Antibiotic Resistance in the European Union Associated with Therapeutic Use of Veterinary Medicines

Report and Qualitative Risk Assessment by the Committee for Veterinary Medicinal Products

14 July 1999
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At its meeting in January 1997 the Committee for Veterinary Medicinal Products (CVMP) agreed that antibiotic resistance in veterinary medicine and its possible transfer to man merited a detailed investigation. In March 1997 the Committee set up an ad-hoc group of scientific experts drawn from the European Community to investigate this matter and report to the CVMP. In the light of the findings and recommendations of the ad-hoc group the CVMP would consider ways of managing any problems that were identified.

The terms of reference of the ad-hoc Working Party on Antimicrobial Resistance were: To investigate the prevalence and changes in antibiotic resistance in animals, its effect on therapy and potential risk to human health.

The members of the ad-hoc group were:

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Dr G. Kienersberger (until April 1998) and Dr B. Cyrus from the European Medicines Evaluation Agency acted as secretary to the group.

Dr M. Wooldridge, head of department of risk research, Central Veterinary Laboratories, UK, carried out the qualitative risk assessment (annex IV of this report).

The group met on 8 occasions. The Fédération Européenne de la Santé Animale (FEDESA) was invited to attend one of the group’s meetings to present information.

The group would also like to express its regards to those CVMP members who supported their work by forwarding necessary information or revising parts of the report.
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CHAPTER I: INTRODUCTION

1.1 **Definition of Antibiotics**
(Sande and Mandell, 1985; Bywater, 1991)

1.1.1 Natural Antibiotics

Natural antibiotics are chemical substances produced by various species of microorganisms (bacteria and fungi) that are able to suppress or kill the growth of bacteria. Hundreds of natural antibiotics have been identified, and nearly 100 have been developed to the stage where they are of value in the therapy of infectious diseases. The first identified natural antibiotic was benzylpenicillin. Other examples are streptomycin, chloramphenicol, tetracyclines and macrolides.

1.1.2 Semi-synthetic antibiotics

Semi-synthetic antibiotics are derivatives of natural antibiotics. They are obtained by small alterations in structural formulas of natural antibiotics.

For example, soon after the introduction of benzylpenicillin, a small variation in the growth medium for the *Penicillium* altered one side chain of its structure by a single oxygen atom, resulting in phenoxyethylpenicillin. This derivative is acid-stable and is suitable for oral administration. After chemical identification of natural antibiotics many derivatives have been, or are still produced and tested for their antibacterial activity. Other examples of semi-synthetic antibiotics are the penicillinase resistant semi-synthetic penicillins such as nafcillin, cloxacillin and flucloxacillin.

1.1.3 Synthetic antibiotics

Synthetic antibiotics formerly called chemotherapeutics are chemically synthesised. The first compound with chemotherapeutic activity that was used therapeutically was Prontosil, an azo dye structurally related to sulphanilamide. Soon afterwards the sulphonamides were developed, and they still play an important role in therapy of infectious diseases. More recent examples of synthetic antibiotics are the nitrofurans and the quinolones.

1.1.4 Mechanisms of action

The diverse sites of action of antibiotics are summarised in figure 1. Their mechanisms of action fall into four categories:

- inhibition of cell wall synthesis (β-lactam antibiotics, vancomycin, bacitracin);
- damage to cell membrane function (polymyxins, polyenes);
- inhibition of nucleic acid function (nitroimidazoles, nitrofurans, quinolones, rifampicin) or intermediate metabolism (sulphonamides, trimethoprim);
- inhibition of protein synthesis (aminoglycosides, folic acids, lincosamides, macrolides, streptogramins, pleuromutilins, tetracyclines).
**Fig. 1** Action sites of antibiotics (Prescott and Baggot, 1988)
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1.2 BACKGROUND AND HISTORY

Antibiotic products are used by veterinary surgeons in the treatment and control of many types of infectious disease such as mastitis, enteritis, peritonitis, pneumonia and septicaemia as well as for local infections in a wide variety of food and companion animal species. If one or a number of animals in a group have overt signs of disease, both sick and healthy animals may need to be treated with an antibiotic product. This is intended to cure the clinically affected animals, reduce the spread of disease and prevent clinical signs occurring in the remaining animals in the group. Antibiotic products may be authorised for the treatment of an individual animal, e.g. by injection, intramammary infusion or by bolus, or for the treatment of groups of animals’, e.g. by oral medication in food or water.

Certain antibiotic substances are also used for growth promotion in food producing animals to increase the rate of weight gain and reduce the amount of feed per unit of gain. They are administered in feed at subtherapeutic doses during the growing period, particularly in pigs and poultry.

The modern era of chemotherapy of infection began with the clinical use of sulphanilamide in humans in 1936. Antibiotic therapy began with the production of benzylpenicillin for clinical trials in 1941 and was followed by the development of streptomycin (1944), chloramphenicol (1947), chlorotetracycline (1948), semi-synthetic penicillins (1958 onwards), cephalosporins (1960s) and fluoroquinolones (1980s).

Since the 1950s and parallel to development in the use of antibiotics to control disease in man, veterinary use has provided similar control in both farm animals and domestic pets. This has contributed to improvements in animal health and welfare and to the marked increase in productivity of livestock destined for human consumption. In the 1950s and 1960s antibiotics such as penicillin and the tetracyclines, in addition to being used for therapy, were used in many countries at subtherapeutic doses for growth enhancement without veterinary prescription. During the 1960s concerns developed about the increase of antibiotic resistance in strains of Salmonella associated with calf disease. The emergence of multiple antibiotic resistance led in the United Kingdom (UK) to the setting up of a Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. In its report to the UK Government, this Joint Committee (1969) recognised that the administration of antibiotics particularly at subtherapeutic levels posed certain hazards to human and animal health. The Committee concluded that these hazards could largely be avoided and recommended that antibiotics available without prescription in animal feed should be of economic value in livestock production but should have little or no application as therapeutic agents in man or animals and should not impair the efficacy of prescribed therapeutic drugs through the development of resistant strains of the organism.

As a result of the Committee’s recommendations, the principle of using different antibiotics for therapy or for growth promotion became established in the European Union (EU). Penicillin and the tetracyclines were no longer permitted at subtherapeutic levels for growth promotion, and they, together with the sulphonamides, became available only on veterinary prescription at therapeutic levels for the treatment of animal diseases. The macrolides tylosin and spiramycin were, however, permitted for growth promotion until their ban in June 1999, although the related antibiotic erythromycin is an important therapeutic drug in human medicine and the same drug is used for therapy in veterinary medicine.

1.2.1 Recent Developments

During the last 5 years the debate on antibiotic resistance has intensified. Sweden, which had not permitted the use of growth promoters in food animals since 1986, joined the European Community on 1 January 1995 and was granted derogation until the beginning of 1999 not to use these products. Also Finland which joined the European Community at the same time had a derogation not to allow the use of avoparcin, tylosin, spiramycin or related antibiotic feed additives. Concerns have also been raised about the risks to public health of fluoroquinolone therapy in animals. The multidisciplinary and international
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aspects of antibiotic resistance have led to a number of meetings to address the issues. In 1995, a Task Force in the USA recognised the emergence of antibiotic resistance as a serious problem and made recommendations about the establishment of a national surveillance system, better education to reduce inappropriate usage and more research to develop new products and reduce reliance on antibiotics. The World Health Organisation held meetings in October 1997 (WHO, 1997) on the “medical impact of the use of antibiotics in food animals” and in June 1998 on the “use of fluoroquinolones in food animals and potential impact on human health” (WHO, 1998). In September 1998, a meeting of European Chief Medical Officers in Copenhagen recognised that the major contributor to antibiotic resistance in human pathogens was clinical usage in human medicines but that the overall reduction of antibiotic resistance required the pursuit of common principles in both human and veterinary medicine (Copenhagen Recommendations, 1998). In the European Union, the Council of Agriculture Ministers decided in December 1998 to ban four antibiotics (virginiamycin, spiramycin, tylosin phosphate and zinc bacitracin) used at subtherapeutic levels as growth promoters from 1 July 1999 (Council Regulation (EC) No 2821/98).

The CVMP’s Working Group on Antimicrobial Resistance was set up in 1997 to carry out an assessment of the risk to public health of antibiotic use in animals with particular reference to antibiotics authorised for therapy in animals and to report to the CVMP.

1.2.2 Authorisation of Antibiotics in the EU

The authorisation of all veterinary medicines in the EU including antibiotic products for animal therapy is part of a harmonised procedure as established in the Council Directives 81/851/EEC and 81/852/EEC. A key feature of the legislation is that a marketing authorisation is required before any product can be sold or supplied. Marketing authorisations are granted only after the product has undergone rigorous assessment on the criteria of safety, quality and efficacy. Safety includes the safety of the treated and in contact animals, the user of the product, the environment and the consumer of products from the treated animals.

Since 1 January 1998 there have been two main procedures for granting marketing authorisations in the EU - the centralised and the decentralised system. Furthermore, products marketed in one country only can be authorised on a national basis. All three procedures, however, operate on the basis of the EU legislation and guidelines, which set out the nature of the data to be provided by the applicant.

Volume VII of The Rules Governing Medicinal Products in the European Union (European Commission, 1995) covers the pre-authorisation testing of veterinary medicinal products. This guidance identifies the essential topics to be covered in the efficacy dossier including pharmacodynamic, pharmacokinetic and clinical trials under experimental and field conditions. It is used by sponsors in the preparation of their dossiers and regulators in the assessment of new drugs. Susceptibility patterns of target bacteria are requested, the extent to which resistance can develop and the possibility of resistance being acquired by the normal flora.

The need to investigate the dynamics of resistance in intrinsically susceptible bacterial populations during a treatment is currently under debate.

All new and existing active substances used in veterinary medicinal products for food producing animals require a maximum residue limit (MRL) to be established under Council Regulation (EEC) 2377/90. This is used to set a withdrawal period for the product to prevent potentially harmful residues of veterinary medicine reaching the consumer in food. For antibiotic substances, microbiological MRLs are set and there is extensive surveillance for the presence of residues in animal products and food from animal origin. It is generally recognised that residues of antibiotics do not present a risk to the consumer in terms of antibiotic resistance.

The main risk to public health from the use of antibiotics in animals is presumed to be the development of resistance in animal bacteria. This resistance could lead to therapy failure in animal treatment and subsequently to a possible risk of transferring resistant zoonotic organisms (e.g. Salmonella) or other resistant bacteria to man.
Growth promoters have been authorised on a European basis since the 1970s under Council Directive 70/524/EEC. The data presented for authorisation must meet the requirements of Council Directive 87/153/EEC (as amended) which addresses the question of resistance. The topics to be covered include minimum inhibitory concentrations (MICs), cross-resistance to therapeutic antibiotics and effects on the gut microflora including the shedding of pathogenic microorganisms.

In addition to the above, antibiotic products are used not only for veterinary medicinal purposes and for growth promotion but also as disinfectants, preservatives or pesticides, but these are the subject of separate legislation. Products used for the control of coccidiosis in animals may also have antibiotic activity.
1.3 Antibiotic Resistance

The term antibiotic resistance can be used in two ways, either as microbiological resistance or as clinical resistance.

1.3.1 Microbiological resistance

Resistant organisms from a microbiological point of view are those that possess any kind of resistance mechanism or resistance gene. This term may be qualified in a quantitative way as "moderately or highly resistant" or as "low-level or high-level resistance".

The MIC of an antibiotic gives quantitative information about bacterial susceptibility. An organism is usually classified as susceptible, when its MIC is less than the breakpoint concentration (see also chapter: methods of determination of resistance).

1.3.2 Clinical resistance

From a clinical point of view the classification of bacteria as susceptible or resistant depends on whether an infection with the bacterium responds to therapy or not.

Before treatment, information on susceptibility may be obtained from the laboratory.

Although for many bacterial species knowledge of their antibiotic susceptibility is considered to be indispensable in rational therapy, the MIC for a specific bacterium does not fully reflect the antibacterial activity of the antibiotic under clinical conditions. The *in vitro* measurement of susceptibility is determined under arbitrarily established conditions and it may bear little relationship to the conditions achieved in infected tissues.

In a clinical setting the successful use of antibiotics depends not only on the dosage regimen and the pathogenic agent, but also on the pharmacokinetics of the antibiotic in the different animal species. Furthermore, other factors such as the status of the patient’s defence mechanisms can influence the success of antibiotic therapy. In the majority of patients, antibiotics probably do not kill all pathogenic microorganisms *per se*, but assist the patient’s immune system in its attempt to eliminate the infection.

In addition, the anatomical location of the infection and the concentrations of an antibiotic achievable in different organs/tissues may vary considerably. For example, high concentrations of antibiotics can be achieved in the urinary tract, when the main route of elimination of the compound is via the urine. This might affect the level of the breakpoint MIC used for urinary infections to classify the bacterium as resistant.

Therefore the susceptibility of bacteria should be expressed as quantitative MIC values rather than classifying them as either sensitive or resistant in the clinical manner.

1.3.3 Resistance distribution in bacterial populations

Frequency distributions of MIC values of one antibiotic in different bacterial species can be significantly different. A bimodal population reflects the difference between susceptible and resistant sub-populations. Often these population distributions are more complex, because intermediately resistant populations can also exist.

In unimodally distributed populations the difference between (clinically) susceptible and resistant can only be determined by the breakpoint MIC value.

In bimodally distributed populations often only a small number of bacteria is resistant. Therefore, methods to detect susceptibility or resistance should be well defined and standardised in order to avoid a failure of tracking this resistant sub-population. During therapy (selection pressure) the resistant sub-population will not be inhibited by the antibiotic, and may be responsible for therapy failure.

Resistance in a bacterial population may or may not be reversible, depending on the antibiotic, the bacterial species, the selection pressure and other factors. Generally, long-term resistance trends are unpredictable, and specific information on reversibility of resistance in a bacterial species or population to a new antibiotic will usually not be known until the compound has been in widespread use for an extended period.
1.4 Genetics of Resistance

Antibiotics have been used for more than 60 years. During this period a tremendous selection pressure has been exerted on bacterial eco-systems in humans and animals and has led to the emergence of resistant bacteria. Looking at the history of antibiotic agents, development of bacterial resistance has been an expected but rather an unpredictable phenomenon (Huovinen et al., 1997). Bacteria isolated from patients 60 years ago had virtually no resistance genes (Hughes and Datta, 1983). Similarly, strains resistant to new antibiotic agents have not been seen among several species of bacteria until these agents had been used for years or decades.

The most reliable information on the characteristics of bacteria from the pre-antibiotic era comes from studies of the "Murray-collection", microbial pathogens that were collected between 1914 and 1950. These organisms are completely susceptible to the common antibiotic agents. Even though sulphonamides were introduced into clinical practice in the mid-1930s, the "Murray collection" is susceptible to this class of drugs. Nevertheless, many of the "Murray strains" carry plasmids and are capable of promoting conjugative transfer (Davies, 1997).

When antibiotics were first introduced for treatment of common bacterial infections, development of antibiotic resistance during therapy was not expected, because the frequency of mutation to resistance in bacteria was thought to be too low. It was at that time unknown that even in nature, bacteria can collect and exchange genetic information with extraordinary ease and lack of species specificity (Davies, 1994).

However, antibiotic resistance exemplifies "par excellence" Darwinism. Resistance has developed rapidly, as has been observed after the introduction of most of the "new" antibiotics.

Table 1: Antibiotic discovery and resistance development
(Ronald et al., 1966, Kliebe et al., 1985; von Eiff et al., 1997; Davies, 1997; O’Brien, 1997; Soussy, 1998, Wiedemann and Heisig, 1999)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Discovered</th>
<th>Introduced into clinical use</th>
<th>Resistance identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1940</td>
<td>1943</td>
<td>1940 (Methicillin 1961/5)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1944</td>
<td>1947</td>
<td>1947, 1956</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1952</td>
<td>1956</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1955</td>
<td>1956</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1972</td>
<td>1987</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1960</td>
<td>1962</td>
<td>1966</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1963</td>
<td>1967</td>
<td>1970</td>
</tr>
<tr>
<td>Third generation cephalosporins</td>
<td>NA</td>
<td>1980</td>
<td>1985</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>1978</td>
<td>1982</td>
<td>1985</td>
</tr>
</tbody>
</table>

NA = date not available

All resistance has a genetic basis, which might either be a fixed part of the bacterial genome or be transferable between bacteria. Whenever antibiotics are used, bacteria will inevitably develop resistance, either by mutation, gene acquisition, or a combination of both. Surviving resistant strains have emerged under protection and selection by the antibiotic.

Bacterial resistance to antibiotics may be intrinsic (natural) or acquired. Intrinsic resistance (nonsusceptibility) is a characteristic of bacterial species that are homogeneously resistant to a particular antibiotic, either because they lack the cellular mechanisms by which that particular antibiotic exerts its action or because the bacterial wall is impermeable to the antibiotic. The latter is commonly encountered in Gram-negative species.

Acquired resistance can originate from chromosomal mutation or from the acquisition of transferable genetic material already present within related or unrelated bacterial populations. It can be found in every pathogenic bacterial species as well as in the commensal flora of man and animals, but the
prevalence varies considerably between bacterial species and even between subspecies. For example, Gram-positive bacteria except staphylococci and enterococci, often lack the ability to acquire plasmids containing resistance genes (R-plasmids).

1.4.1 Chromosomal resistance

This type of resistance develops from mutations in the nucleotide sequences of the bacterial chromosome resulting in the synthesis of proteins or other macromolecules that differ sufficiently from the original chemical entities to interfere with the antibiotic activity. Mutations can occur continuously and irrespective of the presence of antibiotics, but are generally lost or "repaired" by cellular mechanisms. Transfer of mutations takes place during multiplication (vertical transfer). The mutation frequency is low, usually in the range of $10^{-6}$ to $10^{-10}$ per generation. Mutations with increased antibiotic resistance are advantageous to the bacterium only when antibiotics are used. Only susceptible bacterial sub-populations will be eliminated while the antibiotic remains in the environment in concentrations above the MIC. Development of resistance resulting from mutations is usually specific to the selecting antibiotic agent or closely related antibiotics.

Chromosomal resistance is inherited clonally. Development of chromosomal resistance within a population exposed to an antibiotic is usually a gradual, step-wise process effected by several successive mutations, but for some antibiotics a single mutation may produce resistance resulting in a dramatic increase in the MIC. Resistant mutants emerge less frequently in vivo than in vitro, probably because mutations leading to resistance are often associated with other cell changes, which may be disadvantageous to the bacterium. In general the number of the resistant mutants will decrease after cessation of exposure. Therefore, some scientists regard the development of resistance in many bacterial species caused by chromosomal mutations as a smaller problem than transferable resistance. Nevertheless, this depends mainly on factors like survival capacity of the mutants, cross-resistance and co-resistance to other antibiotics or substances and their use.

1.4.2 Transferable resistance

Bacteria have extremely efficient genetic transfer systems capable of exchanging and accumulating resistance genes. Certain bacterial genes, including genes encoding for resistance, can move between chromosomal and extra-chromosomal DNA elements in bacteria. They may move between bacteria belonging to the same or different species or to bacteria of different genera (horizontal transfer). Antibiotic resistance genes on plasmids and transposons flow to and from Gram-positive and Gram-negative bacteria, and among bacteria which inhabit vastly different ecological niches (Levy, 1997). Inter-species transfer implies that once transferable resistance genes have developed, bacteria carrying these genes will remain potential gene donors for other bacteria.

Resistance genes commonly occur in the natural bacterial flora and not all transferable resistance has been induced by use of man-made antibiotics. The transfer of antibiotic resistance genes in natural environments has a very broad host range and can happen even between phylogenetically distinct bacterial genera, such as between Gram-positive and Gram-negative bacteria ("trans-Gram-conjugation/promiscuity", Courvalin, 1994). The first report in the mid-1950s of transferable antibiotic resistance genes was in Japan (Davies, 1997).

The most important vehicles for transfer of resistance genes in bacteria are plasmids, transposons and integrons. Because of their mobility, transferable resistance elements are more likely to persist at a low level in an eco-system even in the absence of antibiotic selection pressure than chromosomal resistance.

1.4.2.1 Plasmids

Plasmids are extrachromosomal, replicable circular DNA molecules that may contain resistance genes. They replicate independently of bacterial chromosomal DNA. Plasmids are important in bacterial evolution, because they affect replication, metabolism, bacterial fertility as well as resistance to bacterial toxins (bacteriocins), antibiotics and bacteriophages, thus providing a better chance of survival and propagation. Nevertheless, in general plasmids are not necessarily required by the bacterium for its
survival. They have been identified in most bacterial species may have the capacity to be transferred (conjugative plasmids) or co-transferred (non-conjugative plasmids) from one bacterium to another, thus resulting in wide spread dissemination of plasmid-encoded characteristics within a bacterial ecosystem. Genes encoded by plasmids are intrinsically more mobile than chromosomal genes because plasmids can be transferred within the same and between different species. R-plasmids are plasmids containing resistance genes. The acquisition of new resistance determinants can occur much more readily by R-plasmids than by genetic mutation. A single R-plasmid may code for resistance to up to 10 different antibiotics simultaneously. Many different R-plasmids have been identified. Plasmids from human and animal isolates seem to be very similar.

Dissemination of plasmids may occur by clonal distribution and by intra-species and inter-species transfer resulting in a gradual increase of the proportion of microorganisms within a bacterial community carrying one or more R-factors. Although some resistance plasmids are non-conjugative, they may often be transferred (mobilised) to a recipient if they co-inhabit a cell with a conjugative plasmid. In contrast to mutation-based chromosomal resistance, acquisition of an R-plasmid generally confers resistance to clinically achievable levels of an antibiotic in a single step.

In Gram-negative bacteria the transfer/acquisition of further plasmid-mediated characteristics, such as virulence and enterotoxin production, is in some instances facilitated by the presence of R-plasmids. Furthermore, a single bacterial cell can contain many different plasmids and each plasmid can carry more than one resistance gene.

1.4.2 Transposons

Transposons (jumping genes) are short sequences of DNA that can move between plasmids, between a plasmid and the bacterial chromosome or between a plasmid and a bacteriophage (bacterial virus). Unlike plasmids, transposons are not able to replicate independently and must be maintained within a functional replicon (e.g. plasmid or chromosome).

