Journal of Applied Microbiology Symposium Supplement 2002, **92**, 85S–89S

Antimicrobial resistance and the food chain

C.J. Teale
Veterinary Laboratories Agency Shrewsbury, Shrewsbury, UK

1. SUMMARY

The extent to which antibiotics given to animals contribute to the overall problem of antibiotic resistance in man is still uncertain. The development of resistance in some human pathogens, such as methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Mycobacterium tuberculosis*, is linked to the use of antimicrobials in man and there is no evidence for animal involvement. However, there are several good examples of transfer of resistant bacteria or bacterial resistance genes from animals to man via the food chain. A bacterial ecosystem exists with simple and complex routes of transfer of resistance genes between the bacterial populations; in addition to transfer of organisms from animals to man, there is also evidence of resistance genes spilling back from humans into the animal population. This is important because of the amplification that can occur in animal populations. The most important factor in the selection of resistant bacteria is generally agreed to be usage of antimicrobial agents and in general, there is a close association between the quantities of antimicrobials used and the rate of development of resistance. The use of antimicrobials is not restricted to animal husbandry but also occurs in horticulture (for example, aminoglycosides in apple growing) and in some other industrial processes such as oil production.

Correspondence to: Mr C.J. Teale, Veterinary Laboratories Agency Shrewsbury, Kendal Road, Harlescott, Shrewsbury, SY1 4HD, UK (e-mail: C.Teale@vla.defra.gsi.gov.uk).

2. INTRODUCTION

The most important factor in the selection of resistant bacteria is generally agreed to be usage of antibiotic-type antimicrobial agents and in general, there is a close association between the quantities of antimicrobials used and the rate of development of resistance (Aarestrup and Seyfarth 2000). Antimicrobials have been used in animal husbandry both for therapeutic reasons and as growth promoters, and both will provide a selective pressure on certain bacteria of animal origin, dependent on the spectrum of activity of the antimicrobial in question. Therapeutic usage of antimicrobials in animals is important to prevent the epidemic spread of animal disease and to protect animal welfare. It can also prevent the transfer of zoonotic disease from animals to man (Ungemach 2000). In the UK, use of antimicrobials as growth promoters was restricted following the recommendations of the Swann Report of 1969, which recommended that antimicrobials used in human medicine should not be used as growth promoters unless they had limited application in human medicine and also, that their use resulted in no cross-resistance to antimicrobials used in human medicine. During the 1970s, the main problems encountered in human medicine were resistance in Gram-negative bacteria, and antimicrobial compounds with a Gram-positive spectrum were authorized as growth promoters, since the use of related compounds in human medicine was at the time more limited. Following the emergence of multi-resistant Gram-positive pathogens in human medicine, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci, these compounds with a Gram-positive spectrum assumed a much greater importance, with the result that by 1999, use of avoparcin (a glycopeptide which gives cross-resistance to

vancomycin), virginiamycin (a streptogramin), bacitracin (also used in human medicine), spiramycin and tylosin (both macrolides) had been banned for growth-promoting purposes throughout the EU. It is important to note that in some other countries outside the EU, the recommendations of the Swann Report have not been adopted and a range of antimicrobials, including penicillin and tetracyclines, may still be used as growth promoters. In addition to the potential problems for both human and animal health that resistant bacteria of animal origin may pose, a low level of resistance in the intestinal flora of food animals has been recommended for consideration as a distinguishing safety and quality mark for the food produced from such animals (Van den Bogaard and Stobberingh 1996).

This paper will look at some of the evidence that exists for transfer of resistant bacteria or resistance genes from animals to man, and will discuss the amplification of organisms that can occur within animal populations. The transfer of resistant organisms from human to animal populations may be a critical control point for reducing transfer of organisms to man via the food chain.

3. TRANSFER OF RESISTANT BACTERIA FROM ANIMALS TO MAN

3.1. Apramycin/gentamicin resistance

Apramycin is an aminoglycoside which was introduced as a veterinary antimicrobial in the early 1980s to France. Resistance to both apramycin and gentamicin was detected in 1984 in *Escherichia coli* from diarrhoeic calves in France, initially in a few herds and then from many independent cattle herds and sheep flocks. In late 1985, resistance to apramycin/gentamicin was detected in *Salmonella typhimurium*. It was found that the resistance was plasmid-mediated, transferable and conferred via possession of the enzyme aminoglycoside acetyltransferase AAC(3)IV, which confers resistance to both apramycin and gentamicin (Chaslus-Dancla *et al*. 1986).

From 1985–1988, strains of *E. coli*, *Salm. typhimurium* and *Klebsiella pneumoniae* were recovered from hospitals in Belgium with similar resistance. It was found that the plasmids containing AAC(3)IV in some human and animal bacterial strains had a high degree of genetic homology (Chaslus-Dancla *et al*. 1989). Spread from animals to man was, however, rather limited, as was spread within hospitals and to the wider human population. Experiments also showed that plasmid-bearing AAC(3)IV could be transferred from *E. coli* to *Salm. typhimurium* in calves treated with apramycin (Hunter *et al*. 1992). The situation was similar in the UK, and an increase in apramycin/gentamicin resistance in *Salm. typhimurium* DT 204c was noted. During the 1980s, *Salm. typhimurium* DT 204c was a common serotype in calves, though it is now very rare in UK livestock (Wray *et al*. 1986; Threlfall *et al*. 2000).

Although the antimicrobial apramycin was in use in animals, it was possible that use of gentamicin in man had led to the emergence of this particular resistance plasmid. Several pieces of evidence point to the conclusion that AAC(3)IV emerged because of veterinary use of apramycin rather than use of gentamicin in man; these include the incidence of resistance in human and animal populations, and the observation that AAC(3)IV was not widespread in other human Enterobacteriaceae. However, the most significant piece of evidence was the discovery that the AAC(3)IV resistance gene was linked to a gene (*hph*B) encoding the enzyme hygromycin B phosphotransferase, which conferred resistance to the veterinary drug hygromycin B (also an aminoglycoside, but one which is active as an anthelmintic). Hygromycin B was not used in human medicine, which strongly suggested that the resistance plasmids had emerged in animals (Salauze *et al*. 1990).

3.2. Nourseothricin resistance

Nourseothricin is a streptothricin antimicrobial that was used as a growth promoter in farm animals in the former East Germany during the 1980s. No equivalent antimicrobial was used in human medicine over this period in East Germany. Resistance to streptothricin was mediated by plasmids containing a transposon which encoded the enzyme streptothricin acetyltransferase. Streptothricin was introduced in 1983 and by the second year after introduction, resistance was detected in *E. coli* from pigs. In successive years, resistance was detected in *E. coli* from pig farmers, subsequently in *E. coli* from man in the wider community and finally, in isolates of *Salmonella* spp. and *Shigella* spp. from man (Hummel *et al*. 1986; Witte *et al*. 2000). Shigellae are pathogens of man and primates, and do not generally affect domestic animals (although odd opportunistic infections have been reported). Therefore, these observations strongly support the spread of resistance genes from animal bacteria to human pathogens.

3.3. The ROB-1 beta-lactamase

Beta-lactamase-producing strains of the human respiratory pathogen *Haemophilus influenzae* appeared in 1974 and in most of these strains, the beta-lactamase produced by the organism is TEM-1. A few strains were found to possess a different beta-lactamase (initially differentiated by having a different iso-electric point) and this was designated 'ROB-1'. It was then found that some isolates of *Actinobacillus pleuropneumoniae*, a respiratory pathogen of the pig, also carried this gene on a plasmid, conferring resistance to ampicillin. Hybridization studies and RFLP showed

the genes and plasmids from both *H. influenzae* and *A. pleuropneumoniae* to be very similar (Medeiros *et al.* 1986). It is interesting to speculate on the origin of this plasmid, since it is possible that the resistance originally developed in pigs and spread to man, or emerged in man and spread to pigs.

3.4. Multi-resistant *Salm. typhimurium* DT104

There has been clonal spread of *Salm. typhimurium* DT104 to many parts of the world. The organism is commonly resistant to five antimicrobials, namely ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (the so-called 'penta-resistance ACSSuT pattern'). These resistance genes are all located on the bacterial chromosome. All isolates of DT104 of the ACSSuT phenotype contain the same gene cassette, irrespective of source (food animal or human) or the country of origin (Ridley and Threlfall 1998). The organism can, however, also acquire plasmids, for example conferring resistance to trimethoprim and apramycin (AAC(3)IV). The origin of DT104 is not known with certainty; the gene conferring resistance to ampicillin (*bla*-PSE-1) is similar to a carbenicillin resistance gene detected in *Pseudomonas aeruginosa*, and carbenicillin has never reportedly been used in agriculture. Two other resistance genes in DT104 (to tetracyclines and to the antimicrobial florfenicol) are very similar to resistance genes found in certain fish pathogens, leading to the suggestion that aquaculture may have played some part in the emergence of this strain (Chaslus-Dancla *et al.* 2000).

Since the spread of this organism has been clonal, the organism is very similar in man and animals. However, this can be an advantage, since *Salm. typhimurium* DT104 organisms with unusual mutations can provide good epidemiological tracing tools. In Denmark, a cluster of 25 cases of *Salm. typhimurium* DT104 occurred, each of which had the same rare mutation in the gyrase gene. The cases included an abattoir worker and a nurse. Many of the human cases had been treated with antimicrobials before acquiring the salmonella. The outbreak could be traced back to pigs on a single pig farm (Mølbak *et al.* 1999). It seems likely that the abattoir worker may have been infected by direct contact, other cases by food-borne infection and the nurse by secondary spread, and this underlines the dynamic situation of the spread of organisms from animals to man.

4. SPREAD OF RESISTANCE FROM MAN TO ANIMALS

There is also evidence for transfer of bacterial organisms and resistance genes in the opposite direction from man to animals via the environment, and there are various mechanisms by which such transfers might occur. For example, at times of flooding, grazing pastures may be flooded by waters containing sewage or human effluent; animals may graze watercourses into which sewage outflows pass, and there is also potential for spread of organisms from sewage plants or outflows to domestic animals via birds such as seagulls. