better targeting of antimicrobial treatments with minimisation of the unnecessary use of these drugs and limitation of the need for broad spectrum or combination therapy.

8.2.1.5. Programmes should be developed for education of healthcare professionals (at undergraduate and postgraduate levels), farmers and associated food and feed producers, industries and consumers regarding the existence of this problem and the rationale and importance of interventions proposed. In particular, education should focus on how all these groups may contribute to reducing the unnecessary use of antimicrobials by better understanding of the role of such agents in the management of infectious diseases. These principles should be incorporated into codes of best practice whenever there is a commercial interest involved in the use of antimicrobials.

8.2.1.6. Regarding the use of antimicrobials as growth promoting agents, the use of agents from classes which are or may be used in human or veterinary medicine (i.e., where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished. Efforts should also be made to replace those antimicrobials promoting growth with no known risk of influencing intestinal bacterial infections by non-antimicrobial alternatives. It is essential that these actions are paralleled by the introduction of changes in animal husbandry practices which will maintain animal health and welfare during the phase-out process. Thus, the phase-out process must be planned and co-ordinated since precipitous actions could have repercussions for animal health. Meanwhile, it should be reiterated to manufacturers and farmers that the continuous feeding of AMGPs to food animals for the purpose of disease prevention is a contravention of EU regulations and represents misuse; more effective enforcement measures should be adopted.

8.2.1.7. The use of antimicrobials from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) for the purpose of plant protection should be discouraged

8.2.1.8. While there is no evidence that antibiotic resistance marker genes have transferred from genetically modified plants to pathogenic

micro-organisms, and whereas the possibility of such an event has been argued to be remote, it is considered appropriate to recommend that marker genes should be removed from plant cells before commercialisation whenever this is feasible; failure to remove markers should be justified by the manufacturer. Companies should avoid the use of marker genes which might have the capacity to express and confer resistance against clinically important antibiotics.

8.2.1.9. Use of genetically modified micro-organisms for commercial purposes either for contained usage or for environmental release was not part of the mandate. However, it is recommended that consideration be given to the potential for development of antimicrobial resistance which might arise from the release of such organisms into the environment.

8.2.2. Prevention of Infection and Containment of Resistant Organisms

These strategies should indirectly contribute to an overall reduction in antimicrobial usage *via* minimising the need for antimicrobial therapy in man, in animals, and in agriculture through the prevention and control of infection and optimal management of infection when it occurs. Measures for consideration include:

8.2.2.1. There should be agreement and collaboration on the implementation of EU-wide standards of infection control in all types of institutions caring for the unwell and infirm, such as hospitals, nursing homes and day care centres. Policies regarding measures to be taken when transferring patients between units and between institutions should be agreed across the EU.

8.2.2.2. There should be action to reduce the risk of infection in individuals and in the population as a whole by encouragement of uptake of immunisations, education regarding home hygiene, attention to public health issues, and by the maintenance and/or improvement of housing and social conditions.

8.2.2.3. There should be a focus on education of veterinarians, farmers, owners of companion animals, food producers and consumers with regard to disease preventive methods in animals and the prevention of zoonotic infections in man and animals.

8.2.2.4. Efforts should be made to reduce the need for herd treatments by improved husbandry, vaccination, and infectious disease control and eradication. In this regard, herd treatment use of antimicrobials should only be allowed if no other alternative is available and should be regarded as a failure of preventive measures which requires evaluation and investigation.

8.2.2.5. Similarly, health control programs and other disease preventive methods should be devised and implemented in animal production

systems in order to reduce the need and demand for the routine addition of antimicrobials to animal feedstuffs

8.2.3. New Modalities of Prevention and Treatment for Infections

8.2.3.1. There should be cooperation and coordination between academic departments, the pharmaceutical industry and medical and veterinary research bodies in order to ensure that the necessary appropriate research is conducted which may facilitate the development of truly novel agents and of effective alternatives to antimicrobials as well as preventive therapies.

8.2.3.2. The identification of novel ways to control and contain resistance may be furthered by investigations into how quickly and to what extent resistance is reversible when antimicrobial use decreases. Other related areas of research include evaluation of the means and likelihood of pathogenic organisms acquiring resistance from normal host flora in vivo, and vice versa, since this may lead to means of interrupting such transfers.