Transposons in Gram-negative bacteria are non-conjugative, in Gram-positive bacteria and Bacteroides spp. they can either be conjugative or non-conjugative. However, if a transposon in Gram-negative bacteria is part of the DNA of a conjugative plasmid, horizontal transfer is possible. Transposons, including those carrying resistance genes, are easily acquired by plasmids and then incorporated into bacterial DNA. Often several transposons are clustered on the same plasmid, resulting in the transfer of multiple resistance determinants with a single conjugation (Burns, 1995). Plasmids of different origin may also carry several sets of identical resistance genes.

The intracellular transfer of transposons between plasmids, between bacterial chromosomes and plasmids as well as an inter-bacterial transfer of plasmids and conjugative transposons can result in rapid development of resistance within several bacterial populations. The major impact of transposons on the emergence of antibiotic resistance is that they can expand the host range of bacteria species to which resistance can be spread.

Expression of resistance genes located on transposons, e.g. production of specific enzymes, may require the presence of the antibiotic(s) in question. Furthermore, the presence of the antibiotic will promote transfer of resistance. Antibiotics create an environment in which possession of resistance determinants is advantageous and, in addition, the rate of transfer of resistance genes will increase.

1.4.2.3 Integrons and gene cassettes

Integrons are naturally occurring gene expression elements. They are composed of two conserved regions and an interposed variable region, which contains gene cassettes for antibiotic resistance. Gene cassettes are elements that include a single gene and a recombination site. More than 40 cassettes have been identified and all but five contain resistance genes (Hall, 1997). One of the conserved regions of the integron contains the integrase gene, which is responsible for the site-specific insertion of the cassettes. The expression of the cassettes is driven in a co-ordinated way by two randomly arranged promoters (Hall, 1997). Integrons can be located in the chromosomal DNA, but are more often located in plasmids or transposons and are therefore mobile. The characteristic resistance pattern in the
chromosome of *Salmonella* Typhimurium DT104 is associated to the presence of integrons (Carattoli, 1998).

**1.4.3 Mechanisms for inter-bacterial transfer of resistance**

Several mechanisms have been identified for transfer of genetic material, including resistance genes, between bacteria. An important mechanism is bacterial conjugation, whereby a plasmid or other genetic material is transferred from the donor bacterium to the recipient via a cytoplasmatic bridge. Conjugation may occur between bacteria of the same species, within species of the same genera or between species of different families. Other ways of inter-bacterial transfer are transduction (transmission by bacteriophages) and transformation (direct transfer of free DNA originating, for example from lysed bacteria).
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1.5 METHODS OF DETERMINATION OF RESISTANCE

Since Fleming's discovery of penicillin many laboratories have been searching for in vitro methods to study the effect of antibiotics on bacteria, and consequently many antibiotic susceptibility tests have been proposed. Table 2 presents the relevant antibiotics for each bacterial species recommended being included in susceptibility tests. The susceptibility of bacteria to antibiotics can be tested in a quantitative or a qualitative way. Quantitative methods will result in data that can be related to actual concentrations of antibiotics inhibiting the growth of bacteria, e.g. MIC-values. Qualitative methods will categorise bacteria as susceptible, intermediate or resistant.

The cornerstone of the antibiotic susceptibility test is the minimum inhibitory concentration (MIC) of an antibiotic that can be determined by agar- or broth dilution methods (macro- and micro dilution). In both methods antibiotic concentrations (usually two-fold dilutions) are mixed with growth media. For a specific bacterium the MIC is the lowest antibiotic concentration that inhibits bacterial growth.

1.5.1 Agar/ Broth Dilution Methods (quantitative tests)

In the broth dilution method the MIC refers to the antibiotic concentration of the most diluted tube without any bacterial growth. Equally, in the agar dilution method, the MIC refers to the antibiotic concentration of the most diluted agar plate without bacterial growth.

Procedures for standardisation of the antibiotic susceptibility test are described by different organisations. Examples are the US National Committee for Clinical Laboratory Standards (NCCLS, 1993), the British Society for Antimicrobial Chemotherapy (BSAC, 1991), the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM, Soussy et al., 1994), the Deutsches Institut für Normung e.V. (DIN 58 940, 1992) and the Spanish Society for Chemotherapy of the Society for Clinical Microbiology and Infectious Diseases (MENSURA, Baquero et al., 1997). These procedures give detailed instructions on preparation of antibiotic dilutions, preparation of inocula, breakpoints used (see below), and any other aspect that may affect the reproducibility of the results. Dilution methods are internationally accepted as the reference standard when other methods are tested.

Irrespective of the method used, interpretative criteria have to be agreed upon before the tests are performed. Additionally, dilution methods are time-consuming and need good laboratory infrastructure (staff or automation), because many test tubes, micro titre trays or petri dishes must be prepared for each test and many parameters must be controlled. For these reasons, these methods are in general not used for routine tests for antibiotic susceptibility in clinical laboratories. Moreover, MIC determinations are expensive compared to agar diffusion tests.

1.5.2 Interpretative criteria (breakpoints)

To interpret the results of quantitative susceptibility tests, criteria (breakpoints) have to be agreed upon. These breakpoints can be used to categorise bacterial strains as "susceptible", "intermediate resistant" or "resistant" to a specific antibiotic substance. Breakpoint MIC values are calculated for each antibiotic. Based on the antibiotic's pharmacokinetics, protein binding and half-life, and a factor by which the maximum concentration in plasma should exceed the MIC (usually a factor of 4), breakpoint concentrations of the antibiotics are calculated.

Because in veterinary medicine specific breakpoints have not been agreed upon, human breakpoints are used instead. For veterinary products without human equivalent no official breakpoints exist.

1.5.3 Agar Diffusion Method (qualitative test)

Kirby, Bauer, Sherris and others developed the agar diffusion method in the 1960s (Bauer et al., 1966). In this method agar plates are overlaid with a bacterial suspension. Paper discs or tablets impregnated with defined concentrations of antibiotics are placed on the agar surface. The antibiotic diffuses in the agar around the discs/tablets and inhibits bacterial growth, which forms circular images on the plates. The diameter of these inhibition zones relates to the MIC values of the appropriate antibiotic, and thus interpretative criteria can be agreed upon, based on the breakpoint MIC values. With adequate interpretative criteria, the laboratory
technician can classify the bacterium as sensitive, intermediate or resistant by measuring the inhibition zone diameter around an antibiotic disc/tablet.

The main parameters of diffusion methods (inocula, agar media, antibiotic discs/tablets, interpretative criteria) are also well standardised and make it possible to compare the results of laboratories using the same method. However, results obtained with the agar diffusion method at different laboratories should be interpreted with care, because many factors may affect the size of the inhibition zone diameter.

Advantages of the agar diffusion method are their simplicity and low costs, since up to six antibiotic discs/tablets can simultaneously be tested on a 90 mm petri dish.

1.5.4 Other Tests

The E-test is a gradient-diffusion method for the determination of MIC. Commercial strips with a continuous antibiotic gradient are used instead of discs, and allow accurate determination of the MIC.

Another method is the breakpoint susceptibility test. In this method fixed concentrations of antibiotic agents, representing confirmed cut-off points of bacterial susceptibility or resistance, are incorporated into agar or broth. Test bacteria and control bacteria are inoculated and their growth/non growth is recorded after incubation. This method is basically a truncated version of the agar or broth dilution method.

Commercial kits are available to facilitate MIC determination. These kits use a limited number of antibiotic concentrations (from one to eight) in a micro titre tray for MIC determination.

1.5.5 Molecular techniques

Molecular techniques are powerful tools to detect antibiotic resistance (Bergeron and Oulette, 1998). With these tools DNA fragments encoding for resistance (resistance genes) can be detected. However, molecular methods are not routinely used in clinical laboratories yet.

The methodology is mainly based on the polymerase chain reaction and DNA-DNA hybridisation (southern blot) technique. These molecular techniques are used to study bacterial isolates (like Neisseria meningitidis, Enterococcus faecalis/faecium, Streptococcus pneumoniae, Helicobacter pylori, Campylobacter jejuni, Mycobacterium tuberculosis or coagulase-positive and -negative staphylococci). They are also used to a lesser extent to detect resistance genes in clinical samples directly (clarithromycin resistant Helicobacter pylori from gastric biopsy samples, methicillin-resistant Staphylococcus aureus (MRSA) in endotracheal aspirates, rifampin resistant Mycobacterium tuberculosis in broncho-alveolar lavages or vancomycin resistant enterococci (VRE) in faecal samples).

However, the molecular approach is not free of difficulties. Especially in Gram-negative bacteria the diversity of resistance mechanisms will complicate the detection of the resistance genotype and not all resistance genes will be expressed phenotypically. Nevertheless, these methodologies will find a place in the clinical microbiology laboratory in the near future.
Table 2: List of relevant antibiotics (+) for each bacterial species.

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<tr>
<td>Miscellaneous</td>
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<tr>
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<tr>
<td>Fusidic acid</td>
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<tr>
<td>Rifampicin</td>
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<td>+</td>
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</tr>
</tbody>
</table>

Gram-negative include: E. coli, Salmonella spp., Bordetella spp., Yersinia spp.
Gram positive include: staphylococci, streptococci, Erysipelothrix rhusiopathiae, Listeria monocytogenes
A/P/H: Actinobacillus spp., Pasteurella spp., Haemophilus spp.,
1.6 **MULTIPLE-DRUG RESISTANCE (MULTIRESISTANCE)**

Within the past few years, several divergent organisms have emerged as significant causes of morbidity and mortality in human medicine, including infections caused by bacteria that are refractory to therapy because of resistance to many antibiotic agents (Levy, 1998). Examples are *Salmonella Typhi*, penicillin resistant pneumococci, methicillin resistant *Staphylococcus aureus* (MRSA) and multiresistant mycobacteria.

Simultaneous resistance in one bacterium to three or more classes of antibiotics by various resistance mechanisms generally encoded by different genes is defined as **multiresistance**. Exceptions are for instance multi-drug-resistance-genes, which encode for different resistance phenotypes by using the same mechanism (e.g. efflux). **Cross-resistance** is defined as resistance to different antibiotics by the same resistance mechanism. In general these antibiotics belong to the same class.

Multiresistance in bacteria is generally attributed to the acquisition of transposons, integrons and/or plasmids bearing genetic determinants for different mechanisms of resistance. If a bacterium is multiresistant with genetically linked resistance determinants, it will not easily lose its resistance to a particular antibiotic, even when this drug is not used for a long period of time. One reason for this would be that the gene, which encodes for resistance to that antibiotic could remain present as a result of the use of other antibiotics to which the determinant is genetically linked (co-selection). Another explanation would be that the plasmid encoding the gene is not counter-selected in the absence of the antibiotic.

Because of the intensive use of antibiotics in hospitals and animal production, hospital strains of bacteria and bacteria in farm animals tend to "collect" resistance genes. As a result, in these environments in general a larger number of multiresistant bacteria can be detected than in environments with less selection pressure.

### 1.6.1 Gram-positive bacteria

#### 1.6.1.1 General

In some bacteria large numbers of transposable elements have been discovered carrying virtually all possible combinations of known resistance genes. In hospitals, as a result of selection by antibiotic use, nosocomial infections caused by multi-/methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase negative staphylococci and glycopeptide-resistant *Enterococcus faecium* (McDonald et al. 1997) are new Gram-positive challenges (Mouthon and Mainardi, 1996). Outbreaks of multiresistant *Mycobacterium tuberculosis* in HIV-infected patients in the USA and Europe have focused international attention (Anonymous, 1998).

Community-acquired infections with multiresistant *Streptococcus pneumoniae* and *Shigella sonnei* also cause treatment problems both, in the developed and the developing world.

Isolates of methicillin-resistant *Staphylococcus aureus* which are also resistant to all penicillins including β-lactams, cephalosporins and carbapenems are frequently also resistant to other antibiotics, especially macrolides, quinolones, aminoglycosides, lincosamines and trimethoprim-sulfamethoxazole. These multiresistant strains cause serious therapeutic problems (Voss et al., 1994, Eiff et al., 1997). The glycopeptides, vancomycin and teicoplanin presently remain the cornerstone of treatment (‘last resort’ reserve antibiotics) for all MRSA infections in human medicine. Therefore, clinical acquisition of vancomycin resistance by MRSA would be catastrophic (Segal-Maurer et al., 1996). However, recently infections with vancomycin (glycopeptide)-intermediate resistant *S. aureus* strains (VISA/GISA) in Japan and the USA have been reported (Sieradzki et al., 1999; Smith et al., 1999).

#### 1.6.1.2 Glycopeptide-resistant enterococci

Multiresistant / glycopeptide-resistant enterococci are currently emerging nosocomial pathogens. They have already become the second most common bacterium recovered from nosocomial infections, and the third most common cause of nosocomial bacteraemia in the USA. One of the
Chapter I: Introduction

major reasons why these organisms have thrived in the hospital environment in the USA is their intrinsic resistance to several commonly used antibiotics and their ability to acquire resistance to all currently available antibiotics as shown in table 3 (Moellering, 1998).

<table>
<thead>
<tr>
<th>Table 3: Multiple drug resistance in <em>Enterococcus faecium</em>:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrinsic resistance</strong></td>
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<tr>
<td>β-lactams (particularly cephalosporins, penicillinase-resistant penicillins)</td>
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<tr>
<td>Aminoglycosides (low concentrations)</td>
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<tr>
<td>Fluoroquinolones</td>
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<tr>
<td>Trimethoprim-sulphonamides</td>
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<td>Clindamycin</td>
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<sup>1</sup> PBP: Penicillin binding protein

The emergence of vancomycin resistant enterococci (VRE) mainly results from the intensive use of glycopeptides, especially the parenteral and oral use of vancomycin in hospitals. The acquisition of or colonisation with VRE by hospitalised patients has also been associated with a number of other factors like the length of hospital stay, underlying disease, intensity of antibiotic exposure, additional use of broad-spectrum drugs such as cephalosporins etc. A recent survey demonstrated greater mortality in patients associated with VRE bacteriaemia compared to patients with vancomycin-susceptible enterococcal bacteriaemia (36.6% versus 16.4%) (Segal-Maurer et al., 1996).

VRE with the vanA-gene have been isolated extensively in animals and food products in Europe. The glycopeptide avoparcin, which can induce cross-resistance to vancomycin and teicoplanin has been used as an antibiotic feed additive in food producing animals in the EU for nearly 20 years. However, in Europe only a few clinical outbreaks of infections with VRE have occurred in humans in comparison to the USA, where infections occurred despite the fact that avoparcin has not been authorised for use in animals in the USA (McDonald et al., 1997). Moreover, European strains of VRE are generally less multiresistant.

The streptogramin-combination of quinupristin and dalfopristin is a promising new reserve-antibiotic for treatment of infections with MRSA and VRE. However, the use of virginiamycin as feed additive has already selected for resistance in enterococci of animal origin in Europe (Bogaard et al., 1997; DANMAP, 1998; Hammerum et al., 1998).

1.6.2 Gram-negative bacteria

Gram-negative bacteria are very important pathogens of humans and animals. The first well-documented bacterial outbreak involving multiresistant bacteria was an epidemic of typhoid fever caused by *Salmonella* (S.) Typhi (which is solely a human pathogen) in Mexico in the early 1970s with more than 10 000 confirmed cases in 1972. The S. Typhi strain involved carried genes encoding for resistance to chloramphenicol (the former drug of choice for treatment of this infection), ampicillin, streptomycin and sulphonamide (Amáble-Cuevas et al., 1995).

Recently multiresistant (including fluoroquinolone-resistant) enteropathogenic bacteria responsible for community-acquired infections, with zoonotic and pathogenic *Salmonella* and *Campylobacter* (Acar and Goldstein, 1997; Gold and Moellering, 1996) and *Escherichia coli* (*E. coli*) have been described (Kim et al., 1994; Murphy and Echeverria, 1998).

Zoonotic infections like salmonellosis are examples of the development and spread of multiresistant bacteria from animals to man via the food chain. Recent data from several European countries show a decreasing prevalence of susceptible *S. Typhimurium* strains. This is caused by the clonal spread of multiresistant *S. Typhimurium* DT104, which can be present in all species of farm animals, especially...
in poultry, cattle and pigs. This phage type of \textit{S.} Typhimurium causes severe illness, particularly in cattle (Angulo, 1997; Liesegang et al., 1997; Van Pelt and Leeuwen; 1998; Wall, 1997; Wray, 1997). The use of antibiotics in food animal production may be one of the factors contributing to the rapid dissemination of multiresistant \textit{S.} Typhimurium DT104 (Wegener, 1998). Nevertheless, other factors might be important as well, which can be illustrated by the example of \textit{S.} Enteritidis PT4, where a global clonal spread of a bacterium occurred that is in general very susceptible to all antibiotics. Resistance genes causing so-called ACSSuT type resistance (resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines) occur as a group of chromosomally integrated resistance determinants with a tendency to acquire further resistance determinants (i.e. trimethoprim and fluoroquinolones). Resistance to fluoroquinolones can also be acquired by mutation. This latter resistance type can be found in \textit{Salmonella} and causes marked concern because of the chance of therapy failure. In many countries fluoroquinolones are considered the first choice drug in humans for treatment of acute gastrointestinal infections.

In 1996 more than 95\% of \textit{S.} Typhimurium DT104 strains isolated from humans and sent to the Public Health Laboratory Service (PHLS) Laboratory for Enteric Pathogens in the UK (Threlfall et al., 1997) and about 90\% of the \textit{S.} Typhimurium DT104 registered in the National \textit{Salmonella} Reference Centre in Germany (Liesegang et al., 1997) were multiresistant to at least four antibiotics. In these countries \textit{S.} Typhimurium DT104 is the second most important \textit{Salmonella} causing infections in humans.
1.7 **EVOLUTION OF RESISTANCE**

Whenever antibiotics are used bacteria will inevitably develop resistance, either by mutation, gene acquisition, or a combination of both. Resistant strains have survived as a result of selection by the antibiotic. Little is known how those resistance genes first emerged or why it took quite a long period for many of them to emerge. Some of these genes may have awaited mutations necessary to evolve from ancestral genes within the species. Other genes may have already existed in environmental bacteria and have been transferred to species that are of more (veterinary) medical interest (O’Brien, 1997).

Extra chromosomal resistance plasmids were already present in bacteria isolated in the pre-antibiotic era. Most resistance genes in bacteria are identical or homologous to those found in antibiotic-producing microorganisms in the soil (see table 4). Antibiotic resistance must be as ancient as antibiotic synthesis and resistance genes were already present in natural environments in soil and water long before the therapeutic use of antibiotics commenced 50 years ago with the advent of penicillin. Their presence is believed to be related to the production of antibacterial agents by saprophytic organisms such as actinomycetes to protect these antibiotic-producing species against self-destruction (Burns, 1995; Bergogne-Bérézin, 1997). For instance, plasmids in *Streptomyces* often include resistance genes to the same antibiotics these organisms produce. Therefore, soil and the natural environment might constitute a huge reservoir for antibiotic resistance genes.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance mechanisms</th>
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<td>Penicillins, Cephalosporins</td>
<td>β-lactamas, Penicillin-binding proteins</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Acetyltransferases, Phosphotransferases, Nucleotidyltransferases</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Acetyltransferases</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Efflux system, Ribosomal protection</td>
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<td>Ribosomal RNA methylation</td>
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<td>Streptogramins</td>
<td>Esterases</td>
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<td>Lincosamines</td>
<td>Phosphotransferases, Acetyltransferases</td>
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<td>Glycopeptides</td>
<td>VanA-ligase</td>
</tr>
</tbody>
</table>

However, widespread emergence of genes expressing resistance to drugs with antibiotic properties and selection of new resistant strains occurred only after the agent became widely used in humans and animals (Danziger and Pendland, 1995). Within the European Union antibacterial drugs available for clinical use have increased within 36 years from 5 antibiotics (plus sulphonamides) in 1959 to 102 different molecules (Bergogne-Bérézin, 1997) in 1995. Since the first introduction of antibiotics in the late 1940s an inexorable increase of antibiotic resistance genes in bacterial pathogens, zoonotic and commensal bacteria has occurred. In particular, the production and use of large quantities of antibiotics have undoubtedly contributed to the selection of bacterial clones possessing resistance genes. Moreover, DNA sequences containing antibiotic-resistance genes have been found recently in commercial antibiotic preparations (Davies, 1997). The resistance gene pool in the environment is readily accessible to bacteria, which are exposed to strong selective pressures by antibiotic usage in hospitals, in general medical practise, for veterinary and agricultural purposes or as growth promoters in animal husbandry (Davies, 1994).
1.7.1 Resistance mechanisms

Over the years bacteria have developed numerous, and often elegant, ways to escape the action of antibiotic agents. The most common resistance mechanism is antibiotic inactivation, but bacteria utilise four main resistance strategies (Moreillon, 1995):

1. Modification of their permeability: Bacteria modify their permeability either by becoming impermeable to antibiotics or by actively excreting the antibiotic accumulated in the cell.
2. Modification of the antibiotic: Bacteria produce enzymes capable of modifying and directly inactivating antibiotics.
3. Modification of target: bacteria modify the structure of the antibiotic’s target molecule, usually an essential metabolic enzyme of the bacterium or express an alternative target molecule not inhibited by the drug, and thus escape the antibiotic’s toxic effect.
4. Overproduction of the target.

The resistance mechanisms developed by bacteria against the commonly used classes of antibiotics are as summarised in table 5.

Table 5: Main bacterial resistance mechanisms against classes of antibiotics

<table>
<thead>
<tr>
<th>Class of antibiotics</th>
<th>Cellular target</th>
<th>Modification of antibiotic resistance</th>
<th>Over-production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bacterial cell permeability</td>
<td>antibiotic inactivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased influx</td>
<td>Increased efflux</td>
</tr>
<tr>
<td><strong>β-Lactams</strong></td>
<td>Penicillin-binding proteins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td>30S ribosomal subunit</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sulphonamides</strong></td>
<td>Dihydropteroate synthetase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Trimethoprim</strong></td>
<td>Dihydrofolate reductase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Macrolides and Lincosamides</strong></td>
<td>50S ribosomal subunit</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Streptogramins</strong></td>
<td>50S ribosomal subunit</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td>30S ribosomal subunit</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Chloramphenicol</strong></td>
<td>50S ribosomal subunit</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Quinolones (Fluoroquinolones)</strong></td>
<td>DNA gyrase Topoisomerase IV</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Glycopeptides</strong></td>
<td>Peptidoglycan precursor</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
1.7.2 Persistence of antibiotic resistance

Evaluation of the persistence of antibiotic resistance is difficult since the genetic ecology is complex and analysis is usually retrospective.

