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Antibiotic Usage in Animals
Impact on Bacterial Resistance and Public Health

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Abstract

Antibiotic use whether for therapy or prevention of bacterial diseases, or as performance enhancers will result in antibiotic resistant micro-organisms, not only among pathogens but also among bacteria of the endogenous microflora of animals. The extent to which antibiotic use in animals will contribute to the antibiotic resistance in humans is still under much debate. In addition to the veterinary use of antibiotics, the use of these agents as antimicrobial growth promoters (AGP) greatly influences the prevalence of resistance in animal bacteria and a poses risk factor for the emergence of antibiotic resistance in human pathogens. Antibiotic resistant bacteria such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp. and enterococci from animals can colonise or infect the human population via contact (occupational exposure) or via the food chain. Moreover, resistance genes can be transferred from bacteria of animals to human pathogens in the intestinal flora of humans.

In humans, the control of resistance is based on hygienic measures: prevention of cross contamination and a decrease in the usage of antibiotics. In food animals housed closely together, hygienic measures, such as prevention of oral-faecal contact, are not feasible. Therefore, diminishing the need for antibiotics is the only possible way of controlling resistance in large groups of animals. This can be achieved by improvement of animal husbandry systems, feed composition and eradication of or vaccination against infectious diseases. Moreover, abolishing the use of antibiotics as feed additives for growth promotion in animals bred as a food source for humans would decrease the use of antibiotics in animals on a worldwide scale by nearly 50%. This would not only diminish the public health risk of dissemination of resistant bacteria or resistant genes from animals to humans, but would also be of major importance in maintaining the efficacy of antibiotics in veterinary medicine.

Since the 1970s many reports have been published about multiresistant bacteria, but it has only been recently reported that organisms resistant to all clinically available antibiotics have emerged.[1,2] Despite the fact that hospitals are considered to be major breeding grounds of resistant bacteria, it is becoming accepted that the much larger populations of healthy people living outside hospitals might be an important reservoir of resistant genes as well. Most antibiotics are used outside hospitals, and not only in humans but also in animals.[3] Antibiotic use in animals, as in humans inevitably selects for resistant bacteria. The proliferation of antibiotic resistance genes on transposons and plasmids in animal populations is a direct result of the widespread use of antibiotics in animals and is increased by the ease of dissemination of resistant strains between animals via faecal contact, espe-

cially among animals housed together in large groups, as with intensive farming units.[4,5]

In domestic animals antimicrobial agents are used for 3 major purposes: therapy to treat an identified bacterial infection, prevention of bacterial infections in animals at risk, or as feed additives to enhance performance. Veterinary antibiotic therapy involves treatment of an individual animal or a group of sick animals with one or more antibiotics during a defined period of time and, in most Western countries, only upon prescription from a veterinarian. Similarly, antibiotics can be prescribed by a veterinarian for a defined period of time to prevent bacterial infections in a group of animals at risk or to prevent the spread of an existing infection in a herd. In the situations when antimicrobials are used as therapy or for prevention of disease in a group of animals, antibiotics are mostly dissolved in the drinking water or milk, or mixed in the feed. This is called group or mass medication.

Despite the fact that much attention is being paid to hygiene and prevention of infectious diseases, intensive animal production depends heavily on the usage of antimicrobial agents for veterinary purposes. Apart from veterinary use, antibiotics are added continuously to the feed of animals used as a food source (i.e. 'food animals') for humans (e.g pigs, poultry and nonruminating veal calves), to enhance their performance and increase growth. In this situation they are called antimicrobial growth promoters (AGP). The term growth promoter is used for feed additives, other than dietary nutrients, which increase growth rate and/or improve feed efficiency in healthy animals fed a balanced diet.[6] AGP are more effective in young than in older animals and the general opinion is that the observed growth and feed efficiency responses to the use of AGP are lower under optimal hygienic and animal husbandry conditions compared with poorer environments.[7] However, even under optimal conditions, the positive effects on growth rate and feed efficiency are between 2% and 4%.[8] As a consequence of the improved feed efficiency, the amounts of waste products excreted in faeces and urine by the animals are lowered in proportion to the decreased amount of feed consumed by the animals, that is, by approximately 3 to 4%.[9]

From an economic point of view, responses in feed efficiency are of greater importance than growth improvement or decreased waste production. The mode of action of AGP has been the subject of numerous scientific papers, but most of these deal with effects rather than modes of action. An early and fundamental finding was that germfree animals, that grow approximately 20% faster than conventionally-reared animals, do not respond to dietary inclusion of AGP.[10] Therefore, the growth promoting activity of AGP seems to be related in one way or another to effects on or mediated by modulation of the intestinal flora of the exposed animals. Recently, several reviews on the possible modes of action of AGP have been published.[7,11,12] Other names for AGP are antimicrobial performance enhancers, antimicrobial feed additives, feed savers, digestion enhancers or intestinal flora modulators.

The use of antibiotics for veterinary purposes is strictly regulated in most countries in a similar way to pharmaceuticals for humans. AGP, however, are regulated separately and differently, even if exactly the same drug is also registered for therapeutic use. Despite the fact that APG are not intended for and not registered for prevention of bacterial diseases, a part of their positive effect is most likely caused by prevention or suppression of bacterial infections as was claimed already by the discoverer of the growth promoting effect of antibiotics.[13] This might be an explanation of why they are more effective in young animals and when used under suboptimal conditions. As many people are not very familiar with the use of antibiotics as growth promoters and with the special agents used for this purpose, special attention will be paid in this review to the use of antibiotics as AGP, that is, the compounds used and their antibacterial activity, the effect of their use on the resistance of bacterial flora of animals, and the risk of transfer of resistant bacteria or their genes from animals to humans.

1. Antimicrobial Growth Promoters

Originally, therapeutic antibiotics like tetracyclines and penicillins were used as AGP, but in lower doses than those required for therapeutic use.[14] In the US, oxytetracycline is still used, but for therapeutic applications it is mixed in pig feeds in concentrations between 200 to 800 mg/kg. For AGP use, it is administered in concentrations between 50 to 200 mg/kg. The quinoxaline antibiotics, carbadox and olaquindox, are used in the US in pig feeds at doses of 50 to 55mg per kg for therapy, and at doses of 10 to 25mg per kg as AGP.[15] Therefore, the use of antibiotics as AGP is sometimes referred to as the 'subtherapeutic use of antibiotics'. In contrast, in the European Union (EU) and many other countries, drugs that have been registered for therapeutic use in humans and/or animals are not allowed to be used as AGP. However, many of the compounds used as AGP are analogues of, and show cross resistance with, therapeutic antibiotics. Table I shows the most important compounds authorised as AGP in the EU, together with the concentrations in which they are added to animal feeds and the minimum inhibitory concentration (MIC) range of susceptible indicator bacteria, namely, *Enterococcus* spp., *Clostridium perfringens* and *Escherichia coli*.[15-18]

For AGP that are not used for therapy, no official breakpoints to differentiate between resistant and susceptible strains have been established. As cross resistance to therapeutically used antibiotics occurs, the break-points of the therapeutic counterpart have been used. Adsorption from the intestinal tract of most of these drugs is very limited or nonexistent, therefore, the concentrations used are high enough to ensure that the levels of AGP reached in the intestinal tract of exposed animals are inhibitory to susceptible bacteria, as shown in table I. This inhibitory effect is confirmed by the preventive effect of AGP against certain intestinal diseases in animals such as necrotic enteritis in poultry (which is caused by *Clostridium perfringens*), swine dysentery (a contagious diarrhoea in pigs caused by *Serpulina hyodysenteriae*), and weaning diarrhoea in swine caused by *E. coli*.[15,19]

In Sweden, which prohibited the use of AGP in 1986, a higher incidence of bacterial infections in poultry and pigs was observed immediately after the ban, with a resulting increase in the veterinary use of antibiotics by approximately 20%.[20] However, improvement of hygiene and animal husbandry systems, and reformulation and modification of feeds has solved nearly all of these problems. Consequently, the incidence of clostridia enteritis in poultry in Sweden is now much

Table I. Authorised antibacterial performance enhancers

Compound			Minimum inhibitory concentration range (mg/L)	
Name	Chemical group	Dose in animal feeds (mg/kg)	Clostridia	Enterococci
Avilamycin	Everninomycins	18-40	<0.25-0.5	0.06-0.075
Avoparcin[a]	Glycopeptide	5-100	0.5-2	1-2
Bacitracin	Polypeptides	5-100	<1-4	<0.5-16
Flavomycin	Bambermycins	1-25	<1-8[b]	0.25-4
Monensin	Ionophore	10-40	0.5-4	1-2
Spiramycin	Macrolide	5-80	0.25-8	0.5-4
Tylosin	Macrolide	4-40	<1	1-4
Virginiamycin	Streptogramins	5-80	0.25-1	0.25-8
Salinomycin	Ionophore	15-60	No data	0.5
				Escherichia coli
Carbadox	Quinoxaline	20-50	≤0.25	≤2
Olaquindox	Quinoxaline	15-50	No data	≤16

a Registration temporarily suspended.

b *Clostridium perfringens* is naturally resistant.

lower than before the ban on AGP. On pig farms, mainly those with a suboptimal management, there are still problems with bacterial infections in recently weaned pigs. Zincoxide is now added routinely to the feed of young pigs for a short period after weaning for the control of these infections. As a result, the total amount of antibiotics used in animals is now (10 years after the ban on AGP), 55% lower than that before the ban. Furthermore, the total amount of antibiotics used for veterinary prescriptions decreased by 33%, despite an increase in the total number of animals.[20] This decrease in antibiotic use was reflected in a recent study in which faecal samples of Swedish pigs were compared with samples of Dutch pigs for the prevalence and degree of resistance of faecal indicator bacteria: enterococci and *E. coli*. The prevalence and degree of resistance was not only significantly lower for AGP, but also for veterinary administered antibiotics.[21]

Despite their undisputed economic value in animal husbandry, there is much concern about the effect of AGP on the selection of resistant bacteria and resistance genes, not only in zoonotic pathogens, but also in the endogenous microflora of animals, as these bacteria might reach humans either directly or via foods of animal origin.[22] According to the advice of the Swann committee in 1968[23] (adopted by most European countries at that time), no antibiotics should be used as AGP which are also (or closely related to) therapeutically used antibiotics. In the EU, this is only true for monensin, salinomycin, flavomycin and quinoxalines. Other compounds such as spiramycin and bacitracin are used in human medicine, and avoparcin, tylosin and virginiamycin are closely related analogues of valuable human therapeutic antibiotics: vancomycin, erythromycin and pristinamycin, respectively.

Moreover, older agents, rejected for therapy and presently used as AGP, are being used as templates for the development of new antibiotics for the treatment of infections with multiresistant bacteria.[24] For example, everninomycin (SCH-27899) is a new antibiotic being developed for treatment of multiresistant cocci, derived from avilamycin by chemical modification of the molecule.[25,26] The mode of action of these new human therapeutics is similar to the AGP ancestor molecules and they show complete cross resistance. As mentioned before, the concentrations in which AGP are used, are high enough to reach levels well above their MIC in the gut. Therefore, the use of AGP results in bacterial subclones or subpopulations resistant against these AGP and related therapeutic compounds.

2. Amount of Antibiotics Used in Animals

Accurate figures on the antibiotic use in humans or animals are hard to obtain. Most data on antimicrobial use in humans and animals are based on the monetary value of sales of antibiotics per annum and show that use in humans is considerably higher.[27] However, as antibiotic use in humans is more expensive per gram of active compound than veterinary antibiotics and certainly than AGP, this might be very misleading.[28] It has also been speculated that the overall use in humans is at least 4 times that in animal husbandry and veterinary med-

Table II. Veterinary use of antibiotics in the Netherlands in 1990 (300 000 kg/year)

Animals	% of total	No. of animals ($\times 10^6$)	Total weight of animals (kg $\times 10^6$)	Total amount of antibiotics used[a] (mg/kg/year)
Pigs	42	15	1000	125
Poultry	26	100	180	430
Cattle	30	5	1800	55
Average[b]				100
Other domestic species	2	No data presented	No data presented	No data presented

a Active substance.

b Average of antibiotic usage in food animals.

icine. This assumption is based on figures that, in humans, most antibiotics are used in individuals below the age of 15 years and above 65 years,[29] and that the older age group does not exist in food animals but is only limited to pet animals. Therefore, it was thought likely that antibiotic use in humans contributes more to resistance in the bacterial population than does nonhuman or veterinary use.[30] However, compared on a bodyweight basis the antibiotic consumption by animals might be as high or higher, because the lifespan of food animals is short, for example, for broilers approximately 7 weeks, and for fattening pigs 6 months. This means that most of the animals present in a population belong to the young age group that is more susceptible to bacterial infections, similar to humans below the age of 15 years. Moreover, in small animals like poultry, the dosage per kg bodyweight is much higher than that in humans.