8.2.3.3. While a connection between the use of antimicrobials in crop protection and resistance adversely affecting humans and animals is less clear, nevertheless the exploration of non-antimicrobials for the prevention and control of plant diseases should be encouraged. In this regard, research is needed to evaluate the potential for the transfer of resistance factors from plant pathogens or environmental micro-organisms to animal and human pathogens.

8.2.4. Monitoring the Effects of Interventions

This report has discussed the fact that there is inadequate evidence to identify with certainty those strategies which may be the most effective in the control and containment of antimicrobial resistance. In particular, it has been mentioned that the data are inadequate to determine which facets of antimicrobial uses and which areas of use are the major contributors to the problem. It has also been pointed out in several chapters that there is a paucity of reliable data regarding the prevalence of resistance across the EU in many pathogenic species, the change in prevalence over time, the incidence of infections due to multiresistant organisms and their clinical outcomes and on antimicrobial consumption within the EU.

While it is recommended above that efforts to control and contain resistance should not await such data since it is felt that the evidence is already compelling that action is needed, nevertheless a baseline should be established regarding resistance and consumption and these issues should then be examined systematically over time. Measures for consideration include:

8.2.4.1. There should be an EU-wide co-ordination of organism collection and of susceptibility testing methods to monitor resistance patterns

over time. Such data are needed to establish the baseline, to determine the effects of interventions, and to allow for meaningful comparisons between countries and regions. This surveillance should involve academic departments, industry (as part of post marketing surveillance) and governments (as part of disease prevention programmes).

8.2.4.2. Research is needed into methods which might allow for determining and quantifying the impact of antimicrobial resistance on human mortality and morbidity.

8.2.4.3. There should be EU-wide requirements for monitoring the consumption of antimicrobial agents in humans, animals, plant protection and in the environment; data by prescriber should be available for personal feedback and individual recipient records should be kept where appropriate to species. In particular, it is recommended that all antimicrobials administered on farms should be used only as part of a comprehensive veterinary health programme. Furthermore, all antimicrobials used on farms, including antimicrobials in AMGPs, should be a matter of record which is kept available for inspection

8.2.4.4. The effects of all interventions should be kept under constant review. An appropriately constituted EU-wide forum could be assigned the task of monitoring and assessing the outcomes of interventions and of advising on any necessary changes. This body could also serve as a major channel of communication and collaboration with non-EU countries and global bodies including the WHO.

8.2.4.5. Resistance to antimicrobials is a global problem and interventions in the EU alone might be less effective unless action is also taken in non-EU countries. Therefore, monitoring the efficacy of EU-wide measures must take into account external factors. In this regard, it is possible that regulatory action may need to be considered in order to control access of animals, meat or foods from non-EU countries should there be a significant threat perceived or detected for importation of resistant bacteria.

9. ANNEXES

ANNEX 1

The regulatory framework for the use of antimicrobials in humans, animals and plants

(1) Medicinal products for human use

A medicinal product for human use must be authorised prior to being placed on the market. Within the centralised procedure, mandatory for products developed by some biotechnological processes, the authorisation is given by the Commission in accordance with a standing committee procedure. The scientific evaluation is carried out by the Committee for Proprietary Medicinal Products (CPMP) at the European Agency for the Evaluation of Medicines (EMEA) (Council Regulation N (EEC) 2309/9).

For nationally approved products (Council Directive 65/65/EEC), the mutual recognition procedure is in place for medicines to be placed on the market in more than one Member State (Council Directive 75/319/EEC). The authorisation specifies, amongst others, the therapeutic indications and the posology. Authorisations are valid for 5 years and are renewed upon request and submission of all necessary information. A market surveillance of all market products is carried out by the marketing authorisation holder and by the competent authorities.

(2) Veterinary medicinal products

Veterinary medicinal products are also the subject of marketing authorisation. In addition to biotechnology (high technology products), any new medicinal products which are intended primarily for use as a performance enhancer in order to promote growth of treated animals or to increase yields from treated animals must be subject to the centralised authorisation procedure (Regulation (EEC) N° 2309/93).

Evaluation of residue data, which might lead to the establishment of maximum residue limits (MRLs), is mandatory for veterinary medicinal products for food-producing animals, in accordance with Regulation (EEC) N° 2377/90. The evaluation of applications for a Community authorisation in the centralised procedure and in the residue data evaluation, is carried out by the Committee for Veterinary Medicinal Products (CVMP) at the EMEA. Both the Community marketing authorisations for veterinary medicinal products and the adoption of the results of residue evaluation are adopted in accordance with a standing committee procedure.

Veterinary medicinal products subject to a national authorisation (Council Directive 81/851/EEC), must be authorised by the mutual recognition procedure, if they are to be placed on the market of more than one Member State.

Each authorisation specifies the animal species in which it may be used, the therapeutic indications, the dosage and the withdrawal period for food-producing animals (time between the last dose and the slaughter and/or obtaining food products provided by treated animals). Authorisations are valid for 5 years and are renewed upon request and submission of all necessary information.

A market surveillance of all market products is carried out by the marketing authorisation holder and by the competent authorities.

(3) Feed additives

Within the EU the use of feed additives is regulated by Directive 70/524/EEC as amended. Community authorization of an additive is given only if inter alia it affects favourably the characteristics of feedingstuffs or of animal products and satisfies the nutritional needs of animals, or improves animal production. Further conditions are that an additive may not adversely affect human or animal health or the environment nor harm the consumer by altering the characteristics of livestock products, that its presence can be monitored, that at the level permitted, treatment or prevention of animal disease is excluded (this condition does not apply to additives belonging to the group of coccidiostats and other medicinal substances). An additive may not be authorised, if for serious reasons concerning human or animal health its use must be restricted to medical or veterinary purposes. Provisional authorization may be given for the use of a new additive or a new use of an additive already authorized, provided that the above mentioned conditions are met but the effectiveness of the additive has not yet been demonstrated. Until 30 September 1999 Member States are free to authorise or not to authorise on a national level provisionally authorised additives. As from 1 October 1999 the provisional authorisations will be valid in the whole Community but limited in time, up to a maximum of four years.

Additives belonging to the group of antibiotics, coccidiostats and other medicinal products, and growth promotors authorised before 1 January 1988 are provisionally authorized as from 1 April 1998 and will be re-evaluated. After the re-evaluation they will - no later than 1 October 2003 - be linked to a person responsible for putting them into circulation for a period of 10 years. For antibiotics, coccidiostats and other medicinal products, and growth promoters authorised after 31 December 1987, such a re-evaluation procedure is not foreseen. For them “brand specific” authorisations will be granted already before 1 October 1999 for a period of 10 years.

A EU ban on four antibiotics as additives (bacitracin zinc, spiramycin, virginiamycin and tylosin phosphate) will take effect on 1 July 1999. Another ban on the growth promoters olaquindox and carbadox with take effect on 1 October 1999. Sweden, which had a total ban on antibiotics as additives since 1986 and which was permitted to keep its ban until 1998 made, end of 1998, use of the safeguard clause of Directive 70/524/EEC in order not to be obliged to permit the use of the four remaining authorised antibiotics flavophospholipol, monensin sodium, salinomycin sodium, avilamycin on its territory. The Commission has now to examine the

scientific grounds cited by Sweden and to decide whether to invite Sweden to lift its ban or to withdraw the Community authorisations.

Antimicrobials in feed additives are used at lower dosages than in therapy. They are however absorbed to a greater or lesser extent and are therefore distributed to the tissues. As for veterinary drugs, withdrawal periods may be required.

(4) Plant protection

Plant Protection Products are subject to marketing authorisation under Directive 91/414/EC. The authorisation contains two elements:

(1) The authorisation of the active substance by means of a Union-wide review process.

(2) The authorisation of the formulated sales product, which remains under the authority of Member States. Annex 6 to Directive 91/414/EC, the Uniform Principles provides harmonised authorisation criteria for sales products in all Member States.

All new active substances are reviewed under this procedure. Existing active substances are subject to re-review in a work-sharing process among Member States. A first list of 90 substances is currently undergoing this process. More substances are soon to follow. All antimicrobials on the market today for plant protection purposes fall under the category of existing substances. There were no applications for new active substances since the adoption of Directive 91/414/EC. Also as none of the antimicrobials used today is among the 90 substances currently re-reviewed, the authorisation of antimicrobials is maintained under the legislation of the Member States.

Several Member States have restricted authorisation for antimicrobials. The use is only permitted in emergency situations, for a limited time period, for a specific purpose and using a defined quantity. Such legislation is currently applied in Germany and Austria, for example. Other Member States have restricted authorisation to specific uses and use rates, but do not require a special permit (e.g. Spain and Greece).

Antimicrobials registered for uses in plant protection in the EU include: kasugamycin (ES, EL, NL); octhilinone (IRL, UK, E); oxytetracycline (EL); polyoxins (E, GR); streptomycin (BE, NL, EL, A); validamycin (NL). In the UK, antimicrobials are used only on ornamentals. In Italy, the use of plant protection products mainly containing antimicrobials is forbidden since 1971; this prohibition was promulgated to avoid the problem of resistance to antimicrobials.

ANNEX 2

Amounts of antimicrobials used in animals in the EU, and comparison with consumption in humans

Table 1

Estimated antimicrobial sales volumes (1997) in the EU. The consumption was the following for each group of antimicrobials

	Tons of active ingredient at 100% purity
Penicillins	322
Tetracyclines	2294
Macrolides	424
Aminoglycosides	154
Fluoroquinolones	43
Trimethoprim/Sulphas	75
Other therapeutics	182
Growth promoters *	1599
TOTAL	**5093**

*Coccidiostats (ionophores and other) are excluded.

Data from information provided at the 1998 FEDESA *(European Animal Health*), Copenhagen, 1998.

Table 2

Estimated annual usage of antimicrobials in humans and animals in the EU (1997) (Data from same source as Table 1)

	Tons of active ingredient at 100% purity	(%)
Human use (hospital and general practise)	5400	(52)
Veterinary (therapeutic) use	3494	(33)
Animal feed additives	1599	(15)
TOTAL	**10493**	**(100)**

ANNEX 2 (continued)

TABLE 3 – Use of Antibiotics in EU and in Finland in 1997

USE OF ANTIBIOTICS IN EU AND IN FINLAND IN 1997						
	EU		**FIN**		**FIN ****	
	tn	**%**	**tn**	**%**	**tn**	**%**
Human Medicine	5400	52	43.50*	68	43.50*	59
Veterinary Medicine	3494	33	16.29	26	16.29	22
Antimicrobial Feed Additives	1599	15	4.01	6	14.06**	19
Totals	10493	100	63.80	100	73.85	100

(Mannerkorpi, 1998)

* Use in 1994

** Antimicrobial Coccidiostats Included

(National Agency for Medicines - Finland)
(Plant Production Inspection Centre)

10. GLOSSARY AND ABBREVIATIONS

Aetiology: the cause of a specific disease

Agar: a solid jelly-like substance capable of supporting the growth of bacteria or fungi

Ampicillin: antimicrobial of the penicillin group which is used to treat a wide variety of infections including chest, bladder and skin infections.

AMGPs: antimicrobials used as feed additives (growth promoters) in accordance with the provisions of Council Directive 70/524/EEC)

Analogue: corresponding or similar to

Antibacterial policy: written guidance of the recommended antimicrobials and their dosage for the treatment of specific infections.

Antibacterial spectrum: the range of antibacterial activity against different types of bacteria

Antibiotic: a substance, produced by or derived from a micro-organism, which destroys or inhibits the growth of other micro-organisms.

Antimicrobial: a drug which, at low concentrations, exerts an action against microbial pathogens and exhibits selective toxicity towards them.

Asymptomatic: not showing any symptoms of disease, whether a disease is present or not.

Audit: organised review of current practices and comparing these practices against predetermined standards. Action is then taken to rectify any deficiencies identified. Later the audit is repeated to see if the standards are now met.

Bacteriophage: a virus that attacks bacteria. Each bacteriophage acts specifically against a particular species or strain of bacterium.

Bacterium: microscopic single-celled organism with a single chromosome of a circle of double-helix DNA

Blood cultures: samples of blood taken from a patient with a serious infection, such as meningitis. These samples are incubated in the laboratory to try and determine the bacterial cause of the illness.

Cell envelope: the outside of a bacterium made up of cell membranes, the cell wall, and in some cases of capsule (exopolymers).

Cephalosporin: a group of antimicrobials effective against a wide range of bacteria. Used for treating many infections including skin, bladder and chest infections as well as meningitis

Cerebrospinal fluid: the clear watery fluid that surrounds the brain and spinal cord

Chemotherapy: the prevention or treatment of disease by the use of chemical substances.

Chromosome: one of the threadlike structures in a cell nucleus that carry the genetic information in the form of genes.

Clostridium difficile: a bacterium which can cause severe diarrhoea or enterocolitis. This most commonly occurs following a course of antimicrobials which has disturbed the normal bacterial flora of a patient's intestinal tract

Cohort nursing: placing together patients with the same infection within an area of a ward to reduce the risk of the infection spreading. Often used when the number of infected patients is greater than the number of single rooms available for isolation.

Colonisation: the ability of some micro-organisms to reside on living tissue but not cause disease, for example normal bacterial flora.

Communicable pathogens: micro-organisms which cause disease and are capable of being passed from a person, animal or the environment to another susceptible individual. Also known as contagious or infectious diseases.

Community: relates to those diseases or health services which occur outside of hospitals.

Compliance: the degree to which patients follow the instructions for taking a course of treatment.

Contaminants (microbial): usually harmless micro-organisms which may be mixed in clinical samples or pollute pure cultures in the laboratory.

Diphtheria: an acute bacterial infection affecting the throat. Vaccination can protect against the disease.

Disinfectant: a chemical that destroys or removes bacteria and other micro-organisms. Used to cleanse surgical instruments and surfaces of equipment or furniture.

DNA: deoxyribonucleic acid. The genetic material of nearly all living organisms, which controls heredity characteristics and is located in the chromosomes.

Empirical treatment: management of diseases, such as drug treatment, based on experience or observation rather than on specific laboratory investigations.

EMRSA: Epidemic methicillin-resistant *Staphylococcus aureus.*

Enterococcus: a bacterium commonly associated with bladder infections as well as skin, blood and wound infections.

Enterocolitis: severe inflammation of the gut especially the colon and small intestine.

Enzyme: a protein that, in small amounts, speeds up the rate of a biological reaction without itself being used up in the reaction.

Epidemiology: the study of the occurrence, cause, control and prevention of disease in populations.

Escherichia coli (*E coli*): A bacterium normally found in the bowel of mammals but which is commonly associated with a wide range of infections including bladder infections and diarrhoea.

Formularium: Latin for list or collection of formulae (=guidelines). In many EU memberstates used for booklet containing the guidelines for prudent antibiotics usage of a hospital or for an animal species.

Formulary: a compendium often used in hospitals to list the drugs readily available for prescribing and sometimes indications as to what seniority of medical staff may prescribe individual agents.

Fungus: Fungi may cause simple infections such as thrush or athletes' foot. They may also cause serious infections in patients whose immune system has been weakened by disease or treatment.

Gene: the basic unit of heredity; a segment of DNA specifying a particular function.

Genus: a category used in the classification of animals and plants. A genus consists of several closely related and similar species.

Glycopeptide: a group of antimicrobials including, for example, vancomycin and teicoplanin, used for the treatment of serious infections such as those due to MRSA.

Gonococcus: a bacterium (*Neisseria gonorrhoeae*) which is the cause of gonorrhoea, a sexually transmitted disease.

GP: General practitioner

Gram's stain: a method of staining bacterial cells with coloured dyes to aid identification when viewed with a microscope and is used in their classification and identification

Gram-positive: bacteria which by Gram's stain appear violet microscopically.

Gram-negative: bacteria which by Gram's stain appear red microscopically.

Growth medium: fluid capable of supporting the growth of micro-organisms such as bacteria and fungi.

Haemophilus influenzae: a bacterium which most commonly causes respiratory tract infections and meningitis. Infection with some strains of *H influenzae* can now be prevented by vaccination.

Helminth: any of the (parasitic) worms including the flukes, tapeworms and nematodes.

ICU: Intensive Care Unit

Immunocompetent: having normal immune responses, as in a normal healthy person.

Immunosuppression: having impaired immunity due to disease, for example cancer, or treatment, for example steroid drugs or radiotherapy.

In vitro: tests undertaken in laboratory apparatus for example test tubes, not in a living human or animal.

In vivo tests undertaken within a living human or animal.

IT: Information technology, such as the use computers.

Locally: used for the route of administration of a drug which is applied directly, or topically, to the part being treated, for example to the skin or eye.

Macrolide: a group of antimicrobials, including erythromycin, which can treat a wide range of infections especially respiratory and skin infections. Often used as an alternative to penicillins.

MDRTB: Multiply drug-resistant *Mycobacterium tuberculosis*

Meningococcus: a bacterium (*Neisseria meningitidis*) which most commonly causes meningitis and septicaemia or blood poisoning.

Metabolic body weight: Bodyweight is transferred to metabolic body weight (metabolic weight = body weight 0.75) according to Brody (1945).

Microbe: any organism too small to be visible to the naked eye. Micro-organisms include bacteria, fungi, viruses and protozoa.

Morbidity: the state of being diseased. Whereas mortality is the state of death.

MRSA: Methicillin-resistant *Staphylococcus aureus*

Mutation: an inheritable change in the genetic material of a cell

Mycobacterium tuberculosis: a bacterium which is the cause of tuberculosis or TB.

Non-pathogen: a micro-organism which may by grown from samples but which does not cause disease.

Normal flora: micro-organisms which normally reside on the skin, in the gut and in the mouth and upper respiratory tract of humans and animals. They usually protect these tissues from diseases and may improve biological functions

Nutrient agar: a solid jelly-like substance made from basic nutritional ingredients capable of supporting the growth of many, but not all, bacteria and fungi.

Parenteral: giving drugs by intramuscular or intravenous injection.

Pathogen: a micro-organism that can cause disease.

Penicillins: a group of antimicrobials, such as ampicillin, which can treat a wide variety of infections. Can be given by mouth or injection. Some people have to avoid these antimicrobials because they are allergic to them.

Penicillinase: an enzyme produced by some bacteria which is capable of antagonising the effect of penicillin thereby making the bacterium resistant to treatment by this antimicrobial.

Plasmid: an extrachromosomal genetic element that is not essential for growth.

Pneumococcus: a bacterium (*Streptococcus pneumoniae*) most commonly associated with pneumonia and meningitis. Vaccination is available to prevent infections due to many strains of pneumococcus.

Prebiotic: a non-digestible feed or food ingredient which passes through the small intestine and is fermented by the endogenous microflora

Probiotics: "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"

Progeny: offspring or descendants

Prophylaxis: any means taken to prevent disease. For example, vaccination against tetanus or measles, or giving antimicrobials when patients undergo procedures which put them at risk of acquiring an infection although they do not have an infection at the time of the procedure. Also includes short-term use of antimicrobials in animals which one knows or has good reason to expect will be exposed to bacterial infection, as during a contaminated operation. The antimicrobials are given only once or maximally up to 3 days.

Protozoa: a single-celled micro-organism, usually bigger than a bacterium, which may be free-living or parasitic. Malaria is a protozoal disease.

Pseudomonas: a genus of bacterium causing a wide variety of infections in animals and plants

Reference laboratory: one which receives samples from other laboratories so that more specialised tests can be carried out. Usually also involved in research relating to their particular area of interest.

Ribosome: a particle, consisting of RNA and protein, that occurs in cells and is the site of protein synthesis in the cell.

Salmonella: a genus of bacteria most commonly associated with diarrhoea and food poisoning and which can also cause disease in farm animals

SPC:

Species: a group of genetically closely related micro-organisms having many features in common.

Staphylococcus: a genus of bacteria which cause a wide variety of infections especially those of skin and wounds in humans and mastitis in cattle.

Streptococcus: a group of bacteria which cause a wide variety of infections including those of the throat, skin and wounds in humans and mastitis in cattle.

Systemic treatment: drugs by or injection or absorbed when given by mouth and distributed through the body via the bloodstream.

Target site: the specific part of a cell upon which a drug such as an antimicrobial acts.

Tetracycline: a group of antimicrobials effective against a wide variety of infections such as respiratory tract infections, acne and genital infections.

Therapeutic use: antimicrobials administered to treat individual humans or animals (or groups of animals) suffering from a bacterial infection.

Topically: used for the route of administration of a drug which is applied directly, or locally, to the part being treated, for example to the skin or eye.

Toxin (microbial): any poisonous substance produced by micro-organisms.

Trimethoprim: an antimicrobial most commonly used to treat urinary infections.

Tuberculosis (TB**:** An infectious disease most commonly affecting the lungs. Treatment with antimicrobials takes many months.

Vaccine: a special preparation of material that can be used to stimulate the development of immunity and thus confer protection against a specific disease or number of diseases. Usually given by injection and started in early childhood. Can be used to prevent many infections including measles, mumps, rubella, whooping cough, hepatitis A or B, and rabies.

Virus: a very small micro-organism of simple structure only capable of multiplying or surviving within a living host cell. Influenza and measles are caused by viruses.

VRMRSA: Vancomycin-resistant, methicillin-resistant *Staphylococcus aureus*

VISA: vancomycin intermediate resistant Staphylococcus aureus.

VRE: Vancomycin-resistant enterococcus

Zoonosis: infection by micro organisms that can be transmitted from animals to humans, for example, salmonellosis and rabies.

11. ACKNOWLEDGEMENTS

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