Although antibiotics achieve selection for a new type of resistance quite rapidly, removal of the antibiotic stopping reverses this trend only slowly. For example, in some areas in the world it was seen that the use of tetracycline, streptomycin (Morell, 1997) or chloramphenicol caused the resistance to decrease very slowly (over the years). As the selected resistant bacteria (for instance by tetracyclines) are just as "fit" as the susceptible flora they continue to propagate and persist (Levy, 1997). Furthermore, in cases where a bacterium is multiresistant with genetically linked resistance determinants, resistance will remain present as long as other antibiotics are used to which the determinant is genetically linked (co-selection).

1.7.3 Factors that influence antibiotic resistance of bacteria

Pharmacokinetic characteristics of different classes of antibiotics may favour the development of resistance as well as dose regimen (e.g. insufficient dose, too short duration of treatment or long-term use), active concentration or route of excretion of the drug (Davies, 1994).

In particular, the long term use of sub-MIC concentrations (subtherapeutic doses) is regarded as one of the major factors responsible for development of resistance, exerting a potent selective pressure for the emergence of resistant clones that already pre-existed in the bacterial population (Corpet et al., 1989, Bergogne-Bérézin, 1997).

The progressive emergence of insensitive bacteria and of acquired resistance in human clinical settings and the veterinary fields reflects the "tuning of these microorganisms to antibiotic polluted" eco-systems (Courvalin, 1996).

The amount of antibiotics used is also a selective force (Prescott, 1997; Swartz, 1997). Some studies have shown that antibiotic usage has directly contributed to an increased prevalence of resistance (Pradier et al., 1997; DANMAP, 1998).

The association of local antibiotic consumption and development of resistance has been shown in many hospital studies (Alos and Carnicero, 1997; Huovinen et al., 1997) and in some studies in veterinary medicine (Rassow and Schaper, 1996; Klarmann, 1997).

Using antibiotics for treatment, therapy, metaphylaxis or prophylaxis in human and veterinary medicine and agriculture has exerted an enormous global selective pressure (Acar and Goldstein, 1997; Cohen, 1997; Levy, 1997). The evolution and dissemination of resistance is clearly associated with use, over- and misuse of antibiotics (Courvalin, 1996).

1.7.4 Evolution of resistance in special antibiotic classes

β-lactamases are enzymes found in most bacterial species, which are able to hydrolyse the β-lactam ring of β-lactam antibiotics. In Gram-negative bacteria and all Enterobacteriaceae chromosomally encoded β-lactamases confer resistance (Davies, 1997). Although these enzymes have protected bacteria against naturally occurring β-lactams long before the introduction of synthetic antibiotic agents, the numbers and varieties of β-lactamases have increased dramatically since the introduction of modern penicillins and cephalosporins. A single base change in the gene for a β-lactamase can change the substrate specificity of the enzyme. Such changes occur frequently, especially in the Enterobacteriaceae.

Stepwise selection of variants within the extended β-lactamases classes has been documented. Most bacterial species can synthesise at least one of the more than 200 described β-lactamases (Bush, 1997; Medeiros, 1997).

In addition, genes encoding the enzymes have been hopping out of the chromosome onto plasmids and then back onto the chromosome, resulting in virtually unrestrained transferability of many enzymes. Also, outbreaks of infections with β-lactam-resistant bacteria can be traced to the introduction of specific classes of β-lactams or the introduction of a specific agent.
Aminoglycosides resistance mechanisms in all types of bacteria have become more complex with the increased usage of aminoglycosides. Combinations of mechanisms occurred widening the spectrum of aminoglycoside resistance in all genera (Miller et al., 1997), for example 53 different inactivating enzymes have been observed in Enterobacteriaceae in Europe.

Sulphonamides and trimethoprim, both synthetic antibiotic agents, were first used in 1932 and 1962, respectively. A dramatic increase in resistance to trimethoprim along with the high-level resistance to sulphonamides has been observed during the past two decades. The number of genes encoding plasmids for resistance to dihydrofolate reductase is already 17; 16 in Gram-negative bacteria and one in S. aureus.

Resistance mechanisms show a remarkable evolutionary adaptation. Most of these genes are transferred by efficient gene cassettes. The genetic linkage of genes for both, trimethoprim and sulphonamide resistance largely invalidates the argument for using the combination of trimethoprim and a sulphonamide to prevent the development of resistance (Huovinen, 1997). However, resistance levels against combinations of these antibiotics seem to be lower than resistance levels against the individual antibiotics; thus it is possible that combinations may be more effective for therapeutic use.

Regarding resistance in macrolides, lincosamides and streptogramins three methylases (ermA, ermB and ermC) have been identified. The corresponding genes are located either on transposons (ermA, ermB) or on small plasmids (ermC). Among these erm genes, ermC has been detected most frequently in Staphylococci from humans and animals (Werckenthin et al., 1997). Other transferable mechanisms of resistance to streptogramins involve inactivating enzymes such as streptogramin-A acetyltransferase (sat-gene) and streptogramin-B hydrolase (vgb-gene) and have been described in staphylococci and enterococci (Zervos, 1997).

The primary target of fluoroquinolone action is DNA gyrase, a type II bacterial topoisomerase composed of two A subunits and two B subunits, encoded by gyrA and gyrB genes, respectively. Topoisomerase IV is another important target, especially in Gram-positive organisms. As quinolones did not exist in nature they had no analogues among microbial biosynthetic products before their development and introduction into human and veterinary medicine. A single mutation in the "quinolone resistance determining region" of the bacterial genome provides an increment for survival, a subsequent "hot spot" mutation produces even higher levels of resistance (Davies, 1997).

The emergence of resistance to fluoroquinolones in all species of bacteria was recognised soon after the introduction of these compounds for clinical use more than 10 years ago and evolved extremely rapidly.

Resistance genes (tet-genes) are known to be the basis for tetracycline resistance in Gram-positive bacteria. These resistance determinants promote efflux of the drug out of the bacterial cell, inactivation of tetracycline or target protection and are transposon-associated. Some of them are found in Gram-positive and as well in Gram-negative bacteria; e.g. Pasteurella spp. can contain tet-genes from Gram-positive and Gram-negative origin or both.

The conjugative transfer, mobilisation and transposition of conjugative transposons that encode tetracycline resistance in some bacteria might also be regulated by the presence of tetracyclines. At least a 100-fold increase in gene transfer was observed in bacteria harbouring the transposon when exposed to low concentrations of tetracyclines. The implications of these findings are alarming. Not only the expression of the antibiotic resistance gene is dependent on the antibiotic, but the antibiotic also provokes the transfer of its own resistance genes.

Subinhibitory concentrations of antibiotics (such as tetracyclines) may also stimulate cell-to-cell contact, and thus gene transfer, by causing subtle changes in bacterial outer membrane structure. The tetracycline group best exemplifies widespread dissemination of resistance genes within the bacterial population. This class of antibiotics has been widely used and the ecology of the tet determinants is a model case for resistance-gene dissemination (Roberts, 1997).
1.8 THE NORMAL/COMMENSAL BACTERIAL FLORA IN HUMANS AND ANIMALS

1.8.1 What is the normal flora?

Indigenous or saprophytic bacteria are abundant on the skin and mucosa of both, humans and animals. They represent the so-called normal bacterial flora or microflora. Any colonised area can be considered a particular ecological niche, where equilibrium exists between the indigenous bacteria and the host. The normal flora is considered to be quite stable, providing that the health conditions of the subject or the environmental conditions remain stable.

1.8.2 The intestinal flora

The bacterial flora of the gastro-intestinal tract is particularly abundant. Several studies have demonstrated that the flora plays an important role in physiology and pathology of humans and animals. Under normal conditions in omnivores and carnivores the acidity of the gastric environment acts as a barrier to the microorganisms ingested with saliva or food. So the acid stomach content is normally sterile and the proximal tract of the small intestine has only a relatively sparse microbial flora predominantly consisting of lactic acid bacteria. However, the colon and caecum harbours a very rich and complex bacterial flora, reaching concentrations of $10^{10}$ to $10^{11}$ bacteria/g of intestinal content. Usually, the term “intestinal bacterial flora” refers to the microbial flora of the colon and, more often, to the faecal flora, as faeces are an easy sample to obtain in comparison to the intestinal content.

1.8.3 Composition of the intestinal flora

The intestinal flora is an exceedingly complex system, comprising more than 200 different bacterial species, some of them are still not named or uncultivable. Due to the low redox potential of the intestinal content, the obligatory anaerobic species outnumber the aerobic or facultative anaerobic species with a ratio of approximately 1:1000. In humans, the Gram-positive *Bifidobacteria* and the Gram-negative *Bacteroides* represent the most common anaerobic genera, while *Escherichia coli* is the most frequent facultative anaerobic Gram-negative species. Enterococci are the most frequent facultative anaerobic Gram-positive species (Finegold et al., 1983).

In ruminants, anaerobic bacteria are prevalent in the rumen and are very diverse, including *Clostridium*, *Ruminococcus*, *Prevotella* and methanogenic bacteria. Although many species are different from the human intestinal microorganisms, the most common anaerobic species, *Prevotella ruminicola*, is a close relative of *Bacteroides*. The intestinal flora matures with age. In very young animals and humans, it is still immature and therefore some of its functions are impaired, especially the colonisation resistance property (see below).

1.8.4 Functions of the intestinal flora

The intestinal flora exerts important physiological functions. In herbivores the fundamental function is the digestion of cellulose (in ruminants in the fore-stomachs, in other herbivores in the caecum). Rumen bacteria represent also the essential source of vitamins and proteins for the animal host. In all animal species, including humans, the microflora affects the morphology and the trophism of the intestinal mucosa and the associated lymphoid system. It participates in the metabolism of fermentable carbohydrate and bile acids, produces vitamins, and exerts protection against the implantation of exogenous bacteria, the so-called “colonisation resistance”.


1.8.5 Modification of the intestinal flora

The intestinal flora of humans is determined by racial and genetic factors as well as by dietary habits. In the individual subject, the composition of the flora tends to be quite stable even in presence of a diet modification of short duration. The ingestion of exogenous bacteria with food does not cause a permanent colonisation, as these bacteria are usually transient and are shed with the faeces for a limited period of time (days or weeks). Also enteropathogenic bacteria can be spontaneously eliminated from the intestines in a few days in normal subjects.

Agents most effective in inducing changes in the intestinal flora are antibiotics, especially non-absorbable or broad-spectrum antibiotics administered orally. Modification of intestinal flora may also take place when antimicrobial agents undergo extensive enterohepatic circulation or when they are excreted into bile. These drugs can reduce the number of bacteria recoverable from faeces by many orders of magnitude. Moreover, antibiotics often alter the ratio between the different species of intestinal bacteria to the detriment of the anaerobic species, which are more beneficial in term of physiological functions and have a low pathogenic potential. While in selected cases the action of the antibiotics on the gut normal flora can be beneficial, e.g. to lower the risk of bacterial infections in large bowel surgery, in many cases this action is detrimental. Such profound modifications of the established flora decrease the colonisation resistance, leading to an increased risk of acquiring exogenous bacteria, including enteropathogens. The most evident effect of the alterations in the microflora caused by antibiotics in humans is the appearance of antibiotic-associated pseudomembranous colitis, a potential life-threatening disease caused by *Clostridium difficile*. These modifications of the normal flora following antibiotic use are known to occur in animals as well and in some species, e.g. in herbivores they can result in a life threatening disease. Moreover, killing of susceptible bacteria in the gut by the antibiotic creates a "vacuum" that can be filled by overgrowth or acquisition of bacteria (and yeast) that are either intrinsically resistant to the antibiotic or have acquired resistant determinants.

1.8.6 The intestinal flora as a resistance gene reservoir

The complex intestinal flora can act as a large reservoir for resistant bacteria and resistance genes. As previously discussed, the use of antibiotics induces profound changes in the normal intestinal flora, favouring the persistence and/or the acquisition of resistant bacterial species. The complex intestinal milieu, where bacteria are tightly packed together, represents an ideal site for the *in vivo* transfer of resistant genes between different bacterial species and genera. This flow of resistant genes, designated "horizontal transfer", opposed to the "vertical transfer" or transfer to the offspring, can be enhanced by the selection pressure exerted by antibiotics (Nikolich et al., 1994). The mechanism is very efficient, as microorganisms can acquire a ready-to-use set of genes coding for multiple antibiotic resistance in a single step through the acquisition of a plasmid or of a conjugative transposon. Conjugative transposons have been described in intestinal *Bacteroides* species: they contain the tetracycline resistance gene together with other resistance genes and their transfer is induced by the presence of tetracycline (Salyers et al., 1995). It is important to stress that antibiotic resistance is not a characteristic of pathogenic bacteria. Resistance genes can be acquired by or selected in commensal bacteria following exposure to antibiotics. Moreover, subjects exposed to environments where antibiotic use is large such as farms (or hospitals), harbour a large number of resistant commensal bacteria in the gut even in the absence of antibiotic intake (Levy, 1978; van den Bogaard et al., 1997).

1.8.7 Transmission of intestinal bacteria and genes between animals and man

Studies conducted in the last decades have shown that bacterial intestinal strains in animals, including strains carrying antibiotic resistance genes, can be transmitted by natural means to humans (Anderson et al., 1973; Levy et al., 1976 A/B; Lyons et al., 1980; Marshall et al., 1990). These animal-adapted intestinal bacteria can spread in the environment and colonise, at least transiently, the intestinal tract of other animal species and of humans. This has been demonstrated in farm workers.
or in people living in proximity of farms. Moreover, intestinal colonisation in humans by vancomycin-resistant *Enterococcus faecium* strains derived from farm animals (pigs and poultry) has recently been demonstrated (Berchieri, 1999). If animal-adapted *E. coli* strains are genetically marked and fed back to animals, they can be recovered in the environment of the farm, in the flies, in the intestines of other animal species and in the faeces of human subjects (Marshall et al., 1990). Some strains can be recovered only transiently from the faeces, while others are able to colonise for a variable length of time: this difference seems to be a strain-dependent characteristic. The factors responsible for an animal- (and also for a human-)adapted strain being a good or poor coloniser of the human intestine are not completely understood. In general, human-adapted strains colonise the human gut better than animal-adapted strains. Although antibiotic resistant strains can spread naturally from animals to humans even in the absence of antibiotic pressure, the selection pressure of the antibiotics and the associated reduction of the colonisation resistance create conditions for these strains to colonise the gut and persist for a longer time (Anderson et al., 1973).

A study performed 30 years ago has clearly shown that animal derived intestinal bacteria, such as *E. coli* harbouring R-factors (transferable plasmids carrying resistance genes), are able to colonise the human gut and transfer resistance genes to the resident *E. coli* strain (Smith, 1969 A). However, low numbers of resistant recipients were isolated from faeces and persisted for only a short time. In these experiments, the human volunteer was not receiving antibiotics. While this type of experiment was not repeated on a larger scale, mainly for ethical reasons, similar experiments were performed using animals as recipients of resistant bacteria. In these experiments, resistant bacteria adapted to a certain animal host were given orally to a different animal species. It was demonstrated that a modification of the flora such as in germ-free or antibiotic-treated animals or in new-born animals, could increase the colonisation ability of the exogenous strains and their ability to transfer resistance determinants (Nijsten et al., 1995; Smith, 1969 B).
CHAPTER II: USE OF ANTIBIOTICS

2.1 ANTIBIOTICS USED FOR THERAPY

Antibiotics are used in animals as in humans for both prevention and treatment of infections. In animal husbandry they are also used as growth-promoting agents mixed with feed. In addition antibiotics are used on a large scale in horticulture and agriculture. For instance in the USA at least 10000 kg streptomycin per year have been used for the control of apple tree diseases. In the EU more than 800 substances are authorised for plant protection which includes many antibiotics (Opinion of the Economic and Social Committee, 1998).

An important question is whether the use of antibiotics in animals contributes to the increase in resistance in relevant human pathogens. To be able to assess the risk of the use of antibiotics on animal and human health, more information is needed on amounts of antibiotics used in animals and humans. Data on amounts of growth promoters in animals and horticultural and agricultural use of antibiotics should be available as well. Ideally information should include comparable data at national and international level and the use in animals should be specified for each animal species.

In order to assist analysis of the current knowledge on antibiotic resistance, this chapter first describes, which antibiotics are used therapeutically in food animals in the EU. Data on antibiotics used in companion animals and other animal species are not included, because only very limited information was available.

Additionally, antibiotics used in human medicine are listed in Annex II.

2.1.1 Antibiotics used in veterinary medicine (see Annex I)

The following information on antibiotics authorised for use in the Member States of the EU in veterinary medicine has been collected:

- Active ingredients used as a single drug or in combination
- Route of administration
- Target animal species
- Year of first authorisation
- Information on substance or a related substance when used in human medicine
- Amounts of antibiotics used

Annex I includes tables (tables 6 to 10) with information on those antibiotics, which are authorised for therapy in veterinary or human use in the EU. The tables do not include information on whether a substance is on the market or not. Although data have been collected since late 1997; the tables should now represent for most Member States those products authorised as of January 1999 due to the ongoing authorisation procedures which have been documented.

Table 6 in annex I summarises all the groups of antibiotics authorised for oral use in 14 EU Member States in cattle, pigs, poultry and fish. As no information has been available from Luxembourg, this Member State has not been included. This table contains also the dates of first authorisation of (fluoro)quinolones (for oral use in food producing animals).

A table with data on antibiotics authorised for all possible routes of administration in the EU is not presented as this information would be very complex. Furthermore, oral use of antibiotics, and specifically oral flock and herd treatment, is the most important factor regarding selection of resistant bacteria and is considered to be the major factor in contributing a potential threat to human health. In contrast to this, use of antibiotics in individual animals - as in companion animals - will only affect the specific animal and is likely to make only a small-scale contribution to a potential human health risk.

Tables 7 to 10 list data on antibiotics authorised for use in cattle, pigs, poultry and fish in 14 EU Member States. For each food animal species the table gives an overview on all routes of drug administration (part A) as well as information on oral administration only (part B).
For all classes of active ingredients with the exception of tiamulin, related or identical substances are used in human medicine (see tables 6 and 10). So as a matter of principle almost all antibiotics used in food animals are potentially able to select for resistant bacteria in animals, which might then be resistant or cross-resistant to antibiotics used in man. However, this does not imply that all therapeutic antibiotics used in veterinary medicine contribute to a potential human health risk.

Significant quantitative differences in the use of antibiotics can exist between the treatment of animals and humans. For instance, third- and fourth-generation cephalosporins are very important antibiotics in treatment of human infection in hospitals. However, in animals this class of antibiotics is generally not used orally.

Antibiotics authorised for use in food animals as both, feed additives and therapeutics are:

- the macrolides tylosin and spiramycin, which are authorised as therapeutics for oral use in cattle, pigs and poultry have been used as growth promoters in almost all EU Member States (Sweden: only in poultry)
- Virginiamycin, which is authorised as a therapeutic for oral use in pigs and poultry in Sweden and has been used as growth promoter in most EU Member States
- Bacitracin, which is authorised as a therapeutic for oral use in cattle, pigs and poultry in Spain, Austria (only pigs) and Germany. Zinc bacitracin has been used as growth promoter in most EU Member States

Feed additive use of these four antibiotics was temporarily banned in the EU in December 1998. Their use will be prohibited in the EU from 1 July 1999; this ban being reviewed by the end of 2000.

### 2.1.1.1 Cattle

In cattle, all classes of antibiotics are authorised for use in all EU Member States (table 7 A/B) except polypeptide antibiotics, first-generation quinolones, fenicolys and some "miscellaneous" antibiotics.

Polypeptides are not authorised in Finland, Ireland, Sweden and the United Kingdom. The first-generation quinolones (e.g. nalidixic acid, flumequin, oxolinic acid, pipemidic acid) are not authorised in Austria, Denmark, Germany, Ireland, Sweden, Finland and the United Kingdom. However, the related fluoroquinolones are authorised in all EU Member States.

In cattle antibiotics are authorised for all possible routes of administration. In general animals are treated individually. Antibiotics for herd treatment will mainly be used in veal calves, which receive the drug orally mixed in milk-replacer. All groups of antibiotics authorised for oral use in cattle may be used for individual animals and for herd treatment.

Regarding oral use in cattle, a striking difference exists in the number of antibiotics representing one class between Member States. For instance, six different aminoglycosides are authorised for use in Spain, while only one is authorised in Sweden and Greece. Fluoroquinolones are authorised for oral use in cattle in all EU-Member States except Sweden and Finland (parenteral use only).

### 2.1.1.2 Pigs

Representatives of nearly all classes of antibiotics are authorised for use in pigs except polypeptide antibiotics, first-generation quinolones, fenicolys and "miscellaneous" antibiotics (table 8). Polypeptides are not authorised in Finland, Ireland, Sweden and the United Kingdom. First-generation quinolones (e.g. nalidixic acid, flumequin, oxolinic acid, pipemidic acid) are not authorised in Austria, Denmark, Germany, Finland, Ireland, Sweden and the United Kingdom.

In most Member States five or more aminoglycosides are authorised for therapeutic use in pigs. In Sweden and Finland however, only streptomycin is authorised for treatment of pigs. In contrast to other Member States, in Spain the macrolides kitasamycin and josamycin are authorised for therapeutic use in pigs. Fluoroquinolones are authorised for oral use in pigs in all Member States except France and Sweden. Virginiamycin is authorised for oral use in Sweden.
2.1.3 Poultry

The most important route of administration of antibiotics in poultry is oral flock treatment (see table 9). Not all representatives of the major classes of antibiotics are authorised for use in all EU Member States. For instance β-lactam antibiotics are not authorised for use in Austria, Denmark, Finland and Sweden, and cephalosporins are only authorised for parenteral use in Belgium and the United Kingdom. In Sweden and in Finland only 3 groups of antibiotics (tetracyclines, fluoroquinolones and virginiamycin respectively tetracyclines, fluoroquinolones and macrolides) are authorised for use in poultry, whilst in other Member States ten or more classes of antibiotics are authorised for this species. However, the poultry industry in Finland and Sweden is relatively small compared to other Member States.

Fluoroquinolones are authorised for use in all Member States and in 1998 a fluoroquinolone (difloxacin) was granted a Community authorisation for oral use in poultry by the centralised procedure in all EU Member States.

2.1.4 Fish

Table 10 gives information on antibiotics authorised for use in fish in the EU and Norway. The main antibiotics used in fish are quinolones and tetracyclines. In almost all Member States with an aquaculture industry first generation quinolones (flumequin and/or oxolinic acid) are authorised. Sarafloxacin is the only fluoroquinolone authorised for use in fish; its use is restricted to Ireland and the UK.

2.1.2 Antibiotics used in human medicine (see Annex II)

Antibiotics used in human medicine are presented in table 11. In most of the antibiotic classes a greater number of substances are used in humans compared to animal treatment. However, in general cross-resistance exists between these substances. Examples are the large number of cephalosporins and fluoroquinolones used in humans compared to veterinary use. However, it is not the number of substances that selects for resistance but the route of administration and the amounts of antibiotics that are used.

Although most of the antibiotics used in humans are not used in animals, cross-resistance with veterinary antibiotics is possible.
Chapter II: Use of Antibiotics

2.2 **Amounts of Antibiotics used in Veterinary Medicine**

In order to evaluate the extent to which the development of resistance in bacteria has occurred, detailed information on the volumes of antibiotics used should be determined. These data should not only focus on therapeutic use in veterinary and human medicine, but also on the use as growth promoters and for horticultural or agricultural purposes. Furthermore, data should be comparable at national and international level and their use in animals should be specified for each animal species.

Regrettably, insufficient information is available in this context. The following chapter summarises the limited data available on antibiotics used in veterinary medicine in the EU.

2.2.1 **Amounts of Antibiotics used for Animal Health in EU Member States**

2.2.1.1 **Estimates from National Authorities**

Legally, companies are not required to provide background information on the marketing of their veterinary medicinal products in all Member States, so data on volumes of antibiotics used is often difficult to obtain. Usage data have been made available only in Sweden, Denmark and Finland and - to a lesser extent - the Netherlands (see table 12).

Little or no information on usage of antibiotics is available from Austria, Belgium, France, Germany, Greece, Ireland, Italy, Luxembourg, Portugal, Spain and United Kingdom. Detailed data on amounts of the specific classes of antibiotics are only available from Denmark, Finland, Sweden and The Netherlands. However, volume data for specific antibiotics or groups of antibiotics by route of administration are available only from Denmark. Volume data on antibiotics used in individual species are rarely available, therefore such data have not been submitted by any country.

**Sweden:**
The total usage of antibacterial and antiparasitic drugs in Sweden has been monitored since 1980 (Björneroth et al., 1996). Detailed information on total usage (kg active ingredient) is available for all antibiotics used. All data are based on the sales statistics in the Central Statistics System of Apoteket AB (National Corporation of Swedish Pharmacies). This system contains registers of all sales from the wholesalers to the local pharmacies. As all pharmaceuticals are distributed from these wholesalers to local pharmacies or to authorised feed companies, these figures represent the total usage in Sweden. The local pharmacies normally have short storage periods for these products, so it can be assumed that the products sold were also used during the respective periods.

**Denmark:**
Since 1996 pharmaceutical companies have been obliged to report to the Danish Drug Agency the quantities of drugs sold. Detailed information on total usage (kg active ingredient) is available for each formulation of antibiotic marketed. Summary data are published by The Danish Zoonose Center in its yearly report for the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 1998). Data concerning antibiotics used for growth promotion are collected by the Danish Plant Directorate.

**Finland:**
Detailed data on antibiotics (active ingredients) used as growth promoters or as therapeutics in medicated feeding stuffs, which are prepared by feed manufactures have been available since 1990 and are provided by feed importers and manufacturers every 6 months. Information (kg active substances) is published annually on the volume of each group of antibiotic (aminoglycosides, tetracyclines, ...), but not for each active ingredient. The responsible authority for data collection is Plant Protection Inspection Centre under the Ministry of Agriculture and Forestry.

In addition to these data provided by manufacturers, wholesalers for veterinary medicinal products have to submit their sales statistics to the National Agency for Medicine once a year (every 3 month for the 2 largest wholesalers).
Chapter II: Use of Antibiotics

Netherlands
Data on antibiotic usage have been reported on a voluntary basis by the industry to the Dutch Institute of Medical Statistics. However, those companies participating represent the majority of the enterprises marketing antibiotics. Since 1990 data have been collected every two years and provide information on the usage of different antibiotic classes as group- and individual medication (kg active ingredients). Data available for 1994 and 1996 include only group treatment.

| Table 12: Total usage of antibiotics in different Member States (kg) |
|-----------------|-----------------|-----------------|-----------------|
| Finland         | 17500           | 18197           | 17286           | 16591           |
| Sweden          | 30342           | 24569           | 20639           | 19655           |
| Denmark         | 78584           | 40172           | 47454           | 53511           |
| Netherlands     | 250200 *        |                 | 251500 *        |                 |

* Only group treatment

2.2.1.2 Estimates from production in feed mills

Following a questionnaire, which was sent to CVMP members in August 1998 only limited information could be provided on volumes of antibiotics used for medicated feed prepared by feed mills.

Germany
Exact quantification of antibiotic products used in Germany is not possible as no legal obligations for reporting such information exist. Nevertheless, some information regarding the use of medicated feed in a large German region with intensive animal husbandry has been provided based on veterinarians' prescriptions. Most antibiotics in this region are used in intensive production systems and are administered by medicated feed, which is in the main (95%) prepared by commercial feed mills. Based on veterinarians' prescriptions for the preparation of medicated feed by feed mills the quantities of antibiotics used have been calculated. Rassow and Schaper (1996) estimated that about 61% of all antibiotics given in medicated feed are used in chickens (mainly for prophylactic purposes) whereas about 38% are used in pigs (with roughly the same amounts being used for prophylactic and for therapeutic purposes).

Finland
Unlike Germany only limited amounts of antibiotics used in medicated feedingstuffs are manufactured by commercial feed mills (about 15% of antibiotics in medicated feed for pigs).

2.2.2 Trends in the use of antibiotics

Trends in the use of antibiotics might be obtained by comparing the different groups of antibiotics used in the Community and their usage in each of the Member States.

Information on such trends over time is available only from the Scandinavian countries and the Netherlands, and is therefore not representative for the whole Community. Nevertheless, an overview is possible (see table 13) with a general trend in the usage of some groups of antibiotics between 1994 - 1997 being available although the picture is very variable.
Chapter II: Use of Antibiotics

Table 13: Amounts of some groups of antibiotics used in some Member States in 1994 - 1997 (kg)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>936</td>
<td>1 067</td>
<td>894</td>
<td>819</td>
</tr>
<tr>
<td>Sweden</td>
<td>1 696</td>
<td>1 342</td>
<td>1 164</td>
<td>1 077</td>
</tr>
<tr>
<td>Denmark</td>
<td>8 641</td>
<td>7 647</td>
<td>7 130</td>
<td>6 137</td>
</tr>
<tr>
<td>Netherlands</td>
<td>5 600 *</td>
<td>3 200 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(Fluoro-)quinolones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>246</td>
<td>200</td>
<td>173</td>
<td>179</td>
</tr>
<tr>
<td>Denmark</td>
<td>1 167</td>
<td>1 163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>11 800 *</td>
<td>8 300 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>4 965</td>
<td>4 193</td>
<td>3 652</td>
<td>3 085</td>
</tr>
<tr>
<td>Sweden</td>
<td>7 730</td>
<td>4 968</td>
<td>2 698</td>
<td>2 558</td>
</tr>
<tr>
<td>Denmark</td>
<td>36 522</td>
<td>9 046</td>
<td>12 850</td>
<td>13 717</td>
</tr>
<tr>
<td>Netherlands</td>
<td>126 600 *</td>
<td>149 100 *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Only group treatment

Sales figures of antibiotics are available for the EU and for countries outside the EU provided by the animal health industry but it is difficult to draw conclusions about the real volumes of antibiotics or even different classes of antibiotics used. Nevertheless, these data differentiate between different types of products used in veterinary medicines or animal species and can indicate trends in usage.

Generally an increase in the sales of all animal health products is evident not only within Europe but also world-wide (Animal Pharm, 1997 and 1998 B). This mainly reflects the increasing importance of the pet sector with the number of companion animals increasing in many countries and more products for pets having been authorised. Furthermore, the BSE crisis and outbreaks of pig diseases in several Member States (swine fever, swine pest) influenced the sales figures in these countries.

Sales data (table 14) on all veterinary health products provided by pharmaceutical companies indicate that antibacterials account for the largest part of the sales of these products (Animal Pharm, 1997).

Table 14: EU sales in 1996 (ECU million)

<table>
<thead>
<tr>
<th>Category</th>
<th>Sales (ECU million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibacterials</td>
<td>705</td>
</tr>
<tr>
<td>Antiparasitics</td>
<td>550</td>
</tr>
<tr>
<td>Biologicals</td>
<td>512</td>
</tr>
<tr>
<td>Metabolics</td>
<td>102</td>
</tr>
<tr>
<td>Others</td>
<td>356</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2225</td>
</tr>
</tbody>
</table>

Nevertheless, in most European countries a decrease in sales of antibacterial substances has been observed in both the growth promoters and the therapeutic sector (Animal Pharm, 1998 A). For Norway a decrease in the usage of antibiotics of 23% has been described between 1995 and 1997 in fish. This was part of an initiative to lower the incidence of production diseases and antibiotic consumption in this species.

However, Animal Pharm (1998, B) reports that the market for injectable antibiotics in Ireland has performed well in 1997, in line with the trend for newer, higher unit cost products. For Denmark an increase of up to 13% in sales of antibiotics has been described for 1997 mainly due to increases in sales of injectable narrow-spectrum penicillins and oral trimethoprim-sulphonamides and chlortetracycline.

World-wide, a slower growth has been described for sales of feed additives (Animal Pharm, 1997) than has previously been the case.
In contrast, an increase in biologicals' sales has been reported in the EU as well as world-wide (Animal Pharm, 1997 and 1998 B). The increase in sales figures in vaccines can be seen in all animal species. Generally, the current trend is for greater use of preventive treatment and for less use of antibiotics.

### 2.2.3 Usage of antibiotics within the EU - data provided by FEDESA

The world-wide use of antibiotics for animal health purposes in 1996 was estimated at 27000 tonnes with about 25% of global usage in the EU. Within the EU 50% of this usage is estimated to arise from prescriptions issued for therapeutic purposes while 25% arose from feed additive usage for growth promotion and another 25% for ionophore feed additives primarily used to prevent coccidiosis in poultry (Boatman/FEDESA, 1998).

In 1997 FEDESA was requested by the European Commission to provide information on actual usage of antibiotics in the EU. Data have been subsequently received and collated from questionnaires completed by 19 corporate members of FEDESA, which represent about 60% of all EU and Switzerland sales in cash terms. These data estimates are shown below in table 15 (Boatman/FEDESA, 1998). Sales of animal health antibiotics (excluding coccidiostatics) in 1997 within EU plus Switzerland were estimated at a total of 5093 tonnes, therapeutics accounting for 3494 tonnes (69% of the total) and growth promoters for 1599 tonnes (31%). Approximately two-thirds of the therapeutic antibiotics were tetracyclines (66%), which were well ahead of the macrolides (12%) and penicillins (9%). The other four therapeutic groups together comprise 12% of the total.

#### Table 15  
Sales volumes of antibiotics in EU and Switzerland in 1997 by therapeutic groups  
(tonnes of active ingredients) (Boatman/FEDESA, 1998)

<table>
<thead>
<tr>
<th>Therapeutic Group</th>
<th>Sales by members of FEDESA</th>
<th>Extrapolation Factor</th>
<th>Estimated Total (%) of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td>161</td>
<td>2.00</td>
<td>322 (9)</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>483</td>
<td>4.75</td>
<td>2294 (66)</td>
</tr>
<tr>
<td>Macrolides</td>
<td>319</td>
<td>1.33</td>
<td>424 (12)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>92</td>
<td>1.70</td>
<td>154 (4)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>32</td>
<td>1.33</td>
<td>43 (1)</td>
</tr>
<tr>
<td>Trimethoprim/Sulphonamides</td>
<td>50</td>
<td>1.50</td>
<td>75 (2)</td>
</tr>
<tr>
<td>Other Therapeutics</td>
<td>91</td>
<td>2.00</td>
<td>182 (5)</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>1228</td>
<td>2.85</td>
<td>3494 (100)</td>
</tr>
<tr>
<td>Therapeutics</td>
<td>1454</td>
<td>1.1</td>
<td>1599</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2682</td>
<td>1.9</td>
<td>5093</td>
</tr>
</tbody>
</table>

1 Factor used for extrapolation of the FEDESA data.

Total sales of animal health antibiotics (excluding coccidiostatics) within each of the European countries are shown in table 16. Out of the estimated total usage of antibiotics within the EU plus Switzerland in 1997 (10493 tonnes), human health antibiotics (estimated at 5,400 tonnes) accounted for 52% whereas therapeutic animal health antibiotics accounted for 33% and growth promoters for 15%.

There is little reason to doubt that for some countries the data provide a fairly accurate picture of the situation for 1997. For example the figure for therapeutic antibiotics quoted for the Scandinavian countries and The Netherlands correspond more or less with those reported above (see remarks 2-5 to table 16). To calculate the total amount of antibiotics used as therapeutics in animals, extrapolation factors were used for each country (table 15). These factors were calculated by FEDESA based on estimated amounts of antibiotics produced by non-FEDESA members. The accuracy of the factors is
unknown, however the author claims the totals are accurate within a range of 15% and all key figures within 25%.

At the EU-conference "The Microbial Threat" in Copenhagen in 1998, the accuracy of the amounts of growth promoters used in the EU as presented by FEDESA as a proportion of the total use in animals was disputed by some participants. They expected it to be a larger proportion than presented. However, table 16 shows that a relation exists between the animal husbandry system and the proportion of growth promoters used. In some countries with intensive animal husbandry such as The Netherlands, Denmark, Belgium or France the proportion of growth promoter use is higher than in countries with less intensive husbandry like Greece, Spain and Italy.

Table 16  Sales volumes of antibiotics used as growth promoters (no coccidiostatics) and therapeutics in different EU Member States in 1997 (tonnes of active ingredients) (Boatman/FEDESA, 1998)

<table>
<thead>
<tr>
<th>Country</th>
<th>Sales of Growth Promoters (% EU market)</th>
<th>Sales of Therapeutics (% EU market)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>23 (1)</td>
<td>8 (&lt;1)</td>
</tr>
<tr>
<td>Belgium + Lux.</td>
<td>110 (7)</td>
<td>125 (4)</td>
</tr>
<tr>
<td>Denmark</td>
<td>75 (5)</td>
<td>60 2) (2)</td>
</tr>
<tr>
<td>Finland</td>
<td>&lt;1 1) (&lt;1)</td>
<td>12 3) (&lt;1)</td>
</tr>
<tr>
<td>France</td>
<td>339 (21)</td>
<td>492 (14)</td>
</tr>
<tr>
<td>Germany</td>
<td>255 (16)</td>
<td>488 (14)</td>
</tr>
<tr>
<td>Greece</td>
<td>15 (1)</td>
<td>110 (3)</td>
</tr>
<tr>
<td>Ireland</td>
<td>34 (2)</td>
<td>22 (&lt;1)</td>
</tr>
<tr>
<td>Italy</td>
<td>100 (6)</td>
<td>389 (11)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>226 (14)</td>
<td>300 (9)</td>
</tr>
<tr>
<td>Portugal</td>
<td>24 (2)</td>
<td>44 (1)</td>
</tr>
<tr>
<td>Spain</td>
<td>198 (12)</td>
<td>616 (18)</td>
</tr>
<tr>
<td>Sweden</td>
<td>&lt;1 4) (&lt;1)</td>
<td>20 (&lt;1)</td>
</tr>
<tr>
<td>UK</td>
<td>191 (12)</td>
<td>788 (23)</td>
</tr>
<tr>
<td>EU</td>
<td>1590 (100)</td>
<td>3474 (100)</td>
</tr>
</tbody>
</table>

1) Data received by the Finnish Authorities: Growth promoters: 0 tonnes respectively 4 tonnes including Olaquindox/carbadox
2) Data received by Danish Authorities: Therapeutics: 54 tonnes
3) Data received by Finnish Authorities: Therapeutics: 17 tonnes
4) Data received by Swedish Authorities: Growth Promoters: 0 tonnes

2.2.4 Relation of volume data and animal numbers

Knowledge of the relationship between the volume of antibiotics used in each class of livestock on a country-by-country basis and the number of animals per country is essential when estimating the prevalence of antibiotic resistance.

Data on numbers of farm animal populations are available from an annual census within all Member States. However, these data represent calculations of animal numbers on a certain day and do not reflect the total number of animals raised in one year and available for slaughter whereas production data (slaughtered animals, milk/egg production) are calculated on a yearly basis for all Member States. EU wide data regarding the weight of carcasses of slaughtered animals differ considerably due to different forms of animal husbandry, breeding, eating customs. For instance, the average carcass weight of pigs slaughtered in Italy is 141 kg whereas it is 79 kg in Greece (Eurostat, 1997).
However, by using a formula generally agreed in meat production (Eurostat, 1997; Meyer et al., 1989) an estimation of the live-weight in relation to the carcass weight can be made:  
\[
\text{Carcass weight} = \text{live-weight} \times 0.45 \text{ (adult cattle)}, 0.6 \text{ (calf)}, 0.47 \text{ (sheep/goat) and 0.8 (pigs)}, 
\]
respectively.

Animal data were received from Eurostat only for 1996 whereas sales data provided by FEDESA are from 1997; however, the animal data are not expected to change dramatically.

Based on the carcass weight the estimated live weight of food animals produced in 1996 and the amount of antibiotics sold in 1997 are shown in table 17.

It is difficult to draw conclusions from these figures. The total live weight produced per country is only an estimate and differences in animal husbandry systems that exist in different countries are very important determinants for use patterns of antibiotics. Hence, for a fair comparison between countries much more information is necessary on specific use in different husbandry systems. For instance, the use of antibiotics in broilers, which are reared in a similar way all around the world might be comparable in different countries. However, the use in slaughter pigs can only be compared in countries with intensive pig industry, while use in cattle needs to be specified for all different types of rearing systems.

Furthermore, antibiotic use in companion animals, horses and other animal species are included in the sales figures, but not in the estimated animal liveweights. The different routes of administration are not distinguished as well as dosage units, which might differ within the countries or the duration of treatment (short-term therapy or long-term prophylaxis with a probably lower dosage). All these aspects might be of different importance for the risk of a development of resistance.

Without this specific knowledge the relation between amounts of antibiotics used in animals and the tonnes of live weight produced per country are only indicative of type of husbandry system.

### Table 17: Relation between live weight of animal slaughtered within the EU in 1996 and estimated volumes of animal health antibiotics marketed in the Member States in 1997 (EUROSTAT, 1997; Boatman/FEDESA, 1998)

<table>
<thead>
<tr>
<th>Member State</th>
<th>Estimated live weight of slaughtered animals in 1996 (x 1000 tonnes) (EUROSTAT)</th>
<th>Antibiotics sold in 1997 (FEDESA) (x 1000 kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pigs</td>
<td>Adult Cattle</td>
</tr>
<tr>
<td>Austria</td>
<td>601</td>
<td>507</td>
</tr>
<tr>
<td>Belgium &amp; Lux.</td>
<td>1337</td>
<td>715</td>
</tr>
<tr>
<td>Denmark</td>
<td>1821</td>
<td>394</td>
</tr>
<tr>
<td>Finland</td>
<td>214</td>
<td>208</td>
</tr>
<tr>
<td>France</td>
<td>2729</td>
<td>3272</td>
</tr>
<tr>
<td>Germany</td>
<td>4545</td>
<td>3138</td>
</tr>
<tr>
<td>Greece</td>
<td>178</td>
<td>131</td>
</tr>
<tr>
<td>Ireland</td>
<td>263</td>
<td>1169</td>
</tr>
<tr>
<td>Italy</td>
<td>1763</td>
<td>2211</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2030</td>
<td>869</td>
</tr>
<tr>
<td>Portugal</td>
<td>374</td>
<td>186</td>
</tr>
<tr>
<td>Spain</td>
<td>2895</td>
<td>1221</td>
</tr>
<tr>
<td>Sweden</td>
<td>400</td>
<td>294</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1248</td>
<td>1517</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20 398</strong></td>
<td><strong>15 832</strong></td>
</tr>
</tbody>
</table>
2.2.5 Conclusions

- Almost all antibiotics used in veterinary medicine are structurally related to human therapeutics. The use of these antibiotics in animals might be able to select for co- or cross-resistance to human therapeutics.
- In human medicine often more substances of one class of antibiotics are authorised for use than in animals. Cross-resistance exists between these substances.
- Data on amounts of antibiotics used in the 1990s are only available from Denmark, Sweden and Finland and - to a lesser extent - from the Netherlands. The amounts are presented as total usage of the antibiotic agents per country and are not differentiated per animal species. Only in Denmark, Sweden and Finland does a legal obligation exist for the pharmaceutical industry to supply these data.
- To obtain reliable data on the amounts of antibiotics used, detailed information should be made available on dosage forms, routes of administration and target species with regards to different animal husbandry systems.
- The accuracy of data on antibiotic usage supplied by FEDESA and the extrapolation factor to estimate the sales of non-FEDESA members cannot be checked by the authors. However, for Denmark and The Netherlands the estimation corresponds with other publications.
- To determine the relation between the emergence and trends in resistance and usage patterns of antibiotic agents it is essential that data on amounts of antibiotics are specifically calculated for those animals from which the resistance data are obtained.
CHAPTER III: DEVELOPMENT OF RESISTANCE

3.1 INTRODUCTION

To gather resistance data on bacteria isolated from animals (mainly food animals) in different EU countries is much more complex in veterinary medicine than it is in human medicine, not only because of the variety of bacterial species, but also as different animal species, animal husbandry etc. must be taken into account. Problems in collecting antibiotic resistance data include heterogeneity of the sources of data (denominator), heterogeneity in test methods used, and lack of quality assurance in the data. These problems result in great difficulties comparing data from different countries or even from different sources within one country.

Data on resistance to antibiotics in bacteria isolated from animals can be obtained from various sources:

1. Diagnostic laboratories that analyse clinical samples from diseased animals, or specimens taken at autopsy. These data provide information to the veterinarian who subsequently chooses the most appropriate antibiotic to treat the animal or a group of animals.

2. Statutory monitoring programmes for *Salmonella* in farm animals. Samples are taken from apparently healthy animals on the farm or in abattoirs. However, in most cases the programme is only designed for the detection of *Salmonella* to obtain prevalence data, without performing susceptibility testing.

3. "Ad hoc" epidemiological studies specifically set up to determine prevalence and trends of antibiotic resistance in particular animal species. These screening studies result in the most useful sets of data as all the aspects of the study are usually designed beforehand and carried out consequently.

Annex III gives an overview about surveillance programmes regarding antibiotic resistance in animals in all EU Member States.

The quality of the data initially gathered by the authors from the above mentioned sources was difficult to assess. Usually clinical laboratories do not perform quantitative assays (MIC determination), but rely on the disc diffusion method (qualitative test). Results are reported as resistant, intermediate or susceptible, and the inhibition zone diameters are seldom registered. Not all clinical laboratories strictly follow standardised quality control procedures, e.g. the concurrent testing of reference organisms to check the performance of the assays. Moreover, different antibiotics of the same class might be used and tested in different countries and their potency is not always comparable.

As little scientific data was available from the published literature the authors devised a questionnaire to gather data on the prevalence of zoonotic bacteria, animal pathogens and commensal organisms in their own countries, which included Belgium, Denmark, France, Germany, Italy, The Netherlands, Spain, Sweden and the United Kingdom. Very little information was available on companion animals therefore, the animal species considered were the major food animal species, i.e. cattle, poultry and pigs. Emphasis was placed on resistance to the main classes of antibiotics, and especially on quinolones. The questionnaire also asked about trends in resistance over the years, if available. By means of the questionnaire the authors avoided the collection of a large amount of raw data that might be difficult to analyse due to their large variation. The authors analysed the data and tried to demonstrate common trends in the Member States.

Although some overlap exists between the categories of bacteria studied, the following description was accepted:

- Zoonotic bacteria are pathogenic or non-pathogenic in animals but are pathogenic in humans.
- Pathogens represent bacteria mainly pathogenic for animals and only occasionally pathogenic for man.
- Commensal organisms are by definition not usually regarded as pathogenic in the normal host, but might have a potential for resistance development and transmission. They also may be opportunistic pathogens in certain situations, as this is the case for the enterococci.
3.2 **Zoonotic Bacteria**

The authors decided to focus on *Campylobacter* and *Salmonella* as the main important food-borne bacteria responsible for zoonoses within the EU. Data on resistance in other zoonotic bacteria are only available for a few Member States.

### 3.2.1 Campylobacter

The thermophilic *Campylobacter* (*C. jejuni* and *C. coli*) are part of the normal intestinal flora of a wide range of birds and domestic animals without causing disease. During the slaughtering process, faecal contamination may result in the presence of *Campylobacter* on the surface of the carcasses. As thermophilic *Campylobacter* are not major veterinary pathogens but are organisms fastidious to isolate and to culture, very few countries have established surveillance programs including antibiotic susceptibility regarding *Campylobacter* in animals or in food of animal origin. In addition, a standardised susceptibility test does not exist.

The data available are listed below and, whenever possible, compared with susceptibilities of human isolates in the same geographical area.

#### 3.2.1.1 Denmark

- **Animals**

Reliable resistance data are available for 1996 and 1997 (DANMAP, 1997 and 1998). Resistance to nalidixic acid appears to be low in *C. jejuni* isolated from broilers (2 to 3%) and higher in *C. jejuni* isolated from cattle and in *C. coli*. Resistance to fluoroquinolones (enrofloxacin) was low (0 to 4%) except in pig isolates of *C. coli* in 1996 (Table 18). The general level of resistance was higher for *C. coli* than for *C. jejuni*, mainly as a result of differences in resistance to macrolides (erythromycin), streptomycin and - to some extent - quinolones. However, the number of isolates tested is in some cases very low, thus making accurate assessment of the proportion of resistant isolates difficult.

- **Humans**

Resistance among human isolates (mainly *C. jejuni*) is generally low, although the prevalence of quinolone resistance is remarkable (nalidixic acid 14% and ciprofloxacin 12 to 13%). Quinolone resistance is assumed mainly to be of foreign origin.

#### Table 18: % Resistance in *Campylobacter* from Denmark (1996 and 1997)

<table>
<thead>
<tr>
<th></th>
<th>Campylobacter jejuni</th>
<th>Campylobacter coli</th>
<th>C. spp. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broilers</td>
<td>Cattle</td>
<td>Broilers</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>2</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Enrofloxacin 1)</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

1) In humans ciprofloxacin was tested  
2) Mainly *C. jejuni*

#### 3.2.1.2 Germany

- **Animals**

In regional investigations in the years 1995 - 1997, 1906 isolates of *C. jejuni* and *C. coli* from animals, humans and food were tested for resistance to nalidixic acid, erythromycin, tetracycline, streptomycin and kanamycin (Thurm et Dinger, 1998). Only 51% of all animal isolates of *C. jejuni* were susceptible to all antibiotics tested; 19% were resistant to nalidixic acid, 0% to erythromycin, 19% to tetracyclines, 33% to streptomycin and 3.3% to kanamycin.
Chapter III: Development of Resistance

- **Humans**

  In human isolates of *C. jejuni* 77% were susceptible to all antibiotics tested; 15% of the strains were resistant to nalidixic acid, 1% to erythromycin, 12% to tetracycline, 1.5% streptomycin and 2.2% to kanamycin. Aleksic (1997) reported a rate of 14% for fluoroquinolone resistance in human isolates of *C. jejuni*.

3.2.1.3 **Sweden**

- **Animals**

  A large study was published in 1993 on 809 *C. jejuni* strains isolated from chicken flocks at slaughter (Berndtson et al., 1993). Resistance to erythromycin was found in 2% of strains, to doxycycline in 0.5% and to enrofloxacin in 4.5% of the strains. No information about trends of resistance development is available.

3.2.1.4 **The Netherlands**

- **Animals**

  Endtz et al. described in 1991 a concurrent increase in fluoroquinolone resistance in *C. jejuni* strains isolated from poultry faeces and from human cases of acute gastro-enteritis. Before 1987 (the year when enrofloxacin was first licensed for use in poultry in The Netherlands) fluoroquinolone resistance was not detected, whereas in 1987 - 1988 6% of *C. jejuni* strains isolated from chickens and 7% from humans were fluoroquinolone resistant. In 1989 these rates increased to 11% and 14%, respectively. The authors concluded that the cause of the increasing resistance rate was veterinary use of fluoroquinolones in poultry.

- **Humans**

  In a large epidemiological survey of human clinical isolates of *C. jejuni* in the eastern part of the Netherlands, an increase in ofloxacin resistance was noted from 11% in 1994 to 27% in 1997 (Talsma et al., 1998). The fluoroquinolone resistance had a seasonal pattern, suggesting that foreign import was not a major factor, and also inter-human transmission did not play an important role.

3.2.1.5 **United Kingdom**

- **Animals**

  In 1993, when enrofloxacin was first licensed for oral use in food producing animals in the UK a small study showed that the maximum MIC of enrofloxacin for *C. jejuni* was 1 µg/ml. However, in further studies on isolates from 1994 and 1995 approximately 7% of the isolates had a MIC to enrofloxacin of 4 µg/ml or higher (Wray, personal communication, 1998).

  In a survey performed in the UK in 1993 (Gaunt and Piddock, 1996), fluoroquinolone resistant *C. jejuni* have been isolated from 14% of poultry carcasses imported from non-UK countries compared with less than 2% of carcasses of poultry bred in UK.

- **Humans**

  11% of *C. jejuni* and 22% of *C. coli* isolated at the Public Health Laboratory Service (PHLS) from humans in 1997 were resistant to ciprofloxacin using the breakpoint of 1 µg/ml. Moreover, 11% of *C. jejuni* and 20% of *C. coli* from humans were multi-resistant.

3.2.1.6 **Other countries**

A temporal relationship between the marketing of fluoroquinolones and an increase in resistance of *Campylobacter* isolates has been noted in Austria (Feierl et al., 1999). No data on antibiotic resistance in *Campylobacter* isolated from animals from the other Member States were made available to the authors.
3.2.2 Summary (Campylobacter)

- Few countries report on resistance in animals among *Campylobacter* to antibiotics other than quinolones. Therefore, no general conclusions can be drawn from these data.
- Both nalidixic acid and fluoroquinolones resistance is widespread among *Campylobacter* isolates, both from animal and human origin: Often resistance of 10 to 20% to nalidixic acid and of 5 to 15% and more to fluoroquinolones are reported.
- Three countries (Austria, The Netherlands and UK) indicate a temporal relationship between the marketing of enrofloxacin and the increase of fluoroquinolone resistance in *Campylobacter* isolated from animals. At the same time an increase in resistance of *Campylobacter* isolated from human cases of gastro-enteritis has been noted in The Netherlands.
- Some countries (e.g. Denmark) report that fluoroquinolone resistance among *C. jejuni* in animals is low but relatively high in humans. Therefore, it is suggested that resistant human *C. jejuni* are of foreign origin, acquired from travel abroad or from imported contaminated food. This does not seem to be the case in The Netherlands.
- No data are available for isolates from companion animals.

3.2.3 Salmonella

Special emphasis and special efforts in data collection have been devoted to *Salmonella* since salmonellosis is one of the most prevalent zoonoses in Europe. Although some *Salmonella* serotypes are host specific, e.g. *S. Typhi* and *S. Pullorum*, most serotypes can be isolated from a large number of animal species and man.

Most data on resistance among *Salmonella* isolates were made available by diagnostic laboratories, including the National Reference Laboratories for *Salmonella*. Furthermore, most Member States have statutory monitoring programmes for *Salmonella* in farm animals, feedingstuffs and food of animal origins (see Annex III for details on antimicrobial resistance surveillance programmes in the EU), and these may include antibiotic susceptibility testing. As a consequence of the different ways the data had been collected, most of the strains from cattle and pigs were isolated from diseased animals (clinical isolates) and those from poultry were from apparently healthy animals.

3.2.3.1 Belgium

- Animals

All *Salmonella* data are collected by the National Reference Laboratory for *Salmonella*, Veterinary and Agrochemical Research centre from the Ministry of Small Enterprises, Traders and Agriculture and, unless otherwise indicated, refer to year 1997.

Data for poultry refer to samples from the official sanitary monitoring (no clinical isolates). Overall, the following resistance is noted: 36% to ampicillin, 12% to spectinomycin, 36% to tetracycline, 27% to nalidixic acid and 17% to chloramphenicol. *S. Enteritidis* strains are mainly (90%) sensitive, while only 22% of *S. Typhimurium* strains are sensitive with 70% of the isolates sharing the resistance profile ASTC. About 80% of *S. Hadar* strains are resistant to nalidixic acid. A significant increase in the number of *S. Typhimurium* isolates with resistance profile ASTC was observed in recent years, i.e. from 4.1% in 1992 to 23% in 1994 and 38% in 1996. Only a minority of *S. Typhimurium* with this resistance profile was phage typed, and results confirmed that most strain belong to DT104 type. In 1997 no resistance to enrofloxacin and nalidixic acid was noted in *S. Typhimurium* isolated from poultry (n=78).

Data for cattle refer to clinical isolates. The following resistance was demonstrated: 46% to ampicillin, 65% to spectinomycin, 73% to tetracycline and 12% to nalidixic acid. No resistance to enrofloxacin was noted among the bovine isolates. All strains of *S. Enteritidis* were sensitive whereas *S. Dublin* strains were resistant to spectinomycin (81%) and to nalidixic acid (24%).

16% of *S. Typhimurium* (n=83) in 1997 were sensitive with 70% showing the resistance profile ASTC. The percentage of *S. Typhimurium* isolates with this resistance profile fluctuated from 53% in 1992 to
60% in 1994 and 48% in 1996. Resistance to nalidixic acid in S. Typhimurium was about 5%; no resistance to enrofloxacin was found amongst these isolates.

Data for pigs refer to clinical isolates. The following resistance was demonstrated: 29% to ampicillin, 18% to spectinomycin, 49% to tetracycline and 2.4% to nalidixic acid. No resistance to enrofloxacin was observed. Most of the S. Derby strains (77%) and all S. Enteritidis strains were sensitive. As for S. Typhimurium strains (n=187 in 1997) only 25% were sensitive with 28% having a resistance profile ASTC. S. Typhimurium isolates with this resistance profile showed an increase over the last years: from 15% in 1992, to 20% in 1995 and 31% in 1996. About 2% of S. Typhimurium isolates showed resistance to nalidixic acid, no strains were found with resistance to enrofloxacin.

Nalidixic acid resistance, but not enrofloxacin resistance, is frequently found in Salmonella isolated from cattle and poultry. This resistance is less frequent in isolates from pigs.

3.2.3.2 Denmark

- Animals

Reliable data on resistance in isolates from food animals are available for the last decade. Resistance data from 1993 and 1997 for S. Typhimurium are shown in table 19. Low-level ampicillin resistance and moderate level streptomycin resistance (not in the table) are found in isolates from all three major food-producing species. A general increase in resistance appears to have taken place from 1993 to 1997, mainly confined to "old" antibiotics, i.e. spectinomycin and streptomycin (not in the table), sulphonamides and tetracyclines. The increase is most pronounced among isolates of porcine origin. In 1997 all multiresistant isolates were of swine origin. Resistance to quinolones is low and does not appear to have increased during this period; resistance to fluoroquinolones was not detected (table 19).

The serotype contributing most to resistance among S. enterica is S. Typhimurium which made up approximately 80% of all porcine Salmonella isolated in 1997 versus 18% of bovine isolates and 28% of poultry isolates. The prevalence of S. Typhimurium DT104 in Danish production herds is unknown before 1996. Routine phage typing of S. Typhimurium isolates was started in 1996 by the Danish Veterinary Laboratory. Since then infections with S. Typhimurium DT104 have been diagnosed in approximately 30 swineherds and in a few cattle herds but not in poultry flocks. All DT104 strains isolated up to 1998 have shown the resistance pattern ACSSuT. Three isolates were also resistant to fluoroquinolones.

The resistance level among isolates of S. Enteritidis (present mainly in poultry) and S. Dublin (widespread within the cattle population) is low and no increasing trend is evident.

Table 19: Resistance data (%) of S. Typhimurium in different species in Denmark

<table>
<thead>
<tr>
<th></th>
<th>Poultry (n=98)</th>
<th>Pigs (n=99)</th>
<th>Cattle (n=48)</th>
<th>Humans (n=228)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Colistin</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>1</td>
<td>28</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Humans

Resistance data for isolates of human origin are similar to those found in isolates of swine origin. The majority of human S. Typhimurium infections in Denmark are assumed to be acquired by food of animal origin (pork). Phage typing of human isolates of S. Typhimurium was started in 1994. In 1995 and 1997 there was no increase in DT104 isolation (about 6% of human isolates). In 1998, due to a common-source outbreak in 1998 (pork originating from one infected herd) the relative frequency of this serotype was higher than during 1995 - 1997.
3.2.3.3 France

- Animals

Susceptibility testing of 15878 *Salmonella* strains from non-human sources (animals, food, environment) isolated in 1994 - 1995, has been performed for a panel of 12 antibiotics (Brisabois et al., 1997). 57.9% of the strains isolated from animals were susceptible to all antibiotics tested (in 1992 - 1993 the percentage was 59.9%). Resistance to only one antibiotic was found in 17.4% of animal strains (14.3% in 1992). Since 1992 - 1993 an increase of resistance to ampicillin, chloramphenicol and streptomycin can be seen whereas the level of resistance to nalidixic acid and trimethoprim-sulfamethoxazole in animal isolates remains constant.

In bovine isolates, many different serotypes are reported, with predominance (since 1986) of *S. Typhimurium* isolates, among which a majority is of the ACSSuT phenotype, suggestive of DT104. As compared to 1992 - 1993, in 1994 - 1995 a decrease of resistance can be noted to kanamycin, neomycin, nalidixic acid and trimethoprim-sulfamethoxazole. However, in 1995 Brisabois and Martel (1997) described resistance in bovine *S. Typhimurium* strains (clinical cases, n=158) by as follows: nalidixic acid 13%, flumequin 6% and oxolinic acid 9%. No resistance to enrofloxacin had been noticed.

Avian strains have been isolated mainly from healthy animals during control procedures of laying flocks. *S. Enteritidis* was predominant but highly susceptible (95% of full susceptibility). *S. Typhimurium* was the second most frequently isolated serotype, with about 50% of strains resistant (phenotype ACSSuT). Resistance to nalidixic acid was 4%. *S. Saintpaul* was the most resistant serotype (only 23% of fully susceptible strains). No high level resistance to new fluoroquinolones was observed in animal strains, as confirmed by the specific bovine surveillance network RESABO.

Moury et al. (1997) presented data on 5 655 strains analysed in 1996, among which 1 741 have been isolated from animals, i.e. 73% from poultry and 20% from cattle. 63% of all strains were resistant to at least one antibiotic (68% in animal health, 60.6% in food hygiene, 59% in environmental samples). Resistance to cefoperazone in the ACST type among *S. Typhimurium* increased from 21.7% in 1994 to 31.4% in 1996. In poultry, multiresistance was also demonstrated by *S. Regent*, *S. Hadar*, *S. Saintpaul* and *S. Newport* isolates, whereas resistance of *S. Enteritidis* to tetracycline reached 18% (17/94 isolates).

- Humans

In a multicentre study carried out in 1997 by 77 public hospitals (Breuil et al., 1998) 2451 strains of *Salmonella* from humans have been examined. The predominant serotypes were *S. Typhimurium* (40.5%) and *S. Enteritidis* (36%). *S. Typhimurium* was the most resistant, with a high frequency of multiple resistance to five drugs (ACSSuT). The highest frequencies of resistance were to ampicillin (73%), tetracycline (83%) and chloramphenicol (56%). Resistance to nalidixic acid was recorded in 5% of *S. Typhimurium* strains, with no ciprofloxacin resistance being recorded. Complementary studies by molecular hybridisation on a sample of pentaresistant *S. Typhimurium* confirms the presence of an integron similar to the structure described in DT104. *S. Enteritidis* remained susceptible to most drugs (resistance to amoxicillin 7%, to the other drugs less than 5%). The prevalence of *S. Hadar* increased (6% of strains against less than 1% in 1994); this serotype showed 72% of resistance to amoxicillin and 6% of resistance to ciprofloxacin. In all strains of other serotypes only one ciprofloxacin resistant strain was observed. According to the authors, comparison with data from 1994 shows a general stability of the resistance frequencies, with only a slight tendency to increase.

3.2.3.4 Germany

- Animals

At the National Reference Laboratory for *Salmonella* in Berlin about 2000 to 3000 *Salmonellae* are typed annually. More than 20 000 strains mainly from diagnostic laboratories and veterinary medicinal institutions of the federal Länder, of universities and private laboratories were examined for their resistance in the period from 1986 to 1997.
An increase in the overall resistance to at least one antibiotic was observed for Salmonella strains isolated from animals. In cattle the resistance increased from 31.8% (1986) to 84.3% (1990), 41.4% (1993) and 67.3% (1997). In pigs resistance increased from 14.3% (1986) to 17.9% (1990), 41.2% (1994) and 67.9% (1997). In poultry an increase in resistance was observed from 33% (1992) to 61.2% (1996); resistance was higher in turkeys than in chickens.

The fluoroquinolone resistance of all serotypes investigated was 2.7% in 1989 and 14.5% in 1990. Since 1991 the incidence decreased to 2.2% (1991), 1.1% (1993), 0.2% (1994/95) and 0.1% (1996). In Salmonella isolates from cattle, a high fluoroquinolone resistance rate was observed in the years 1990 - 1992 (linked to the phage type 204c) which showed in addition resistance to tetracycline, ampicillin, chloramphenicol, kanamycin and trimethoprim (Helmuth and Protz, 1997). Fluoroquinolone resistance in cattle declined from 30% in 1992 to 1% in 1996 (all Salmonella serotypes) and in S. Typhimurium from 75% in 1992 to 38% (1993), 3% (1994), 6% (1995) to 0% (1996), respectively. In poultry, fluoroquinolone resistance showed a tendency to increase from 0.1% in 1992 to 1.5% in 1996.

The isolation rate of S. Typhimurium DT104 increased in all the major food animals from 1992 to 1998. Since 1992 S. Typhimurium DT104 isolates showed also an increasing rate of multiresistance with the phenotype ACSSuT. Quinolone resistance in S. Typhimurium DT104 is very rare until now in Germany (Rabsch et al., 1997; Schroeter et al., 1998). Enrofloxacin resistance in S. Typhimurium in cattle decreased from 75% (n=64) in 1992 to 38% (1993), 3% (1994), 6% (1995) to 0% (n=99) in 1996. In poultry enrofloxacin resistance was described with 1% in 1992, 12% in 1993 (n=99) and 0% in 1994 - 1996. In pigs enrofloxacin was resistance was described with 7% in 1992, 28% (1993), 3% (1994), 1% (1995) and less than 1% in 1996 while resistance to nalidixic acid was 4% in 1995 and 6% in 1996 (National Salmonella Reference Laboratory). Other investigations (Trolldenier, 1996) confirmed the trend of enrofloxacin resistance in cattle from 11% in 1992 (n=136) to 13% in 1993 (n=136), 9% in 1994 (n=122), 6% in 1995 (n=127) to 3% in 1996 (n=86).

• **Humans**

The National reference centres for human isolates of Salmonella are the Robert-Koch-Institute (Branch Wernigerode) and the Institute for Hygiene (Branch Hamburg). S. Enteritidis is still the most frequent serotype in humans, although S. Typhimurium DT104 has gained importance. In 1996, resistance of S. Enteritidis was low to ampicillin (2%), cotrimoxazole (0.1%) and oxytetracycline (2%), while sulfamethazine-resistance was 44 to 72%. Fluoroquinolone-resistance was 0%. In the same year, the resistance of S. Typhimurium was 47.7% for ampicillin, 62.1% for oxytetracycline and 72.2% for sulfamethazine.

The epidemic strain S. Typhimurium DT104 was ranking second of the most prevailing serotypes in 1996. Its broad multiresistance (ACSSuT) is appearing in 90% of the isolates (Liesegang et al., 1997). A slight increase in fluoroquinolone resistance (from 0 to 0.2%), can be detected in 1996 - 1997 in S. Typhimurium including S. Typhimurium DT104. The fluoroquinolone-resistance in other Salmonella serotypes was 0.4 to 0.5% in 1996 - 1997 (Tschäpe, 1997).

3.2.3.5 **Italy**

• **Animals**

Strains of S. Typhimurium isolated from avian sources (mainly poultry, but also quails and turkeys) in local laboratories of north-east Italy have been tested for susceptibility to various antibiotics in the years 1996 and 1998. In this area of Italy poultry production is particularly relevant, representing one third of the national poultry meat production. Susceptibility tests were performed by a disc-diffusion method. In 1996, out of 125 strains examined, 27% showed the resistance type ACSSuT and further 11% had an additional resistance to nalidixic acid (resistance type ACSSuTNal). Overall, 38% S. Typhimurium isolated from poultry had a resistant phenotype suggestive for the phage type DT104. This observation was further confirmed by the finding of the 60 kDa virulence plasmid in the multiresistant strains. In 1998, out of 78 strains, mainly from avian sources, 60% had the resistance type ACSSuT and 20% the resistance type ACSSuTNal. Overall, 80% of the strains had a phenotype characteristic of the multiresistant clone DT104. There was an increase in the frequency of this resistant pattern and also an
increase in the frequency of the association with nalidixic acid resistance (Ricci et al., personal communication, 1998).

- Humans

In a survey of serotype and phage type distribution of Salmonella from human and animal sources in Italy from 1973 to 1995, Fantasia and co-workers noted in recent years an increase in the number of multiresistant strains, mostly belonging to S. Typhimurium DT104 (resistance type ACST) and 193 (resistance type AST) (Fantasia et al., 1998).

None of the S. Typhimurium strains isolated in central Italy up to 1996 was resistant to nalidixic acid, while nalidixic acid resistance was found frequently in isolates of S. Enteritidis and S. Hadar (Luzzi, personal communication, 1998).

3.2.3.6 Spain

- Animals

Preliminary data about resistance patterns in Salmonella isolated between January and June 1998 are reported by the network for surveillance of antibiotic resistance in bacteria from animals (VAV, 1998), and include 47 strains of Salmonella isolated from different animals species (sheep, poultry, pig, cow, cat, etc.). Resistance was low or moderate for ampicillin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole and tetracycline. Resistance to nalidixic acid was 8% and 4% to enrofloxacin.

3.2.3.7 Sweden

- Animals

The incidence of Salmonella infections in Sweden has always been very low, according to the Swedish National Veterinary Institute. The resistance pattern of Salmonella including S. Typhimurium isolated from animals in 1988 to 1991, show moderate resistance to streptomycin (7 to 38% according to species and source), very low resistance to ampicillin (0 to 4%) and tetracycline (0 to 3%) and no resistance to gentamicin, co-trimoxazole and enrofloxacin. The resistance patterns of more recent isolates have not changed significantly, according to the National Veterinary Institute (1999).

3.2.3.8 The Netherlands

- Animals

At the National Institute of Public Health and the Environment in Bilthoven, which is the National Reference Laboratory for Salmonella in the Netherlands, Salmonella strains from humans and animals are tested for their susceptibility by agar diffusion test (Stobberingh et al., 1998). All data reported refer to the period 1994 - 1997.

In Salmonella from healthy pigs the proportion of strains resistant to ampicillin (7 to 23%), tetracycline (36 to 58%) and trimethoprim (10 to 30%) was more or less stable during period of the study. Neomycin resistance was detected in 1995 only (2%) and flumequin resistance was not observed.

In Salmonella isolated from healthy cattle the proportion of strains resistant to ampicillin (8 to 21%), tetracycline (22 to 33%) and trimethoprim (4 to 11%) was slightly lower than in pigs. However, resistance to neomycin (3 to 7%) and flumequin (2 to 3% in 1994 and 1996, respectively) was slightly higher.

In Salmonella from healthy poultry the proportion of strains resistant to ampicillin (2 to 16%), tetracycline (10 to 43%), trimethoprim (0 to 8%) and neomycin (0 to 1%) was comparable to the proportion in cattle and pigs. However, flumequin resistance was more prevalent in poultry.

The proportion of resistant Salmonella in poultry varied from 34% in 1994 to 7% in 1997. The observed decreasing resistance tendency should be confirmed throughout the years, if the same Salmonella serotypes are tested.

In S. Typhimurium strains isolated from diseased veal calves and pigs, the same resistance trend can be observed (Animal Health Service, 1998), although in general the proportion of resistant strains is higher.
in diseased than in healthy animals. Flumequin resistance in \textit{S. Typhimurium} isolates from veal calves tended to decrease from 42% in 1994 to 15% in 1995 and 0% in 1996, although the number of strains tested are low. No resistance to flumequin or enrofloxacin was observed in this period in pig clinical isolates of \textit{S. Typhimurium}, an animal species for which enrofloxacin was not licensed at that time. Resistance to enrofloxacin in \textit{S. Typhimurium} in veal calves was 3% in 1994 and 0% in 1995 and 1996.

- **Humans**

In human isolates of \textit{S. Typhimurium} (gastro-enteritis cases) the proportion of resistant strains is comparable to that from animals (Stobberingh et al., 1998). However, in 1994 - 1997 the proportion of strains resistant to ampicillin (15 to 46%), tetracycline (53 to 66%), trimethoprim (9 to 25%) and neomycin (0 to 31%) was slightly higher than the proportion in food animals. Flumequin resistance was stable (1%) over the time period observed (1994 - 1997).

\textit{S. Typhimurium} DT104 was tested for quinolone susceptibility starting from 1991 in isolates from human and non-human sources. No resistance to quinolones was observed in 1991 - 1995, whereas 1.2% of all isolates (n=83) tested in 1996 showed resistance to flumequin (Van Leeuwen et al., 1997). In human isolates of \textit{S. Enteritidis}, resistance was in general much lower than in \textit{S. Typhimurium} with the exception of flumequin resistance, which was higher (8 to 10%). This same resistance rate was observed in other \textit{Salmonella}.

3.2.3.9 United Kingdom

- **Animals**

All \textit{Salmonella} data are from 1997 unless specified and derived from "\textit{Salmonella} in Livestock Production 1997", published by the Veterinary Laboratories Agency (1998). In 1997 as in previous years 98.2% of all \textit{S. Dublin} were sensitive to all 16 antibiotics tested and 74.8% of all other \textit{Salmonella} strains (except \textit{S. Typhimurium}) were sensitive to all test antibiotics. Only 11.4% of \textit{S. Typhimurium} strains were sensitive to all tested antibiotics; this sensitivity had increased slightly from 7.6% in 1995 to 10.8% in 1996. 73.7% of \textit{S. Typhimurium} tested were DT104. The most common resistance pattern was ACSSuT; however, a further 16% were resistant to nalidixic acid (9% in 1996) and 13.8% were resistant to sulfamethoxazole / trimethoprim (16% in 1996). During 1990 to 1997 an increase in resistance to nalidixic acid in \textit{S. Typhimurium} isolates from different sources has been noted from 0 to 1% (1990 - 1994) to 3% (1995), 9% (1996) and 13% in 1997. In strains isolated from poultry, resistance in \textit{S. Typhimurium} DT104 to nalidixic acid has increased to 78% in 1997 in turkeys (61 isolates tested) from 75% in 1996 respectively 16% in chickens (31 isolates tested) from 6% in 1996. In \textit{S. Typhimurium} DT104 from cattle, resistance to nalidixic acid has increased from 5% in 1996 to 11% (597 isolates tested). In \textit{S. Typhimurium} DT104 from pigs resistance to nalidixic acid was 5% in 1997 (88 isolates tested) compared to 7% in 1996, and 1% in 1995.

- **Humans**

Most human isolates of \textit{S. Enteritidis} received by the PHLS’s Laboratory of Enteric Pathogens in 1997 were fully sensitive to all tested antibiotics. Resistance to ciprofloxacin was recorded at 0.3% for these isolates. Ciprofloxacin resistance in \textit{S. Typhimurium} has risen from 1% in 1994 to 13% in 1997. This resistance predominates in \textit{S. Typhimurium} DT104. The incidence of \textit{S. Typhimurium} DT104 has dropped by 20% compared with 1996. It must be emphasised that the breakpoint of ciprofloxacin resistance used at the PHLS is lower than in other Member states (1 against 4 \( \mu \text{g/ml} \)).
3.2.4 Summary (Salmonella)

- Most *Salmonella* strains, independent of their origin (human or animal) are mainly sensitive to the antibiotics tested.
  - Exceptions are serotypes Hadar and Typhimurium, which are multiresistant, i.e. profiles ampicillin - tetracyclines - nalidixic acid and ampicillin - spectinomycin/streptomycin - tetracyclines - chloramphenicol, respectively. In France, human *S.* Hadar isolates resistant to ciprofloxacin have been reported.
  - *S.* Enteritidis and *S.* Dublin are generally susceptible, but both serotypes show high nalidixic acid resistance in some countries.
  - Some countries report resistant *S.* Virchow isolates.

- In 9 Member States of the EU the highest level of resistance in *Salmonella* of both animal and human origin is observed in *S.* Typhimurium DT104. At this moment it is not clear if its spread is due to specific virulence factors of the strain, to antibiotic use, farming practice or increased isolation rates.
  - In some countries (Netherlands, UK) resistance in *Salmonella* and spread of *S.* Typhimurium DT104 has increased in the first part of this decade and by now appears to have reached a plateau or a peak. In other countries (Germany, Belgium, Italy, Spain) resistance and spread of DT104 still seems to be on the rise. The reasons for this difference are not clear.
  - Although resistance to fluoroquinolones (enrofloxacin, ciprofloxacin) is in general low or even absent in *S.* Typhimurium DT104, resistance to nalidixic acid is on the rise in most countries, especially in strains isolated from poultry (Italy, UK, Germany, etc.). Resistance to nalidixic acid indicates that the strains have already acquired a mutation in the target of quinolones. This could mean that resistance to the related fluoroquinolones might be expected in the near future. This applies also to strains isolated from human.
Chapter III: Development of Resistance

3.3 Pathogenic Bacteria

Most resistance data available for pathogenic bacteria in animals can be obtained for *E. coli*. Only a few Member States include other animal pathogens in their surveillance programmes (see annex III). For this reason, the authors agreed to focus on *E. coli* as example for pathogenic bacteria in animal species, i.e. intestinal *E. coli* in cattle and pigs, and septicaemic *E. coli* in poultry.

3.3.1 *E. coli*

3.3.1.1 Belgium

Data on cattle and pig strains are from laboratory findings on clinical isolates analysed in 1997 at the Veterinary and Agrochemical Research centre of the Ministry of Small Enterprises, Traders and Agriculture. Unless otherwise noted all data refer to 1997. From only a few isolates the serotype is available.

- **Cattle**
  
  In 1997 approximately 21% of the intestinal *E. coli* from calves are sensitive, 32% have the profile ACSuT and 15% the profile ACST. About 13% of strains are resistant to more than 10 antibiotics. Most *E. coli* strains isolated from cattle older than one year (64% of cases) seem to be sensitive.
  
  Regarding quinolone resistance among bovine strains analysed in 1997, about 34% are resistant to nalidixic acid, and 24% to enrofloxacin. Especially strains isolated from calves are quinolone resistant. A significant increase in resistance to both nalidixic acid and enrofloxacin is apparent in 1997 as compared to 1996; preliminary data for 1998 confirm that this trend is going on.

- **Pigs**
  
  Only about 10% of the strains isolated from neonatal pigs are sensitive. The most frequently encountered profile is ACT (26%). As for quinolone resistance, about 21% of isolates are resistant to nalidixic acid, and 11% to enrofloxacin. In finishing pigs, only 7.4% of the isolates are sensitive, and the profile ACT was detected in 41% of cases. Nalidixic acid resistance was found in about 16%, and enrofloxacin resistance in 9.3% of strains.
  
  Compared with previous years, a significant increase in resistance to both nalidixic acid and enrofloxacin is apparent in strains isolated in 1997 and 1998 (preliminary data) as opposed to 1996.

3.3.1.2 Denmark

Pathogenic *E. coli* include *E. coli* from poultry belonging to serotype O2 and O78, *E. coli* from pigs belonging to serotype O149, and fimbriae type F5 positive *E. coli* from cattle.

- **Cattle**
  
  A high percentage of *E. coli* strains resistant to the main antibiotics was detected in 1994, 1996 and 1997, and at a relatively constant level, e.g. ampicillin up to 83%, streptomycin up to 85%, sulphonamides up to 91%, and tetracycline up to 78%. Whereas quinolone resistance was not found in 1994, up to 9% nalidixic acid and 5% enrofloxacin resistance was detected among the 1997 isolates.

- **Pigs**
  
  The same general conclusions can be drawn as for cattle, although the percentage of resistant pig strains in 1994 - 1997 is generally lower, e.g. streptomycin 65 to 71%, ampicillin 26 to 29% and tetracycline 57 to 62%. However, nalidixic acid resistance is higher than that found in cattle strains i.e. up to 19%.

- **Poultry**
  
  A high percentage of resistance to sulphonamides (64%) and nalidixic acid (32%) was registered in pathogenic *E. coli* strains, isolated in 1997. Resistance to enrofloxacin was not found.
3.3.1.3 France

- Cattle

Information is available from a study that is based on the work of the RESABO Network (Martel et al., 1995) on clinical bovine strains. In general, isolates from adult cattle were much more susceptible than isolates from calves, with resistance ranging from 0% for gentamicin to 34% for tetracycline in adults as compared to 12% and 88% respectively for the same antibiotics in calves. In calves, isolates expressing a virulence determinant such as antigen K99 were more resistant than isolates devoid of it.

3.3.1.4 Germany

The following data on *E. coli* isolates (clinical cases) are collected by the BgVV from different laboratories and summarised by Trolldenier (BgVV, 1995; Trolldenier, 1996).

- Cattle

*E. coli* in cattle is the bacterial pathogen with the highest rate of multiresistance: more than half of the strains tested in the period 1992 to 1995 are resistant to broad spectrum antibiotics like tetracycline (65 to 70%), ampicillin (65 to 70%), chloramphenicol (65%), aminoglycosides (neomycin 55%, streptomycin 70%), gentamicin (19 to 35%) and sulphonamides (60 to 90%).

In the period 1992 to 1996 an increase in enrofloxacin resistance of *E. coli* from cattle (7 to 16%) is apparent.

- Pigs

A high rate of resistance in porcine *E. coli* strains is demonstrated (chloramphenicol 50%, Sulphonamides 75 to 85%, aminoglycosides/streptomycin 75%, macrolides 95 to 100%) in the period 1992 to 1995. A tendency towards increasing tetracycline resistance (75 to 87%) and ampicillin resistance (50 to 74%) from 1992 to 1995 is demonstrated.

In the period 1992 to 1996 an increase in enrofloxacin resistance of *E. coli* from pigs (2 to 8%) is apparent.

- Poultry

Resistance in *E. coli* from chickens in the period 1992 to 1994 was 59 to 66% for tetracycline, 77 to 44% for Sulphonamides, 34 to 48% for ampicillin, 15 to 10% for gentamicin and 10 to 12% for enrofloxacin. *E. coli* strains from turkeys in the same period showed higher (multi-) resistance rates than other poultry species (tetracycline 75%, chloramphenicol 55%, ampicillin 36 to 50%).

In the period 1992 to 1996 an increase in enrofloxacin resistance of *E. coli* from turkeys (18 to 24%) is apparent. According to Klarmann (1997) the resistance rate was 30% in poultry (chicken and turkeys together) in the district Weser-Ems, a large German region with intensive animal husbandry.

3.3.1.5 Italy

- Pigs

*E. coli* strains isolated from neonatal piglets with colibacillosis in two regions of Italy (Lombardia and Emilia Romagna) from 1990 to 1994 have been studied for susceptibility to aminoglycosides and quinolones. There was no trend toward an increase in resistance for all the aminoglycosides tested, although yearly variations could be observed with resistance ranging from 35 to 70%. Resistance to nalidixic acid was stable around 40% from 1991 to 1994, while resistance to enrofloxacin increased from 1.3% in 1990 to 9.8% in 1994 (Guadagnini, 1995).

Data obtained from Regional Veterinary Laboratories (Istituti Zooprofilattici Sperimentali) in the years 1996 to 1997 on clinical pig isolates confirmed the trend of previous years: 47% of the strains were resistant to nalidixic acid and 19% were resistant to enrofloxacin. Moreover, resistance to ampicillin was 70% and to aminoglycosides ranged from 40 to 60%.
3.3.1.6 The Netherlands

At the Animal Health Service in the Netherlands clinical isolates of *E. coli* are tested for their susceptibility by agar diffusion test. The resistance percentages are based on nationally agreed breakpoints, similar to those used in other countries.

- **Cattle**

  In clinical isolates of K99-positive *E. coli* from calves isolated from 1994 - 1996, resistance to amoxicillin (79 - 83% of the isolates) and tetracycline (75 to 93% of the isolates) is very common. Resistance to trimethoprim-sulfamethoxazole varies from 25 to 40% of the isolates. In that period only one flumequin resistant strain was detected.

- **Pigs**

  In clinical isolates of enteropathogenic *E. coli* isolated from clinical cases of diarrhoea in pigs, isolated from 1994 to 1996, the proportion of strains resistant to amoxicillin (52 to 66%), tetracycline (83 to 84%) and trimethoprim-sulfamethoxazole (49 to 60%) is also very high. Flumequin resistance is stable at a level of 2% of the isolates.

- **Poultry**

  In clinical isolates of *E. coli* from poultry in the period 1981 to 1992 the proportion of resistance to tetracycline (63 to 75%), sulphonamides (71 to 75%), chloramphenicol (17 to 27%) and neomycin (2 to 7%) has remained stable, although at different levels. Resistance to ampicillin and trimethoprim-sulfamethoxazole has increased from approximately 15% in the early 1980s to 56% for ampicillin and 34% for trimethoprim-sulfamethoxazole in 1992.

  In clinical isolates from poultry flumequin resistance was first detected in 1983 at a very low level (0.3%) increasing to 49% in 1992. Enrofloxacin resistance was first detected in 1987 (0.08%). The level rapidly increased to 9.2% in 1992.

3.3.1.7 Spain

- **Cattle**

  A limited comparative analysis of *E. coli* strains from diarrhoeic calves can be made using data of Blanco et al. (1993) and Orden et al. (1999) reporting results obtained with disc diffusion and agar dilution, respectively. Antibiotics studied in both reports are cephalotin, cefotaxime and nalidixic acid, and resistance figures are as follows: cephalotin 16% to 32%, cefotaxime 0% in both studies and nalidixic acid 1 to 35%. These data suggest increasing resistance to the reported antibiotics among *E. coli* from diarrhoeic calves.

- **Poultry**

  Data on 301 septicaemic strains of *E. coli* isolated during 1992 and 1993 are available (Blanco et al., 1997). Resistance to quinolones was high (48% nalidixic acid, 24% flumequine, 17% ciprofloxacin) as well as to streptomycin (78%), tetracycline (94%), sulfadiazine (88%) and trimethoprim-sulfamethoxazol (63%). Resistance to beta-lactam antibiotics was 35% (ampicillin) and 16% for cephalotin and 0% for cefotaxin and cefoxitin. Resistance to aminoglycosides ranged from 14% for gentamicin to 0% for amikacin. About 25% of the strains were also resistant to chloramphenicol.

3.3.1.8 Sweden

- **Pigs**

  Data are available on the resistance pattern of strains isolated from cases of neonatal diarrhoea (National Veterinary Institute, 1999). A comparison can be made between the periods 1981 to 1982, 1989 to 1991 and 1994. No significant changes are apparent between these periods; resistance is mainly found to streptomycin (40 to 50%) and tetracycline (about 40%). Enrofloxacin resistance is below 1%.
3.3.1.9 United Kingdom

Information is available from Wray et al. (1993). Resistance figures quoted from this source represent the combined data for a five years period. Unpublished data are also available from 1998 concerning enrofloxacin resistance (Teale, personal communication, 1999).

- **Cattle**

  Of all bovine strains tested, 12% were sensitive to all test antibiotics. Most resistance was found to ampicillin (64%), tetracycline (74%), aminoglycosides (neomycin 48%, streptomycin 60%) sulphonamides (76%), and trimethoprim (26%). In 1998 1% of clinical isolates (n=1197) from cattle showed resistance to enrofloxacin (Teale, personal communication, 1999).

- **Pigs**

  Although a similar percentage of strains were sensitive to all test antibiotics (15%), resistance among these strains was lower compared with bovine strains, especially to ampicillin (25%) and neomycin (17%). In 1998 3% of clinical isolates (n=163) from pigs showed resistance to enrofloxacin (Teale, personal communication, 1999).

- **Poultry**

  In general, resistance of poultry *E. coli* isolates to individual antibiotics was lower than in pig strains. In 1998 6% of clinical isolates (n=183) from poultry showed resistance to enrofloxacin (Teale, personal communication, 1999).

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### 3.3.2 Summary

Due to the different time periods during which the pathogenic *E. coli* were analysed in the various European countries, the available data cannot be compared. Furthermore, the clinical data on *E. coli* presented here should be interpreted with care, since other characteristics of the isolates, that may be responsible for an eventual clonal spread, are not taken into account, e.g. virulence factors and serotypes. To draw more correct conclusions, these data should be compared to similar data from *E. coli* isolates from the slaughterhouse. This hampers the description of the actual position on resistance among these isolates in Europe.

The available data indicate, however, that in the population of pathogenic *E. coli* only a low level of sensitive strains is present. This is supported by the following points:

- The number of sensitive isolates from cattle, pigs and poultry, when reported, varies between about 10 and 20%.
- Multiresistance is a common finding among pathogenic *E. coli*.
- Resistance to β-lactams, aminoglycosides, tetracycline and sulphonamides is frequently observed
  - **β-lactams.** Percentages of resistance to ampicillin or amoxicillin of 60 to 80% or more are reported in various countries, especially in cattle and pigs.
  - **Aminoglycosides.** Streptomycin resistance among cattle (70 to 80%) and pig strains (up to 70%) is frequent, but also gentamicin resistance in cattle (12 to 34%) and neomycin resistance (55% in cattle) was found.
  - **Tetracycline.** Between 50 and 90% of cattle, pig and poultry strains are found resistant.
  - **Sulphonamides.** Resistance to sulphonamides alone, or in combination with trimethoprim is frequently found in all species
  - **Quinolones.** A significant increase is reported in fluoroquinolone resistant poultry strains in recent years, particularly among turkeys. Nalidixic acid and enrofloxacin resistant strains are also persistently found recently in cattle and pig isolates.
3.4 COMMENSAL BACTERIA

Commensal bacteria are part of the normal intestinal flora of animals and are generally regarded as non-pathogenic. They are present in healthy animals in large numbers (see introduction) and therefore can easily contaminate carcasses during the slaughter process. Commensals can readily acquire resistance genes, and are considered as indicators of the level of antibiotic resistance carried by an animal. They also may be opportunistic pathogens in certain situations, as is the case for enterococci.

The commensal bacteria studied are *E. coli* and *Enterococcus* spp., which represent respectively, the most common Gram-negative and Gram-positive (non-anaerobic) species in the gut. Samples of commensal bacteria are obtained from faeces or intestinal contents of healthy animals at the slaughterhouse or just before slaughter. Therefore, the study of antibiotic resistance in animal commensals requires particular monitoring programmes. So far only Denmark has implemented such a programme, and this country made data available for 1996 and 1997 (DANMAP, 1997 and 1998).

3.4.1 Denmark

Most commensal *E. coli* strains isolated from cattle in 1996 appear to be sensitive to all of the antibiotics tested. A tendency toward an increase in resistance to ampicillin (10%), streptomycin (21%), sulphonamides (25%), and tetracycline (17%) was observed in 1997. No strain was resistant to nalidixic acid.

Commensal *E. coli* strains from pigs were more resistant, especially to streptomycin, sulphonamides and tetracyclines, with a tendency to increase from 1996 to 1997. However, resistance to ampicillin was stable at 10% and resistance to nalidixic acid or enrofloxacin was not detected.

Commensal *E. coli* strains from broilers appear to have a moderate level of resistance to ampicillin (12%), streptomycin (9%), and tetracycline (9%). Resistance to sulphonamides was 34% in 1996 and 25% in 1997. Resistance to nalidixic acid was 15% in 1996 and 8% in 1997. No resistance to enrofloxacin was found.

Resistance data on *E. coli* isolated from food of animal origin seem to reflect those reported in animal strains. Examining resistance rate from 1997, animal and meat data are quite similar in poultry, whereas strains isolated from beef and pork have lower resistance compared to those isolated from cattle and pigs.

The most common species of *Enterococcus* (E.) isolated from cattle, pigs and broilers was *E. faecium*. *E. faecalis* strains were studied only from pigs.

*E. faecium* isolated from the three major species of food animals were sensitive to ampicillin and to gentamicin. Resistance to streptomycin was low in broilers and cattle (5% and 13%, respectively) and increasing in 1997. Resistance was high in strains from pigs (40%). Macrolide resistance was high in strains from all animal species (cattle 38%, broilers 65%, pigs 91%) with a tendency to decrease in 1997. Tetracycline resistance was moderate in strains from cattle and broilers and high in pigs (69% in 1996 and 86% in 1997).

*E. faecalis* strains isolated from pigs show the same resistance patterns as *E. faecium* in pigs.

3.4.2 Other countries

Very few data are available from other countries, where only limited studies (e.g. at a single slaughterhouse or animal farm) have been conducted.

In The Netherlands, a study was conducted from June 1991 to April 1992 at a pig farm in three groups of pigs to determine the prevalence and the degree of antibiotic resistance in *Enterobacteriaceae* (Nijsten et al., 1994). The prevalence of resistance to the most commonly used antibiotic agents was high (range amoxicillin 70 - 97%, oxytetracycline 89 - 100%, sulfamethoxazole 88 - 100%, trimethoprim 78 - 100%). The percentage of the *E. coli* strains resistant to oxytetracycline,
streptomycin and sulfamethoxazole ranged from 49 to 68%. Resistance to three or more antibiotics was observed in 43% of the isolates.

In Sweden, a survey of antibiotic resistance in commensals has recently been initiated. Preliminary results in *E. coli* isolated from chickens at slaughter indicate that the resistance is low (0 to 7%) for all the principal classes of antibiotic tested, except for tetracycline (17%). In *E. coli* isolated from weaned pigs resistance to streptomycin is 13% and to tetracycline is 26%. Resistance in *Enterococcus* isolated from chicken at slaughter was absent or low except for macrolides (17%) and tetracycline (64%).

### 3.4.3 Summary (Commensal bacteria)

- Since there is only one country in the EU with a surveillance system adapted for the monitoring of commensals, general EU wide data on resistance in these indicator bacteria to therapeutically used antibiotics are limited.
Chapter IV: Effect of Resistance on Therapy

4.1 Effect of resistance on therapy in human medicine

The development of resistance to antibiotics is one of the main reasons for failure of antibiotic therapy. This is especially true for the critically ill or the immuno-compromised patient as well as for very young or elderly patients, where the susceptibility of the infecting microorganisms to the antibiotic to be used is of paramount importance. The occurrence of multiresistance is of particular concern, because it restricts dramatically the treatment options: in these instances the only effective antibiotics are often the newest and more expensive ones.

In spite of these obvious effects of the antibiotic resistance, few studies have addressed and documented clearly the economic costs of antibiotic resistance. In many studies factors such as virulence of the microorganism, site of infection, immune status of the patient, are variables that can influence the outcome of therapy besides the resistance of the microorganism. One of the recommendations of the EU Conference in Copenhagen (Copenhagen Recommendations, 1998) has been to design an appropriately controlled study which enables investigators to evaluate the consequences of antibiotic resistance both in terms of morbidity and mortality and in terms of economic costs.

One meta-analysis study was performed ten years ago by investigators of the Centre for Disease Control (CDC) and the Infectious Diseases Society of America on 175 published and unpublished reports of nosocomial and community infections with selected bacteria, including Staphylococcus aureus, Salmonella, Klebsiella, Serratia (Holmberg et al., 1987). They found that for both nosocomial and community-acquired infections, the mortality, the likelihood of hospitalisation, and the length of hospital stay were twice as great for patients infected with drug-resistant strains as for those infected with drug-susceptible strains of the same bacterial species.

More recent example of the implications and costs of antibiotic resistance are reported below.

Penicillin resistance in pneumococci has its most dramatic effects in patients with meningitis. Due to the poor penetration of antibiotics in the cerebrospinal fluid, the concentrations reached are ineffective not only against highly penicillin-resistant but also relatively penicillin-resistant strains. This difficulty accounts for the reported treatment failure in such patients (Leggiadro, 1994).

Increased mortality has been demonstrated among patients with bacteraemia due to vancomycin-resistant E. faecium (VRE) in comparison to those with bacteraemia due to sensitive strains. In the first group mortality can approach 50% (Frieden et al., 1993; Shay et al., 1995). Moreover, in patients with VRE bacteraemia more invasive interventions for infective complications are required than in patients with bacteraemia due to sensitive strains (Linden et al., 1996).

Patients infected with MRSA need expensive antibiotics and longer hospital stay (from 13 to 30 days) (Saravolatz et al., 1982; Holmberg et al., 1987). A recent study evaluated that the attributable cost of a patient with MRSA infection was approximately 2500 $ higher than the attributable cost of a patient with an infection due to sensitive staphylococcus (34000 $ versus 31000 $). The higher cost included also the cost of the isolation procedures for the patient (Rubin et al., 1999). Moreover, as shown by a recent multivariate analysis, patients with MRSA bacteraemia have a four-fold higher mortality risk than patients with bacteraemia due to sensitive staphylococcus (Conterno et al., 1998).

Multiresistant tuberculosis has spread especially among HIV infected patients, a population in which it is very difficult to evaluate the impact of single variable (resistance) on the outcome. In a recent study in HIV-sero-negative patients unfavourable response to therapy, including death, was significantly associated with infection with a multiresistant M. tuberculosis strain (Kritski et al., 1997). Reviewing multiresistant tuberculosis in Denmark, Viskum and Kok-Jansen (1997) noted that patients required prolonged hospitalisation and expensive treatment.

Multidrug resistant Salmonella Typhi is now endemic in many developing countries, including Southeast Asia and Africa (Rowe et al., 1997). As these strains are resistant to the traditionally recommended drugs to treat this life-threatening infection (ampicillin, chloramphenicol and co-
trimoxazole) more recent and expensive antibiotics need to be used, which are not readily available or affordable in these countries.

Multiresistant non-typhoid *Salmonella* infections, such as *S. Typhimurium* DT104, pose a less serious problem as the invasive disease is uncommon and only individuals in life threatening situations may require therapy. However, it is obvious that the antibiotic options where treatment is required are becoming very limited (Frost et al., 1995 and 1996; Threlfall et al., 1997 and 1998). Fluoroquinolones and third-generation cephalosporins are indicated as the drug-of-choice for salmonella infections in humans (Keusch, 1994). Surveillance studies in the UK and a recent outbreak of (quinolone-resistant) salmonellosis in Denmark indicate that infections with *S. Typhimurium* DT104 result in a higher rate of hospitalisation and mortality compared to infections caused by other *Salmonella* serotypes (Altekruse et al., 1997; Anonymous, 1998 B). In a review of the outbreaks of *Salmonella* infections in the US, Holmberg et al. (1984) evaluated that the fatality case rate for patients with infections with multiresistant *Salmonella* was higher than the rate associated with antibiotic-sensitive *Salmonella* infections (4.2% versus 0.2%). Moreover, more patients with infections due to resistant *Salmonella* required hospitalisation, compared to patients with infections due to sensitive strains (Holmberg et al., 1987).

### Effect of Resistance on Therapy in Veterinary Medicine

Literature documenting therapy failure in veterinary medicine as result of an infection with antibiotic resistant strains is scarce. Moreover, little relevant pharmacovigilance data on therapy failure caused by antibiotic resistance are available within the EU.

The publications that describe therapy failure are summarised in this chapter together with reports of efficacy trials comparing the effect of antibacterial therapy of susceptible and resistant strains.

It has been stated that agricultural and veterinary use of antibiotics select for antibiotic resistant bacteria. This use of antibiotic agents, at subtherapeutic or therapeutic concentrations, provides a selective pressure that results in an increase in the prevalence of antibiotic resistance (Prescott, 1997; Angulo et al., 1998).

The critical aspects of the use of antibiotics in veterinary practice that select for resistance and therapy failure are prophylactic and metaphylactic use of therapeutics and long-term use of antibiotics at subtherapeutic dosages in the feed or water (Rassow and Schaper, 1996; Straub, 1998).

*In vitro* data on susceptibility of *Brachyspira (Serpulina) hyodysenteriae* appear to be a good indicator of the clinical outcome of antibiotic treatment of swine dysentery (Jacks et al., 1986). Increasing resistance to macrolides has been documented for *B. (S.) hyodysenteriae*. *In vitro* susceptibility data show that the therapeutic potential is now limited (Ronne and Szancer, 1990; Gunnarson et al., 1991, Fellström et al., 1996, Molnar, 1996) which may pose a potential threat to the pig industry (Buller and Hampson, 1994).

Originally tylosin was the drug of choice for the treatment of swine dysentery, but due to widespread resistance it has been replaced by tiamulin (SOU, 1997). For prevention and therapy of swine dysentery tylosin and tiamulin are both mixed with feed. The costs of one ton of medicated feed at the recommended concentration for swine are significantly higher for tiamulin than for tylosin (SOU, 1997).

A similar development has occurred in *Pasteurella* spp. causing respiratory infections in young cattle. Reports on increasing resistance are available from all around the world (van Amstel et al., 1987; Post et al., 1991; Martel and Coudert, 1993). Respiratory infections are of major significance for the cattle industry and loss of therapeutic efficacy of the most common antibacterial drugs may cause serious problems.

The efficacy of sulbactam, a β-lactamase inhibitor, was studied in combination with ampicillin in an experimental infection model in cattle using ampicillin-resistant *Pasteurella haemolytica* (Farrington et al., 1987). In this trial a comparison was made with the efficacy of ampicillin. In cattle treated with sulbactam-ampicillin the mortality was 0%, while in cattle only treated with ampicillin the mortality was 50%. In the untreated control group mortality was 74%.
Chapter IV: Effect of Resistance on Therapy

In another field efficacy study conducted by Risk and Bentley (1987), involving a total of 92 naturally infected pneumonic veal calves, the efficacy of the β-lactamase inhibitor sulbactam plus ampicillin was compared with ampicillin alone in the treatment of bacterial pneumonia. Cultures from nasal swabs and lung tissue during the 10 to 11 day studies were predominantly ampicillin-resistant Pasteurella multocida. The mortality in the ampicillin and sulbactam-ampicillin treated groups of veal calves was 43% and 14%, respectively, and demonstrated that antibiotic therapy of a resistant strain was less successful. Lockwood et al (1996) found that the use of tetracycline for treatment of resistant Pasteurella strains was less effective than a control antibiotic to which the bacteria were susceptible.

Migaki et al. (1993) stated that the efficacy of tylosin in the treatment of Mycoplasma gallisepticum infection depended on the susceptibility of the strains involved. For infections in chicks with tylosin intermediate/resistant M. gallisepticum strains (MIC values 8 µg/ml and 32 µg/ml, respectively), tylosin was significantly less effective than for infections with susceptible strains (MIC = 0.25 µg/ml).

Although the clonal spread of pentaresistant (ACSSuT) S. Typhimurium DT104 in Europe, in the USA and in Canada (Poppe et al., 1998), both in animals and humans is a reason for concern, treatment failure of infections in animals has not been documented. A case report exists of treatment failure of infection with a multiresistant (including fluoroquinolones) untypable S. Typhimurium strain in veal calves in The Netherlands (Bosch and Hartman, 1993). The report describes a very rapid and severe Salmonella infection in a flock of veal calves that started a few days after the onset of the fattening period. The animals were administered enrofloxacin mixed with the milk replacer from day one for infection prevention. Morbidity and mortality was 90% in spite of antibacterial therapy with colistin and furazolidon. It was stated that the preventive use of enrofloxacin might have selected for the resistant clone.

4.3 Recommendations from recent reports

A report prepared by the UK's House of Lords (1998) stated that the use of potent agents important to human medicine, such as the fluoroquinolones are acceptable on an individual basis for short-term-therapy for large and companion animals; but mass-treatment of herds of pigs and flocks of poultry with such agents is not best practice from the point of view of human health. In that report the veterinary profession is challenged to address this problem by introducing a Code of Practice for prescription of such compounds.

In several countries principles for judicious use have been introduced and also guidelines for rational and prudent use of antibiotics for therapy in veterinary medicine, thus minimising therapy failure by antibiotic resistance (American Veterinary Medicinal Association, 1998; BgVV, 1997; Copenhagen Recommendations, 1998, Couper, 1997; Deutsche Veterinärmedizinische Gesellschaft, 1999; WHO, 1997 and 1998; OIE-Conference, 1999).

These guidelines recommend the use of antibiotics following different priorities of choice (1st, 2nd, 3rd) for special indications, pathogens or animal species - taking into consideration the regional resistance situation based on resistance monitoring data of the relevant country (Veterinary Antibiotic Policy Working Group of the Danish Veterinary Laboratory, 1997).

In May 1999 the Scientific Steering Committee of the European Commission DG XXIV published an opinion on antimicrobial resistance (European Commission, 1999). This report based on a comprehensive mandate addresses the subject in the fields of human and veterinary medicine, animal husbandry (including growth promoters), agriculture and other aspects covering the environment and genetically modified organisms. The report makes a number of proposals for further research and some key recommendations, which the CVMP will wish to take account of when finalising its recommendations for risk management once this report is adopted.
4.4 **Conclusions**

- The medical and economic consequences of antibiotic resistance are not well documented but are likely to be considerable. This includes increased morbidity and mortality, extra medical expenditures, extended hospital stay or prolonged absence from work. These effects could be more dramatic in the near future if the rise in antibiotic resistance continues at the present pace.
- Although case reports of therapy failure are poorly documented in animals, the increase in prevalence of resistance will inevitably lead to problems with the choice of antibiotics available and their efficacy.
- Prudent use policies are being developed in different Member States. Prudent use of antibiotics can be defined as maximising the effect of an antibiotic whilst minimising the risk e.g. the development of resistance with the aim of continued efficacy and availability of antibiotic products.
Chapter V: TRANSFER OF ANTIBIOTIC RESISTANCE FROM ANIMALS TO MAN

There is little doubt that much of the problems of antibiotic resistance in human pathogens have arisen because of mis- and overuse of antibiotics in human medicine. Examples of this include the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant pneumococci, multidrug resistant *Mycobacterium tuberculosis*, multidrug resistant nosocomial pathogens such as *Pseudomonas aeruginosa* (Cheng et al., 1996) and multidrug and fluoroquinolone-resistant *Salmonella* Typhi (Wain et al., 1997). Nevertheless it is equally clear that some resistant bacteria and resistance mechanisms have arisen as a result of veterinary use of antibiotics and then these have transferred to man.

In general resistance genes and mechanisms may be transferred to man by:

1) Transfer of antibiotic-resistant zoonotic bacteria.
2) Transfer of antibiotic-resistant commensals.
3) A "hit and run" mechanism whereby an antibiotic-resistant animal commensal or pathogen during its passage through the human intestine transfers its resistance genes to human bacteria.

The critical and unanswered question is how much resistance in animals contributes to resistance important in human medicine (Prescott, 1997), the quantification of the potential transfer of resistance and - as a consequence - to possible therapy failures in humans (Piddock, 1996).

Precisely how the transfer occurs is not entirely clear, for example there does appear to be a greater risk of transferring some bacteria by contact with animals (e.g. to farm-workers or abattoir workers) than via food from animal origin such as meat, eggs and milk or vegetable foods such as salads and other vegetables eaten raw which might be vehicle for indirect transfer from the animate or inanimate environment. Criteria for proof of transfer are also quite variable. Some evidence is produced from experimental feeding studies where antibiotic resistant bacteria are fed to animals and their transfer to in-contact humans is observed. Other evidence is obtained by epidemiological studies of antibiotic-resistant zoonotic pathogens in animals and man. There are occasional studies of antibiotic resistant bacteria in food and even fewer studies examining antibiotic-resistant commensals in animals and their transfer to humans by contact or via food. It is only recently that robust genotypic typing technologies have been available to determine the relatedness of bacterial species and to characterise their antibiotic resistance plasmids and genes. Previously, studies have relied on less precise phenotypic characterisation.

From the above it is clear that absolute evidence for transfer or lack of transfer is difficult to obtain. Most often we rely on circumstantial evidence which of course leads to controversies such as that raging at present.

5.1 TRANSFER OF ANTIBIOTIC RESISTANT ZOONOTIC BACTERIA

The pathogens examined here are *Salmonella* (which can also cause disease in animals), *Campylobacter* spp., *Escherichia coli* O157 and other verotoxin producing *E. coli*, *Yersinia* spp., *Listeria monocytogenes* (which is an environmental contaminant) and *Staphylococcus aureus*.

5.1.1 *Salmonellae*

*S. Typhi* and *S. Paratyphi A* are solely human pathogens. There is no animal reservoir; thus antibiotic resistance in these bacteria must have arisen from antibiotic usage in human medicine. Typhoid is not a problem in developed countries, except for the occasional imported infection. In developing countries it is estimated that there are 12.5 million cases per year with over 300,000 deaths. Multiresistant *S. Typhi* have become a major problem in Asia, for example 69% of the blood isolates of *S. Typhi* in Quetta, Baluchistan, carry plasmid encoded resistance to the three readily available antibiotics (Mirza et al., 1996). In addition because of over-use of fluoroquinolones (the only other effective antibiotics) resistance (chromosomally encoded) has arisen in Vietnam (Wain et al., 1997) and a very large outbreak has occurred in Tajikistan with high mortality and morbidity (Murdoch et al., 1998).
Non-typhoidal *Salmonella* (NTS) are food-poisoning bacteria but person-to-person transfer is also possible. There is little doubt that the principal chain of transfer is from colonised animals (mainly poultry) via food to man with some secondary person-to-person spread. In the United Kingdom, illness caused by DT104 (1997: 2820 human infections) has been associated with contact to farm animals and with consumption of foods, including beef, pork sausages, and chicken: The organism has been isolated primarily from cattle, but also from poultry, sheep, and pigs (Altekruse et al., 1997).

Antibiotic-resistant NTS appear to arise in animals with different resistance patterns both in different NTS serovars and different animal species. The evidence of transfer is strong and largely based on epidemiological studies, tracing cases and outbreak descriptions, and has been reviewed previously (Anderson, 1968; Linton, 1986). Recent evidence includes the parallel increases in UK in *S. Typhimurium* isolates from cattle, poultry and man, with the same resistance profiles including to furazolidon, an antibiotic not used in the UK in human and veterinary medicine (Threlfall et al., 1993). Fluoroquinolone resistance has appeared in NTS in recent years temporally related to the use of enrofloxacin in veterinary medicine with a coincident rise in resistance in isolates from man (Frost et al., 1996). However, it must be stressed that there is also evidence that treatment of humans with salmonellosis with ciprofloxacin can select for fluoroquinolone resistance in *S. Typhimurium*, *S. Enteritidis* and *S. Hadar* (Howard et al., 1990; Ouabdesselam et al., 1996). Nevertheless, transfer of resistant NTS from animals to man is likely to be numerically more important than development of resistance during therapy in man (Heisig et al., 1993; Lontie et al., 1994). Although there are extensive data documenting the isolation of multiresistant NTS in animals and a concomitant rise in isolates from food and from man, precise delineation of the chain of transmission via food is usually assumed rather than proven. For example, in an epidemiological study investigating a large increase in isolations of *S. Typhimurium* DT104 in north west England, food questionnaires demonstrated that no single food was associated with acquisition of infection and that ten of the cases had had close contact with ill cattle (Wall et al., 1995). In the USA tetracycline-resistant *S. newport* were found in a beef cattle herd given tetracycline as a growth promoter. Eighteen cases of salmonellosis occurred in people who had eaten beef burgers made from meat from the cattle. The *S. newport* was tetracycline-resistant and phenotypically the same as that in the cattle (Holmborg et al., 1984). However, this did not result in human treatment failure since tetracycline is not used for treatment of salmonellosis.

Feeding experiments have been used to assess the possibility of spread of antibiotic resistant NTS, through a simulated food chain. These experiments showed that antibiotic (kanamycin) administration, to both donor (poultry) and recipients (rats) increased the frequency of transmission of antibiotic resistant *S. Typhimurium* through the simulated food chain (Gast et al., 1988).

### 5.1.2 *Campylobacter*

In human medicine campylobacteriosis should normally only require antibiotic therapy if there is invasive or persistent disease. The agents used for therapy are either fluoroquinolones such as ciprofloxacin or macrolides such as erythromycin. Thus in terms of transfer of resistant *Campylobacter* these are the only resistances studied and very little has been done of macrolide resistance. Again evidence for transfer comes from increasing isolation of quinolone resistant *Campylobacter* spp. from animals, food and humans and its temporal association with the introduction of enrofloxacin into veterinary practice (Endtz et al., 1991; Perez-Trallero et al., 1993). Although there is some controversy over how fluoroquinolone resistance should be assayed (some use the quinolone nalidixic acid) there is little doubt that resistance is increasing (Piddock, 1995).

### 5.1.3 *Escherichia coli* O157 and other enterohaemorrhagic *E. coli* (EHEC)

There is good evidence that *E. coli* O157 and other EHEC colonise farm animals and are transmitted to man via the food chain or directly. Antimicrobial resistance in such bacteria has not been an issue since there is evidence that antibiotic chemotherapy might release more toxin and thus lead to a greater risk of haemolytic uraemia syndrome and most but not all strains are antibiotic susceptible (Schmidt et al., 1998). Nevertheless, such *E. coli* can carry multi-drug resistance plasmids (Kumar et al., 1994) although some of the large plasmids in EHECs are *traT* deficient that cannot be transferred (Hales et al., 1992).
5.1.4 Yersinia enterocolitica

*Y. enterocolitica* is a cause of human gastroenteritis but is rare in the more temperate parts of Europe. The major source of human infection is the pig. Antibiotic resistant *Y. enterocolitica* have been isolated from pigs and man (Kagawa and Iverson, 1993; Reed, 1997). Antimicrobial chemotherapy is rarely if ever indicated in human yersiniosis.

5.1.5 Listeria monocytogenes

This is transmitted via food but is ubiquitous in the environment and it is likely that food could be contaminated at source (i.e. the animal is a carrier) or from the inanimate environment. Although most strains are susceptible, transmissible antibiotic resistance and multiresistance has been found in *Listeria* spp. isolated from food (Facinelli et al., 1991; Slade and Collins-Thompson, 1990). Whether this is related to veterinary use of antibiotics is unclear.

5.1.6 Staphylococcus aureus

*S. aureus* can be transferred to man from animals via food (in particular milk or milk products). MRSA are rare in animals but widespread in humans. For example, in a survey of over 3000 *S. aureus* strains (Lacey, 1988) isolated from man, 13% were resistant to erythromycin and only 1.7% to tylosin (which is not used in human medicine). MRSA are widespread in human medicine but rare in veterinary medicine (Devriese, 1975). In a recent report of 11 isolates of MRSA from dogs, it was considered possible that these had originated from man (Tomlin et al., 1999).

5.2 Transfer of Antibiotic Resistance via Commensals

It is considered likely that the normal enteric flora forms a major reservoir of antibiotic resistance and that, for example, multidrug resistance plasmids can be assembled in such bacteria via transposons and integrons which may then be acquired during passage through the intestine (e.g. Shears, 1993). *E. coli* is the major aerobic Gram-negative bacterium and *Enterococcus* spp. one of the major aerobic Gram-positive bacteria in the normal flora of man and other animals.

5.2.1 E. coli

Large longitudinal studies of antibiotic resistant *E. coli* in the faecal flora of food animals are few, despite this being a recommendation of, for example, the Lamming report (Lamming, 1992). There are some reports often looking at antibiotics that are rarely (neomycin) or never (apramycin) used in human medicine. Nevertheless, kanamycin, which is closely related to neomycin, shares the same resistance mechanism such as phosphorylase APH(3')II and is still used in human medicine. There have however been a number of experimental, observational and epidemiological studies examining the possibility that commensal antibiotic resistant *E. coli* could be transferred from animals to man.

Some data indicate that transfer can occur via contamination of water or vegetables by animal excreta as shown by Guinee et al. (1970) who reported that vegetarians are more likely to exhibit faecal carriage of antibiotic resistant *E. coli* than non-vegetarians are.

Smith (1969 A) showed that it was possible for antibiotic resistant *E. coli* of porcine, bovine or poultry origin to colonise the gut of one volunteer but not well. Levy et al. (1976 A) incorporated tetracycline into the feed of 3 month old chickens. They found that within 36 hours virtually all of the faecal *E. coli* were tetracycline resistant in the antibiotic supplemented group, whereas in those chickens fed unsupplemented feed, the prevalence was less than 10%. Spread of tetracycline resistant *E. coli* to farm staff was detected in 31% within 4 to 6 months of the introduction of supplemented animal feed. Evidence of transfer of plasmid encoded resistance was obtained using a temperature-sensitive plasmid encoded chloramphenicol resistance gene (Levy et al., 1976 B). However, colonisation of humans was rare and short-lived. Others have produced similar results in chicken and cattle feeding experiments (Hirsch et al., 1974; Marshall et al., 1990).
Several studies carried out in the 1970’s have suggested that antibiotic resistant *E. coli* are more frequently found in the intestinal flora of farmers rearing calves, pigs or poultry (e.g. Moorehouse, 1971; Linton et al., 1972; Wells and James, 1973; Fein et al., 1974; Marsik et al., 1975) than the in "normal" population. A number of studies have shown that resistant *E. coli* can be transferred from animals to man and vice versa (Linton et al., 1977). However this has not been the case in all studies (O’Brien et al., 1993). Recent studies have shown that *E. coli* isolates from poultry and in-contact children in rural Kenya do not share the same resistance plasmids and are usually of very different genotypes (Kariuki et al., 1997 and 1999). Studies from the Netherlands have indicated that resistant porcine *E. coli* could be acquired by in-contact humans (Nijsten et al., 1994 and 1996 A / B), however pig farmers tended to carry more resistant bacteria than their pigs.

Apramycin is an aminoglycoside antibiotic used only in veterinary medicine but related to gentamicin and netilmicin which are both used in human medicine. Although apramycin resistance can be mediated by several mechanisms, expression of the often plasmid-encoded enzyme AAC(3)IV can impart high level resistance to apramycin (MIC ≥ 1024 mg/L) but lower level resistance to gentamicin (MIC 32 mg/L) and netilmicin (Salauze et al., 1990; Hunter et al., 1992).

Apramycin-resistant (mediated by AAC(3)IV) *E. coli* (and *Salmonellae*) appeared in farm animals after the introduction of apramycin into veterinary practice (Chaslus-Dancla et al., 1986 A / B; Wray et al., 1986; Salauze et al., 1990; Hunter et al., 1992, 1993, 1994). Apramycin-resistant *E. coli* obtained from pigs and their stockman have been shown to carry similar sized plasmids encoding the AAC(3) IV enzyme (Hunter et al., 1994). Apramycin resistance was detected in hospital isolates from Belgium (Chaslus-Dancla et al., 1989), a comparative study of plasmids encoding this resistance in human and animal isolates demonstrating a high genetic homology between some of them (Chaslus-Dancla et al., 1991) and there has also been an increase in the proportion of gentamicin- (and apramycin-) resistant *E coli* expressing AAC(3)IV isolated from human urinary tract infections in Liverpool (Hunter et al., 1993).

Although these data suggest that apramycin-resistance has originated in *E. coli* in farm animals and spread to man, it is possible that the opposite is true. Especially since the aminoglycoside modifying enzyme AAC(3)II, which mediates gentamicin resistance appeared in human strains prior to the introduction of apramycin or gentamicin into veterinary practice and was later detected in bovine strains (Chaslus-Dancla et al., 1987).

### 5.2.2 Enterococcus spp.

Enterococci are part of the normal gut flora of many animal species. They are opportunistic pathogens in man, rather than primary pathogens, causing infections in intensive care units, transplant and haematology wards. They are responsible for 5 to 10% of nosocomial bacteraemia. Enterococci tend to be more resistant than other streptococci (e.g. they are intrinsically resistant to penicillin and cephalosporins) and seem to acquire resistance readily (e.g. to gentamicin and glycopeptides). The current debate rages over how vancomycin resistant enterococci have arisen. Glycopeptide resistance was first encountered in European hospitals in UK and France and most vancomycin resistant enterococci (VRE) are *E. faecium* although other VRE such as *E. faecalis* and *E. durans* have been described (Arthur and Courvalin, 1997). Four phenotypic variants of VRE have been found (VanA, B, C and D). The VanA phenotype is transferable, imparts inducible resistance to vancomycin and teicoplanin and is found in a wide variety of *Enterococcus* spp. VanB is also transferable, imparts inducible resistance to vancomycin but not to teicoplanin and has been found in *E. faecium* and *E. faecalis*. VanC and VanD are similar in imparting constitutive resistance to vancomycin but differ in the host species. The VanA phenotype is the most highly prevalent VRE in UK (Morrison et al., 1997) and the only one present in humans, farm animals and food (Witte and Klare, 1995; McDonald et al., 1997). The VanA phenotype is encoded on a 10 kb transposon, Tn 1546 (Arthur et al, 1993). In human medicine there is concern over problems of treating VRE infections (which are increasing) and a possible transfer of vancomycin resistance to MRSA (which has been demonstrated in vitro). The area of controversy rages at the ability of the growth promoter avoparcin, which is a glycopeptide antibiotic to select for vancomycin resistance in animals and its spread to humans. However, like other forms of antibiotic resistance evidence for this is not complete and it should be borne in mind that neither
avoparcin nor vancomycin or any related glycopeptide have been used in veterinary therapy or under veterinary prescription.

- Are VRE present in food animals in the EU?
  Various surveys have revealed carriage prevalences of 0 to 92% of VRE in poultry, pigs, cattle, horses, cats and dogs (Kruse, 1995; Devriese et al., 1996; Van Belkum et al., 1996; Bates, 1997; DANMAP, 1997; Van den Bogaard et al., 1997).

- Is the presence of VRE in animals related to avoparcin administration?
  In Denmark the prevalence of VRE in broilers has declined from 80 to 10% since the ban on avoparcin in 1995, but the level in pigs has remained at 20% (DANMAP, 1997). In Sweden where avoparcin has not been used, VRE was not detected in pigs or poultry (Greko, 1997). In Norway there was a highly significant association (odds ratio: 104) between the use of avoparcin on farms and the presence of VRE (Kruse, 1995). In USA where avoparcin is not used VRE with the VanA phenotype have not been detected in animals or food (McDonald et al., 1997).

- Are VRE present on food for human consumption?
  A number of studies have found VRE on raw meats in UK (e.g. Bates et al., 1994; Casewell et al., 1996; Chadwick et al., 1996), Denmark (Wegener et al., 1997), Finland (Tast et al., 1997), Germany (Witte, 1997; Klein et al., 1998) and Holland (Van den Braak et al., 1998) but not Sweden (Quednau et al., 1998).

- Are VRE causing human infections acquired from animals via the food chain?
  Here the evidence becomes less clear-cut. In the USA there are major problems of nosocomial VRE infections. Intestinal carriage of VRE in non-hospitalised patients has not been detected (McDonald et al., 1997). VRE have not been isolated from food in USA, which has been attributed to not using avoparcin as a growth promoter in USA. Nevertheless, there is recent evidence of VRE being present in animal-food (Schwalbe et al., 1999). In USA it thus appears that VRE causing infections in humans is linked to the use in human medicine of vancomycin (e.g. oral administration in pseudomembranous colitis) and highly potent cephalosporins (Morris et al., 1995). The widespread use of cephalosporins appears as a risk factor for being colonised / infected with VRE as their usage might select for enterococci, which are intrinsically resistant to cephalosporins.

In Europe however, links between the increase in cases of human VRE and the increased prevalence of VRE in farm animals and food have been postulated. The evidence for such a link is circumstantial. In general the VRE from humans, animals and food are diverse both at the species and genotypic levels (Klare et al., 1995; Casewell et al., 1996). Some authors have suggested that the Tn1546 transposon might have arisen under avoparcin pressure and then disseminated to “human” enterococci. However, there is considerable heterogeneity among VanA determinants and a constant correlation between such determinants in animal and human VRE has not been found (Palepou et al., 1998).

### 5.3 "Hit and Run" Mechanism

There is good evidence that antibiotic resistance genes can be transferred between commensals, from commensals to pathogens and vice versa in the intestines of chickens (Sansonetti et al., 1980), cattle (Hunter et al., 1992), mice (Jones and Curtiss, 1970) and men (Anderson, 1975). There is evidence to show that animals' strains of E. coli could transfer resistance plasmids to commensal E. coli in the human intestine (Smith, 1969 A). However, there are no studies to address the question of how frequently this occurs.
5.4 **CONCLUSIONS**

- There is good evidence that antibiotic-resistant non-typhoidal *Salmonellae, Campylobacter* spp. and *enterohaemorrhagic E. coli* (e.g. O157) can be transferred to man from animals via the food chain, although there is also evidence that some antibiotic resistance in some of these pathogens can arise during therapy of the infection in man. The transfer of antibiotic-resistant commensal bacteria from animals to man via the food chain is also possible, but there are conflicting data. There are no data showing how important such a transfer is or whether transfer occurs via the food chain or via contamination of water or vegetables by animal excreta (Corpet, 1988). Again the "hit and run" mechanism of transfer is possible but there are no data on its relative importance.

- Animals undoubtedly represent a source of antibiotic-resistant microorganisms for humans (and vice versa) and a prudent use of antibiotics is therefore needed in veterinary as well as in human medicine. Both doctors and veterinary surgeons need clear guidelines to avoid undesirable selection of resistant microorganisms and a further expansion of the general pool of antibiotic-resistance genes. However, the respective contributions of animals and human therapy to the medical problems resulting from antibiotic resistance cannot presently be assessed. As emphasised by a medical author "by focusing on (antibiotic) use in animals, we might ignore overuse in humans" (Goossens, 1997).
CHAPTER VI: SCIENTIFIC RISK ASSESSMENT

In order to assess the risk of antibiotic resistance in animals to public health it is necessary to carry out a scientific risk assessment. Risk assessment is part of a wider procedure called risk analysis. The components of risk analysis are hazard identification, risk assessment, risk management and risk communication.

**DEFINITIONS**
- A hazard is something potentially harmful.
- A risk is the probability that a hazard will cause a specific outcome.
- Risk Assessment is the process of estimating objectively and transparently the probability that an identified hazard will result in an unwanted outcome.
- Risk Management is the systematic process of defining the acceptable risk and deciding the most appropriate level of protection based on the highest possible level of consensus.
- Risk Communication is the dialogue about risk and benefits concerning certain problems and possible measures between stakeholders.

For the assessment of the risk of transmission of resistance in the food chain to humans the following aspects need to be addressed (OIE, 1999):
- History of antibiotic use in the animals;
- Prevalence of resistance in animals;
- Presence of animal bacteria in food products and transmission by the food chain;
- Transfer of resistance genes to human bacteria;
- Ability of animal bacteria to colonise humans;
- The outcomes of risk assessment could include information on
  - Additional prevalence of resistant bacteria in humans
  - Additional infections in humans due to resistant bacteria
  - Additional morbidity and mortality

Risk assessment can be quantitative or qualitative. Because a quantitative risk assessment takes much time and resources, the ARWP has asked Dr. M. Wooldridge from the Department of Risk Research, Veterinary Laboratories Agency (Weybridge), UK, to conduct a qualitative risk assessment on the following question:

“**What is the risk of adverse human health effects in the EU, consequent upon the development of antibiotic resistance to (fluoro)quinolones in S. Typhimurium which is due specifically to the use of (fluoro)quinolones in livestock**”

The report of Dr. M. Wooldridge is added as annex 4. It describes in detail all aspects of a qualitative risk assessment, based on data obtained from the present report and the scientific literature. A summary of the conclusions is:

- The probability of (fluoro)quinolone resistant *S. Typhimurium* in animals due to use of (fluoro)quinolones in animals is low, with a high degree of uncertainty;
- The probability of a food item of animal origin to be contaminated with (fluoro)quinolone resistant *S. Typhimurium* is very low, although variation with animal species and country exists, and a high level of uncertainty surrounding that estimate;
- The probability of a randomly selected human being in the EU being infected with *S. Typhimurium* is low. Incidence rate varies from 0.002 to 0.055 per 100 humans per year;
- The probability that a human isolate of *S. Typhimurium* is (fluoro)quinolone resistant varies with country (around 1%), but this value is dynamic and may increase in time;
- The probability that in case of salmonellosis due to resistant strains in humans antibiotic treatment is necessary varies from 10 - 36%, with wide uncertainty limits;
- The probability of adverse health effects in humans due to the presence of (fluoro)quinolone resistant *S. Typhimurium* derived from farm animals is low, but with a high degree of uncertainty and much variation by country and animal species.
CHAPTER VII: CONCLUSIONS

- All antibiotics used in veterinary medicine except for pleuromutilins are related to or identical to human medicinal products and can select for cross-resistance or co-resistance.
- Information on amounts of antibiotic agents used in animals in the EU is, with few exceptions, not available.
- Where data on amounts of antibiotics is available, it is presented as kg active substance per country. Adequate denominator data, such as consumption pattern of an antibiotic in a specific sector of food animals is lacking.
- Although national antibiotic resistance monitoring systems exist within the EU, the majority are not specifically aimed at antibiotic resistance development in animal pathogens or zoonotic and commensal bacteria in animals.
- Antibiotic susceptibility testing is carried out in veterinary diagnostic laboratories in all EU Member States. However, the results are difficult to compare for the following reasons:
  - Differences in methods exist between laboratories and countries;
  - Most results are presented qualitatively in categories (resistant - intermediate - sensitive), often without information on interpretative criteria (e.g. breakpoints);
  - Little information is available on quality control in these laboratories;
  - No standard sampling strategies are used;
  - Bacterial strains included in the reports are generally clinical isolates and the results represent a worst case scenario. This complicates trend analysis, and the data can only be used as an early warning system within one country.
- In all EU Member States, Salmonella Reference Laboratories test susceptibility of Salmonella spp. isolated from animals and humans to antibiotics. The data are often presented qualitatively in categories.
- Although the data available are few and not easily comparable, a trend towards increasing resistance has been noticed in some zoonotic and pathogenic bacteria. Data on susceptibility of commensal bacteria are generally lacking.
- Antibiotic resistance in humans has lead to increased hospitalisation and costs of therapy. Examples of increased therapy failure as a result of infections with (multi)resistant bacteria in hospital settings have been described.
- The effect of resistance on efficacy of antibiotic products in animals is poorly documented.
- In scientific literature evidence exist of transfer of resistant Salmonella and Campylobacter from animals to humans via the food chain. The main public health risk is the illness caused by these zoonotic bacteria. The emergence of fluoroquinolone resistance in these bacteria is of particular concern and may add to the risk.
- In scientific literature limited evidence exists for transfer of resistance from commensal gut flora in animals to human bacteria. For Enterococci this is circumstantial.
- Quantitative risk assessment of public health risk of antibiotic resistance as a result of the use of antibiotics in animals, properly performed with a well defined model, would provide quantitative information on all aspects that contribute to the final risk. It would thus provide useful information for potential intervention by regulatory authorities.
- Calculated risks will never be zero and will be dynamic. This means that acceptable threshold levels need to be defined for each class of antibiotics and that the calculated risk needs to be re-evaluated.
CHAPTER VIII: RECOMMENDATIONS

The use of antibiotics leads sooner or later to the selection of resistance. This principle accounts for all types of use of antibiotics, whether in human or veterinary medicine, growth promotion in animals or as agricultural or horticultural use.

Based on this report the authors recommend that models are developed to assess the risks of antibiotic resistance occurring in animals and its potential transfer to man. To establish these models further information is necessary such as:

- Regular monitoring of usage of all antibiotic agents in animals in all EU Member States. The data should be presented as kg active substance used in each animal species and specified for each type of animal production, e.g. laying hens, broilers etc.
- National programmes for monitoring antibiotic resistance in animals. Each national programme should include: major food producing species; zoonotic bacteria, animal pathogens and commensal gut flora; random sampling strategies to ensure appropriate statistical analysis of the data; representatives of all relevant classes of antibiotics; standardised methodology.

In addition to this the authors make the following recommendations:

- The authorisation of veterinary medicinal products should take account of pre-marketing surveillance for antibiotic resistance and ensure that recommended dosages are optimum to minimise the development of resistance.
- Susceptibility patterns of populations of target bacteria may need to be monitored post authorisation, on a case by case basis, in accordance with risk management procedures to be agreed by the CVMP.
- Strategies should be developed to maintain efficacy of the veterinary antibiotic product, to ensure the maintenance of animal health and reduce the potential risk to public health.
- The authors endorse the development of prudent use policies in the EU. These policies should include guidelines for antibiotic therapy in animals to ensure continued efficacy and to minimise the risk to public health. In addition such policies should be enshrined in a consistent manner in the risk management recommendations to be considered by CVMP as a result of the findings of this report. These should apply equally throughout the community to antimicrobial products authorised centrally and by the mutual recognition procedure.
LIST OF ABBREVIATIONS AND TERMS

ACSSuT Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines
ACSSuTNal Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines, nalidixic acid
ACST Resistance to ampicillin, chloramphenicol, streptomycin, tetracyclines
ACSuT Resistance to ampicillin, chloramphenicol, sulphonamides, tetracyclines
ACT Resistance to ampicillin, chloramphenicol, tetracyclines
ARWP CVMP Ad Hoc Working Party on Antimicrobial Resistance
AU Austria
BE Belgium
BgVV Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (Germany)
C. Campylobacter
CVMP Committee for Veterinary Medicinal Products
DE Germany
DK Denmark
DT104 Salmonella Typhimurium definite phage type 104
E. coli Escherichia coli
E. faecalis Enterococcus faecalis
E. faecium Enterococcus faecium
e.g. exempla gratia (Latin: for instance)
EHEC enterohaemorrhagic E. coli
EL Greece
EU European Union
F France
FEDESA Fédération Européenne de la Santé Animale
FIN Finland
i.e. id est (Latin: that is to say)
IRE Republic of Ireland
IT Italy
K99 enterotoxigenic E. coli expressing the K99 (F5) adhesion factor
LUX Luxembourg
MIC Minimum Inhibitory Concentration
MRL Maximum Residue Limit
MRSA Multi-resistant Staphylococcus aureus
N Norway
NL The Netherlands
NTS Non-typhoidal Salmonella
O157 E. coli expressing the somatic antigen O157
OIE Office International des Epizooties
PHLS Public Health Laboratory Service (UK)
PO Portugal
S. aureus Staphylococcus aureus
S. Salmonella
SP Spain
spp. subspecies
SW Sweden
UK United Kingdom
VRE Vancomycin Resistant Enterococci
WHO World Health Organisation
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