According to the European Federation of Animal Health Industries (FEDESA),[1] the World Animal Health Product Market was, at manufacturers prices, 11 billion Euro in 1995, of which 44% were therapeutic pharmaceutical products and 41% feed additives. The European market was 3.3 billion Euro of which 48% were therapeutics and 37% feed additives. In 1993, it was estimated that 42% of all pharmaceuticals used in animals worldwide were AGP and 18% therapeutic antibiotics.[31] Prescott[32] considered that some 40% of the total antibiotic production in the US was for animal production, and even 55 to 60% of the production of benzylpenicillin (penicillin G) and tetracyclines was used in food animal feeds. Levy[33] agreed with the figure of 40% of total production, which he considered to be 23 million kg. He also reported that more than 80% of all tetracycline and benzylpenicillin use were in subtherapeutic doses mixed in animal feeds as AGP.

In the EU and Switzerland in 1997,[2] 10493 tons of antibiotics (active ingredient at 100% purity) were used and of this amount nearly 50% was used in animals: 3474 tons (33%) were used in animals for therapy and prevention, and 1590 tons (15%) as AGP. However, large differences in antibiotic use exist between countries. In Belgium, Denmark, France, Ireland and the Netherlands (countries with a large intensive animal farming industry), the use of AGP was of the same size of order as the veterinary use of antibiotics. In Austria, AGP use was 3 times higher than the therapeutic use in animals. In Sweden and Finland, no AGP (<1%) were used, and in Germany, Greece, Italy, Portugal, Spain and the UK, AGP use was considerably lower (15 to 50%) than veterinary use.

These figures are not stable, but tend to change over time. In Finland in 1995 for example, 14 tons of antibiotics were used as AGP and 14 tons for therapeutic reasons in animals (approximately 30% of the human use). In Denmark in 1995, these figures were 94 and 50 tons, respectively.[34] In Norway, figures were 25 000kg in humans and 50 000kg in animals. In 1990 in the Netherlands, 80 000kg of antibiotics were used on medical prescription for a population of approximately 15 million people. This is equivalent to 60 million defined daily doses or 100mg active substance/kg bodyweight/year.[28] The veterinary usage was 300 000kg, which also equated to 100mg active substance/kg bodyweight/year, as presented in table II. The additional use of antibiotics as AGP in animal feeds was estimated (no official figures could be obtained) to be approximately 300 000kg.[28] These figures show that the selection pressure exerted by the veterinary use of antibiotics on the animal bacterial population in the Netherlands is of the same order as medical usage. However, this selection pressure is more than doubled in intensively reared animals because of the use of antibiotics as AGP.

The literature on resistance against AGP is very limited as most of these compounds are not used for therapy, susceptibility testing is not regularly performed, and no recognised breakpoints to differentiate between susceptible and resistant microorganisms exist. Therefore, AGP that are not com-

1 Fedesa Animal Health Dossier 14. Facts and figures about the European Animal Health Industry 1997.

2 Pres. release FEFANA at the invitational EU-conference 'The Microbial Threat' Copenhagen, Denmark, 1998.

monly used in human or veterinary medicine will be discussed briefly.

3. Antimicrobial Growth Promoters

3.1 Avoparcin and Ardacin

Avoparcin and ardacin are glycopeptides and closely related to vancomycin and teicoplanin. Avoparcin has been widely used as AGP in the EU, but has never been registered in the US. However, the EU has suspended the use of avoparcin and ardacin, which was just being introduced, in 1997. Both agents are active against most aerobic and anaerobic Gram-positive bacterial species, but not against most Gram-negatives as these drugs are unable to pass through the outer membrane of these bacteria.[35]

Glycopeptides are compounds of 2 or more substances with similar molecular structures, produced by a variety of soil bacteria. Lactobacilli are intrinsically resistant to glycopeptides. The use of avoparcin selects for an acquired high level resistance in enterococci, that is mostly mediated by the *VanA*-gene cluster located on a transposon Tn 1546 or a self transferable plasmid.[35-37] Transfer of the *VanA*-gene cluster has been shown *in vitro* from *Enterococcus faecium* to *Listeria monocytogenes*, *Staphylococcus aureus* and several *Streptococcus* spp. with frequencies ranging from 10^{-6} to 10^{-9}. Transfer from *E. faecium* to *E. faecium* was more efficient with a frequency of $1:10^{-4}$.[38] The *VanB*-gene cluster is transferable either via Tn 1547 or via plasmids at a low frequency,[39-41] and is induced by vancomycin, but not by teicoplanin or avoparcin,[42] which is consistent with the fact that there are no reports that avoparcin use selects for VanB resistance genes. Transfer *in vivo* of glycopeptide, erythromycin and chloramphenicol resistance on the skin of nude mice from *Enterococcus faecalis* to *S. aureus* has been described by Noble et al.[42]

Use of avoparcin results in a wide spread prevalence of enterococci with high level VanA-mediated resistance to vancomycin (VRE) and other glycopeptides in the enterococcal flora of exposed animals and their environment.[43-45] However, the lack of earlier data precludes conclusions on whether the resistance trait was present in animal populations before or at the time of introduction of avoparcin in animal husbandry,[19] but in countries where avoparcin has never been used, such as the US, or where its use has been suspended for several years as in Sweden, no VRE have been found in the intestinal flora of food animals or healthy people outside the hospital environment.[46,47] The presence of *VanC*, conferring low level resistance is species-specific for *E. gallinarum* and *E. casseliflavus*.[48] More recently, the *VanD* gene which confers low level transferable resistance to vancomycin has been described in enterococci.[49]

3.2 Tylosin, Spiramycin and Virginiamycin

Tylosin and spiramycin, are macrolide antibiotics, while virginiamycin belongs to the streptogramins. Both groups of antibiotics are produced by *Streptomyces* spp. Macrolides and streptogramins are not chemically related, but are often grouped together because of similar antibacterial spectra and mode of action. The lincosamides (linco- and clindamycin) are often included for the same reason, leading to the acronym MLS antibiotics (*m*acrolides, *l*incosamides and streptogramins). They all inhibit protein synthesis by binding to the 50S subunit of the bacterial ribosome and are active against a wide range of aerobic Gram positive bacteria and obligately anaerobic bacteria.[15] Most aerobic Gram-negative bacteria are not susceptible because their outer membrane is not permeable for MLS antibiotics.

Lincosamides are not used as AGP. Tylosin and spiramycin, are commonly used as therapeutic agents in veterinary medicine, and as AGP. They are in contrast to most other AGP readily absorbed from the gut. Virginiamycin is not absorbed and in most countries only used as AGP, but in a few countries it is utilised for therapy in animals or humans. An analogue compound, however, quinupristin-dalfopristin has recently been introduced as an alternative for treatment of multiresistant and/or glycopeptide-resistant *E. faecium*. *E.*

faecalis is intrinsically resistant to streptogramins,[50] and another new streptogramin efepristin (RPR106972) is currently being evaluated for oral medication in humans.[51] Virginiamycin is effective for therapy or prevention of clostridial infections in animals.[15] All streptogramins (also called pristinamycins) are a mixture of 2 different groups of molecules: A and B, which are both bacteriostatic, but act synergistically to express bactericidal activity. Acquired resistance to MLS antibiotics is due to alteration of the ribosomal target, enzymatic inactivation and active efflux.[52,53] Two genes *SatA* and *vgb* encoding streptogramin resistance in *E. faecium* have been detected in streptogramin resistant clinical isolates of *E. faecium*[54] and in faecal isolates from healthy individuals and food animals.[55] *SatA* encodes for resistance to the streptogramin A components while *vgb* confers resistance against the streptogramin B components. *Erm*-genes not only confer resistance to streptogramin B but also to all MLS antibiotics.[56,57]

Transfer of pIP811, a self transmissible plasmid carrying the *ermB*-gene, and also resistance to chloramphenicol, tetracycline and streptomycin from *L. monocytogenes* to *E. faecalis* and vice versa has been observed.[58] Moreover, transfer of Tn 1545, a chromosomally located transposon carrying resistant determinants to macrolides, lincosomides, streptogramin B (*ermB*), kanamycin (*aphA3'*) and tetracycline (*tetM*) from *E. faecalis* to *L. monocytogenes* has been described both *in vitro* and *in vivo* in mice.[59]

3.3 Avilamycin.

Avilamycin, a mixture of oligosaccharides of the orthosomycin group produced by *Streptomyces viridochromogenes*, is mainly active against Gram-positive bacteria by binding to the 30S subunit of the bacterial ribosomes.[60] Other members of this group include curamycin and the everninomycins. Avilamycin has never been used for therapeutic purposes, but a related compound everninomycin (Ziracin®) is being developed for therapeutic use in humans.[25,26] No data have been published on resistance mechanisms against everninomycins, but increased MICs for avilamycin and bacteria resistant to everninomycin have been shown.[61]

3.4 Bacitracin

A mixture of cyclic polypeptides produced by *Bacillus subtilis* and *Bacillus licheniformis*, bacitracin is bactericidal against Gram-positive bacteria and shows limited activity against Gram-negatives.[15] Bacitracin interferes with the formation of bacterial cell wall peptidoglycans. As AGP it is mostly used in complex with zinc which stabilises the molecule. Bacitracin, in concentrations used as AGP, also showed preventive and therapeutic effects on necrotic enteritis in poultry.[62,63] In humans, bacitracin is mainly used topically and for intestinal decontamination in elective colorectal surgery.[64] It is expected that future bacitracin use in humans will increase because of its effectiveness against VRE.[65,66] No information about cross resistance with other antibiotics exists, but several resistance mechanisms of which at least one is plasmid mediated, have been described.[67]

Bacitacin has been shown to induce the expression of the *VanA*-gene in enterococci.[68,69] It has been claimed that bacitracin as AGP 'cures' resistance against other antibiotics[70-73] but because of weaknesses in study design this claim cannot be considered to be substantiated.

3.5 Flavophospholipol

Flavophospholipol, a complex of very similar phosphorus-containing glycolipids, produced by a group of *Streptomyces* spp. is also known as flavomycin, bambermycins or moenomycin. It is used for growth promotion only and is effective against Gram-positive organisms, by inhibiting bacterial cell wall synthesis. However, it is not active against Gram-negative bacteria as it cannot penetrate the lipid membrane of these organisms.[74] Nevertheless, some activity against enterobacteriaceae has been observed.[22,75-77] In some studies a plasmid clearing effect and a decrease in transfer frequency of some R-plasmids has been described, but an increase in frequency

transfer of other plasmids has also been observed.[78-82] No reports on resistance mechanisms against flavophospholipol have been found. Flavophospholipol has been shown to induce VanA resistance in enterococci.[68] Currently, insufficient data are available to assess the risk of flavomycin use as AGP on the prevalence and spread of resistance of this compound and related substances in humans.

3.6 Monensin and Salinomycin

Monensin, like salinomycin, is a carboxylic ionophore antibiotic produced by *Streptomyces cinnamonensis*. Both drugs are bactericidal by altering the membrane permeability which causes passive transport of potassium ions out of the bacterial cell. The drop in intracellular pH as a result of their replacement by hydrogen ions kills the bacterial cells. They are active against Gram positive bacteria, *Campylobacter* spp., *Serpulina* spp., coccidia and Toxoplasma. These drugs are used in poultry feeds for the prevention of clinical coccidiosis in chickens.[15]

In contrast to other AGP that are only used in pigs, poultry and nonruminating veal calves, monensin and salinomycin are also used to enhance feed conversion in ruminants by modulation of the rumen flora. Both are toxic (at the recommended dosages for pigs and poultry) for several other mammalian species such as horses and dogs. Even in poultry and pigs, it has a small tolerability margin and its toxicity is increased by a number of other drugs.[15] No information could be found on resistance mechanisms, transfer and prevalence of resistance in bacteria.

3.7 Carbadox and Olaquindox

Carbadox and olaquindox are quinoxalines, which are synthetically produced compounds with activity against obligately anaerobic bacteria such as *Clostridium* spp., and Enterobacteriaceae and staphylococci.[15,83] In contrast to most other AGP they are readily absorbed from the intestinal tract. Quinoxalines are not used in human medicine. Despite their toxicity they are used therapeutically in some countries against *Salmonella*, *E. coli* and *Serpulina* infections in pigs. Quinoxalines inhibit DNA synthesis and denature preexisting DNA. Resistance to quinoxalines is caused by target alterations, cell wall impermeability or increased efflux of the drug. Plasmid mediated resistance has been described.[84,85]

4. Resistance in Animals

4.1 Animal Pathogens

Many retrospective and prospective studies have been performed concerning the emergence and selection of resistance in bacteria from animals following antibiotic use. Despite large differences in methodology, most results show that after the introduction of an antibiotic in veterinary practice the resistance in pathogenic bacteria and/or the commensal intestinal microflora increases, as in human medicine. However, some bacteria, such as most Enterobacteriaceae, staphylococci and *Pasteurella* spp. become more readily resistant to certain antibiotics than other bacteria such as *Clostridium* spp. and streptococci, of which animal isolates are still fully susceptible to benzylpenicillin.

In veterinary medicine the problem of resistant bacteria is less severe than in human medicine. Broad-spectrum β-lactamases or bacteria that are multiresistant, because of a broad spectrum efflux pump mechanism,[86,87] coded for by a single gene and conferring resistance to several unrelated antibiotics, have never been found in veterinary pathogens. This difference is most likely due to the fact that immunocompromised patients and the accumulation of these patients in special wards in hospitals are uncommon in veterinary medicine. Recently, however, for the first time the first well documented cases of methicillin-resistant *S. aureus* infection in animals have been published, namely, postoperative wound infections in a horse and in dogs.[88,89]

4.2 Faecal Flora

Antibiotic use not only selects for resistance in the target pathogenic bacteria, but also causes the

emergence of resistant populations of bacteria in the endogenous flora of exposed individuals and populations. Resistance genes are mostly associated with plasmids or transposons located on plasmids or on the chromosome and can easily be transferred to susceptible micro-organisms. The high density of bacteria in the gut facilitates this transfer between bacteria.

Transfer of resistance has been described between bacteria belonging to the same species, and the gene pool of Gram-positive cocci has extended to Gram-negative bacteria as well.[90,91] Under antibiotic pressure, resistance genes tend to accumulate in multiresistant plasmids. However, not only does clonal spread of bacteria occur but also resistant bacteria from animal origin may transfer these resistance genes either to bacteria pathogenic for humans or to the endogenous flora of humans. Conjugal transfer of plasmid pAT 191 DNA which confers resistance against kanamycin, from *E. faealis* to *E. coli* in the digestive tracts of gnotobiotic mice have been found with a frequency of 3×10^{-9}.[92] Furthermore, *in vitro* conjugative transfer of VanA vancomycin resistance between Enterococci and listeriae of different species have been found.[93]

Co-transfer or linked transfer, that is, the simultaneous transfer of different genes located on the same mobile element, has been described for genes encoding for different antimicrobial agents and for genes encoding for antibiotic resistance and virulence genes. The *E. coli* plasmid pNV 13 encodes for resistance to carbadox, streptomycin, spectinomycin and ampicillin.[84,85] The transposon Tn 1545 carries resistance genes to kanamycin, macrolides and tetracyclines.[59] Furthermore, co-transfer of antibiotic resistance and virulence genes has been described for enteropathogens.[94] Macrolide resistance and toxin A production, encoded by Tn 5398 can be transferred from *Clostridium difficile* to *B. subtilus*.[95] The greater the number of resistant intestinal micro-organisms in the intestinal flora the greater the chance of transfer of their resistance genes to pathogenic bacteria and dissemination in the environment.

Comparing the populations of 3 cities in 3 different countries, Lester found that the prevalence of resistance in the intestinal flora of each population was not only strongly correlated with the amount and types of antibiotics used, but also indicative of resistances observed in pathogenic bacteria isolated from infections in that particular population.[96] This clearly demonstrates that the endogenous flora is a reservoir for resistance genes and that resistance in pathogenic bacteria is only the 'tip of the resistance iceberg'. Because intensively reared food animals are mostly treated as a group, continuously with AGP and temporarily (but regularly) for therapeutic reasons or for prevention, the endogenous flora of these animals contains a relatively high proportion of resistant bacteria. Furthermore, since hygienic measures in intensive farming are only directed against the introduction of pathogenic micro-organisms from outside the stable or farm, and not against the prevention of spread within a stable, newly introduced resistant bacteria or emerged resistance genes will spread very fast through the whole herd by faecal contact. These resistant micro-organisms can reach humans either directly through direct contact or indirectly via meat products.

As bacteria of the endogenous flora of food animals contaminate foods of animal origins, they might either colonise humans or transfer resistance genes to human endogenous flora and so superimpose an additional load to the reservoir of resistance genes already present in humans. Therefore, it has been proposed that a low level of resistance in the intestinal flora of food animals should be considered as a safety and quality mark for these animals.[24,28] Several studies have shown a correlation between the amount of antibiotics used and the prevalence of resistance in the faecal flora of animals.

In the Netherlands, enrofloxacin, a methylester of ciprofloxacin, is extensively used for therapy in turkeys, but its use in pigs is very limited. In a point prevalence study the prevalence of ciprofloxacin resistant faecal *E. coli* in turkey flocks was 49% and in pig herds 2%.[97] For tetracycline, ampicillin

and furazolidone, which are used regularly for therapy in both animal species, the prevalence of resistance was, for both animal populations, in the same order of approximately 98%, 98% and 16%, respectively. The literature on resistance against AGP is very limited as most of these compounds are not used for therapy and therefore susceptibility testing is not regularly performed.

In a surveillance study on the resistance against AGP of *C. perfringens* from various animal sources conducted from 1979 until 1992 no increase in resistance to AGP was observed.[16] However, Linton et al.[98] found a significant increase in the prevalence of resistance against tylosin and bacitracin in faecal enterococci of pigs and poultry fed these compounds. Virginiamycin use did not result in an increase in resistance. Thal et al.[99] found an increase over time in resistance against quinupristin/ dalfopristin in faecal *E. faecium* in turkey flocks given virginiamycin as AGP. After the introduction of olaquindox in 1982 on farms using olaquindox as AGP the prevalence of resistance in faecal *E. coli* in pigs increased from 0.004% to 6% in 3 years, whereas on farms not using olaquindox the prevalence of resistance increased by a significantly lesser degree, suggesting dissemination of resistant clones.[100]

Ohmae et al.[84,85] noticed an increase of resistance against carbadox in faecal *E. coli* isolates in pigs after its introduction as AGP. All resistant isolates from 6 farms that fed carbadox continuously to pigs either as AGP or for prevention of swine dysentery, carried the same transferable plasmid conferring carbadox resistance. Carbadox is not used in poultry and cattle, and no carbadox resistance was found in *E. coli* isolates from these animal species in the same region. Mills and Kelly[101] also reported an increase in resistance of *Salmonella* isolates from 37% to 61% after the introduction of carbadox. Carbadox, however, was not only used as an AGP, but also for the prevention of swine dysentery and therapy of salmonellosis. Hedges and Linton[102] showed that not only the prevalence but also the degree of resistance increased by olaquindox use. In herds not exposed to the AGP, the degree of resistance varied from 0 to 0.02%, while in herds given olaquindox it varied from 1.3 to 6.5%.

4.2.1 Vancomycin-Resistant Enterococci

Interest in the selection of resistance by AGP increased after the emergence of vancomycin resistant enterococci (VRE) in human infections. It was soon recognised that avoparcin, a glycopeptide like vancomycin and until recently commonly used as AGP in most EU-member states, selects for VRE in the intestinal flora of animals.[44,103] In countries where avoparcin was used as AGP, VRE were not only found in food animals fed avoparcin, but also in the faecal flora of healthy humans and pet animals.[43,103-106] Furthermore, resistance against MLS antibiotics such as erythromycin and the streptogramins is common in enterococci from animals fed related antibiotics as AGP, such as tylosin (a macrolide) or virginiamycin (a streptogramin).[106] Similar figures have been found in other European countries like Denmark,[34] where in 1995 the prevalence of resistance in enterococci isolated from faecal samples of pigs and poultry was 21% and 56% against vancomycin, 91% and 59% against erythromycin, and 53% and 37% against pristinamycin, respectively. In Finland, however, where tylosin is not used as AGP and only limited to veterinary purposes, the prevalence of erythromycin resistance in enterococci is significantly lower, being 18% and 9%, respectively.[107]

Since 1986, Sweden has banned the use of AGP in animal feeds. The prevalence of resistance against AGP or related compounds in faecal samples of Swedish pigs was significantly lower than that in Dutch pigs in 1997. In Sweden and the US, where avoparcin has never been used, no high level VRE (VanA resistance) have been found in faecal samples of food animals or healthy humans outside hospitals.[46] Following the suspension of the use of avoparcin in Denmark in 1995 the prevalence of vancomycin-resistant *E. faecium* in broilers decreased from 5.2% in 1995 to 1.2% in 1997.[34]

In the Netherlands, a significantly higher percentage of VRE per gram of faeces (10 to 100%) was observed in veal calves from farms using avo-

parcin as an AGP than in faecal samples of calves fed bacitracin as AGP (≤1 to 10%).[108] The prevalence of VRE in turkey flocks fed avoparcin was 60% in contrast to 8% in flocks not exposed to avoparcin,[109] and the relative odds ratio was 7.5. In Denmark, Bager et al.[110] found a high correlation between the use of avoparcin on a farm and the prevalence of VRE in the intestinal flora of animals. The likelihood of isolation of VRE from faecal samples of animals (pigs and poultry) was 3 times higher in animals fed avoparcin than in other animals. The relative odds ratio for the use of avoparcin on the presence of VRE in the faecal flora of these animals was 2.9 for poultry and 3.3 for pigs.

It can be concluded that the use of antibiotics for growth promotion in animals, as with veterinary antibiotic use, selects for resistance among susceptible micro-organisms, not only in pathogens but also in bacteria belonging to the commensal intestinal microflora of animals, such as enterococci and *E. coli*. This has been shown for avoparcin, bacitracin, tylosin, virginiamycin, carbadox and olaquindox.

5. Transfer of Resistant Bacteria from Animals to Humans

5.1 Zoonotic Bacteria

Most investigations on the transfer of resistant bacteria from animals to humans concern Gram-negative food infections caused by bacteria like *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp. Transfer of resistant Salmonellae from animals to humans has been described by several authors.[111-114] Because the resistance of salmonella isolates from humans and animals has been monitored for many years, the emergence and dissemination of resistance in this species is very well documented. Before the introduction of antibiotics, salmonella isolates and other Enterobacteriaceae were susceptible to most antibiotics despite the prevalence of plasmids.[115] Humans become infected with salmonella isolates from animals by direct contact with infected animals or animal faeces, but the most important source of human infections are food products of animal origin.

Asymptomatic salmonella infections and carriers are common in food animals in intensive animal husbandry. The salmonellae in the intestinal tract of these animals contaminate during slaughtering and processing of the carcasses, and meat and meat products. Humans can then become infected via the meat and meat products, and also via eggs etc. Humans do not always become ill after a salmonella infection. Deleener and Haebaert[116] showed that the frequency and variation of the different isolated salmonella-serotypes from asymptomatic carriers in a meat packing plant corresponded with the serotypes isolated from the supplied meat and from the produced meat products.

Despite the fact that since the introduction of antibiotics in clinical medicine resistance in human and animal isolates increased in general, the majority of clinical salmonella isolates are still susceptible to most antibiotics.[117] In the Netherlands, the prevalence of tetracycline resistance in human and animal salmonella isolates increased clearly until the ban on tetracycline as AGP,[118] when it started to gradually decline.[119-122] In Great Britain, after the ban on tetracycline as AGP, tetracycline-resistant *Salmonella typhimurium* isolates from calves fell from 60% in 1970 to 8% in 1977.[123] However, the spontaneous ending of epidemics by virulent tetracycline resistant *S. typhimurium* clones might have contributed to this decrease as well.[124] In most EU-member states *Salmonella enteritidis* is the most commonly isolated serotype from human infections, as a result of its extensive dissemination among poultry since 1980. Because this serotype does not, in most cases, cause clinical symptoms in affected flocks, the animals are not treated with antibiotics. Therefore, the selection pressure is low and generally isolates are still susceptible to most antibiotics.

Sporadically however, epidemics of salmonella clones with an enhanced virulence and pathogenicity for animals occur, such as *S. typhimurium* phage type 29 from 1963 to 1969, definitive type

(DT) 204 in 1977, and DT 204 and DT 193 in 1980.[123] The primary reservoir of *S. typhimurium* is calves, but sheep, goats, pigs, poultry and horses can also become infected. During all these epidemics the same phage type with identical resistance profiles was isolated from animal and human infections. Because these strains cause serious disease in affected animals, these animals are treated with antibiotics and as a result of the selection pressure these strains tend to become (multi)resistant. Since 1994, *S. typhimurium* DT 104 has caused an epidemic. This strain was, from the start, resistant to most of the antibiotics normally used to treat enteric infections in animals, but it has acquired additional resistance against trimethoprim and fluoroquinolones,[117,125,126] most likely because affected groups of animals could only be treated with these antibiotics.

The most important reservoir for human campylobacter infections is poultry. Endtz et al.[127] observed that the emergence of fluoroquinolone-resistant *C. jejuni* infections in humans in the Netherlands coincided with the introduction of enrofloxacin for poultry therapy in spring 1987. Enrofloxacin and ciprofloxacin (the latter was introduced in October 1988 for human therapy in the Netherlands), are fully cross-resistant. In 1989, 14% of poultry and 11% of human isolates of *C. jejuni* were resistant to ciprofloxacin. Experimentally, it was shown that in flocks only colonised with ciprofloxacin-susceptible *C. jejuni*, after therapy with enrofloxacin mutants resistant to ciprofloxacin emerged.[128] In Great Britain, enrofloxacin was registered for veterinary use in 1993, and in that year 14% of *C. jejuni* isolated from poultry carcasses imported from the Netherlands were fluoroquinolone resistant compared with 1% from locally raised broilers.[129] In 1997, the percentage of fluoroquinolone resistant *C. jejuni* from English broilers had approached a continental level of about 10%. Transfer of chloramphenicol resistant *Yersinia enterocolitica* strains from animals to humans has been described by Perez Trallero.[130]

6. Disturbance of Colonisation

Another aspect of the use of antibiotics in animals is disturbance of the colonisation resistance of the intestinal flora of animals exposed to certain antibiotics.[131,132] In cases of reduced colonisation resistance not only are the minimal infectious or colonisation doses of pathogenic or resistant bacteria considerably lower, but animals also excrete these bacteria in higher numbers and over a longer period of time compared with animals with an intact colonisation resistance. This enhances not only dissemination of salmonellae or resistant bacteria within a group of animals, but also increases the contamination of carcasses with these bacteria during slaughter. This effect has been clearly demonstrated for most broad-spectrum antibiotics[133] and for certain AGP: avoparcin[134-136] and to a lesser extent, virginiamycin and tylosin. Avilamycin and bacitracin do not appear to disturb the colonisation resistance in the dosages used for growth promotion.[137-141] Furthermore, flavomycin has been shown to provide a certain degree of protection against salmonella infections.[142]

6.1 Indicator Bacteria

As a result of exposure to antibiotics, the level of resistance against antibiotics among bacteria belonging to the normal intestinal flora of humans and animals increases. These bacteria constitute an enormous reservoir of resistance genes for (potentially) pathogenic bacteria.[4] Moreover, the level of resistance in the endogenous flora is considered a good indicator for the selection pressure exerted by antibiotic use in that population and for resistance problems to be expected in pathogens.[96] Resistant bacteria from the intestinal flora of food animals contaminate, like zoonotic bacteria, the carcasses of slaughtered animals and reach the intestinal tract of humans via the food chain.

Investigation of the prevalence of resistance of certain indicator bacteria like *E. coli* and enterococci in the intestinal tract of different populations of animals and humans makes it feasible to compare the prevalence of resistance in different pop-

ulations and to detect a possible transfer of resistant bacteria from animals to humans and vice versa. Because of the inevitable high use of antibiotics in hospitals, selection and dissemination of resistant clones and resistance genes is high in this environment. The emergence of new resistances due to the acquirement of new genes or gene clusters like the *VanA*-gene cluster are not likely to occur in hospitals but must have been introduced from somewhere outside into the hospital setting. Therefore, healthy individuals in the community are a source of resistant bacteria and resistance genes, and can be considered to be a suitable population to study with regard to the possibility of transfer of resistance from animals to humans.

Corpet[143] showed that the prevalence and degree of resistance in faecal *E. coli* flora in humans who used only sterilised food decreased significantly. Furthermore, Nijsten found significantly more resistant *E. coli* in the faecal flora of pig farmers than in faecal (sub)urban residents.[144,145] However, the personal antibiotic use of the farmers was much higher than that of the (sub)urban residents.

Comparison of the prevalence of ciprofloxacin resistant *E. coli* in faecal samples of turkeys and turkey farmers with pig and pig farmers clearly indicated transfer of ciprofloxacin-resistant *E. coli* strains from turkeys to turkey farmers.[97] In the Netherlands, enrofloxacin is commonly used in turkeys but not in pigs because no oral formulation for pigs was available at the time of study. Tetracyclines are extensively used in both animal species, similar to the use of furazolidone in the past. The prevalence of ciprofloxacin resistant *E. coli* was not only significantly higher in turkey farmers and turkeys than in pig farmers and pigs, but also *E. coli* strains were isolated from farmers and turkeys which were completely identical in pulsed field gel electrophoresis (PFGE) after *XbaI* digestion. None of the turkey farmers and urban residents in this study had used antibiotics in the 3 months prior to the study. For the turkey slaughterers the infection risk seemed much lower, despite the fact that ciprofloxacin-resistant *E. coli* strains had been isolated from the turkey carcasses after slaughtering.[109]

In contrast, there was no difference between the prevalence of furazolidone or tetracycline resistant *E. coli* between the 2 animal populations and between the 2 groups of farmers. For furazolidone, as with the fluoroquinolones, horizontal transfer of resistance via plasmids or transposons is not important for dissemination of resistance, but only clonal spread of resistant strains. These results therefore suggest transfer of resistant strains from animals to humans. The extent of transfer seems to be correlated with the prevalence of resistance in the animal population, which is positively correlated with the amounts of antibiotics to which the animal population is exposed. In the same study, VRE were isolated from a turkey farmer and his turkeys which were not only identical using PFGE after *SmaI* digestion, but also had a *VanA*-gene with a unique mutation.[105] This again strongly indicates transfer of resistant strains from animals to humans.

In Sweden, no VRE were found in the faecal flora of healthy humans and animals, and no VRE could be detected in stool samples of healthy volunteers after taking a course of oral vancomycin.[146] However, in Belgium in a similar experiment, all volunteers in which no VRE were found in their stool samples before the study, became positive.[147] This is in concordance with the results of Quednau et al.,[148] who were able to isolate VRE from Danish but not Swedish meat products, and a Dutch study in which VRE could only be isolated from faecal samples of carnivorous humans but not from vegetarians.[149]

7. Transfer of Resistance Genes from Animals to Humans

In 1976 in a prospective study, Levy observed transfer of tetracycline resistance genes between chicken *E. coli*-strains, from chicken to chicken and from chicken to humans, in chickens fed tetracycline.[150,151] A wide dissemination of a tetracycline resistance gene (*tetQ*) was observed by Nikolich et al.[152] They found identical *tetQ* genes

in host-specific intestinal flora bacteria; *Bacteroides* spp. and *Prevotella intermedius* from humans, and *P. ruminicola* from bovines.[152,153] The relationship between the use of an antibiotic and the dissemination of bacterial resistance from animals to humans has been described in detail by Hummel et al.[154]

In 1982 in the former German Democratic Republic (DDR) nourseotricin, a streptotricin antibiotic, was introduced as an AGP for pigs. Streptotricin antibiotics have not been used in human medicine and do not show cross-resistance with other antibiotics. Resistance to nourseotricin became, within 1 year after its introduction, common in faecal *E. coli* from pigs fed this antibiotic. The resistance genes were located on transposon Tn 1825 and within 2 years this transposon was found in faecal isolates from pig farmers and their family members, in urban residents, and in *E. coli* isolated from urinary tract infections in humans. A few years later it was also found in pathogenic bacteria, not only in zoonotic bacteria like *Salmonella* spp. but also in *Shigella* spp., which only affect humans and do not have an animal reservoir. Outside the DDR, nourseotricin resistance has never been found.

Other examples of the dissemination of resistance genes from animals to humans are the dissemination of the *aacC4*-gene (apramycin resistance) and *hphB*-gene (hygromycin resistance) from animals to human bacteria. These genes are co-transferred. Despite the fact that apramycin is only used in animals, these genes have been found in animal isolates or zoonotic bacteria isolated from humans, in Enterobacteriaceae in the environment, the intestinal flora of farmers, and in hospital isolates.[155-158] A high degree of similarity between plasmids encoding for *aacC4* and *hphB* from these sources has been demonstrated.[159,160] A question regarding the similarity of the *VanA*-gene cluster from animals and humans has been addressed in several studies. In a study from Norway, horizontal transfer of the gene cluster was strongly suggested, that is, 9 out of 12 human and 7 out of 10 poultry isolates were identical.[161] In the Netherlands, similarity between a VRE isolated from a turkey and a turkey farmer was demonstrated.[105] Furthermore, in a study from the UK, the novel insertion sequences in the *VanA* genes from VRE isolated from poultry were not found in hospital isolates.[162]

8. Conclusions

In animals, as with humans, the use of antibiotics causes an increase of resistance both in pathogenic bacteria, and in the endogenous flora of these animals. Resistant bacteria from animals, that is, zoonotic bacteria or intestinal flora, can infect or reach the human population by direct contact, and also via food products of animal origin. These resistant bacteria can colonise humans and/or transfer their resistance genes to other bacteria belonging to the endogenous flora of humans. Moreover, the greater the number of resistant bacteria in the intestinal flora, the greater the likelihood that genes encoding resistance will be transferred to (potentially) pathogenic bacteria, and disseminated into the environment from animals to foods of animal origin. In this respect, one might consider the resistance observed in zoonotic and nosocomial pathogens to be just the tip of the iceberg.

As bacteria from the human flora cause not only infections in immunocompromised hosts, but are also considered an important reservoir of resistance genes for human pathogens, it has been proposed that a low level of carriage of resistant strains by humans should be a public health goal in much the same way as a normal blood pressure and a low serum cholesterol level are public health goals.[96] Despite the fact that it is not yet clear to what extent the use of antibiotics in animals contributes to the resistance problems in human medicine, it cannot be disputed that there is a definite link. Because we are now encountering multiresistant micro-organisms in the clinical setting which are difficult to combat with currently available antibiotics, every source of resistance must be controlled as efficiently as is feasible. Consequently, a low level of resistance in the intestinal flora of food animals should be thought of as a distinguishing safety and quality mark for food animals.[24,28]

Moreover, this will not only protect public health, but also safeguard the future efficacy of antibiotics in veterinary medicine.

This goal can only be achieved by reducing the amounts of antibiotics used in animals. The requirement for antibiotics in veterinary therapy and bacterial infection prevention should be minimised by improving methods of animal husbandry, disease eradication, optimal use of existing vaccines and the development of new vaccines. If antibiotics have to be used, the use of agents with a narrow spectrum of activity should be preferred and this use should be according to a sensible veterinary antibiotic policy.[163] Discontinuing the practice of routinely adding AGP to animal feeds would reduce the amount of antibiotics used for animals in the EU by a minimum of 30% and in some countries by up to 50%. In this case, the public health risks should be weighted against the economic profits. The Swedish have shown that modern and profitable animal husbandry without AGP is feasible.[20] A ban on the use of AGP would encourage research into alternatives like pre- and probiotics.

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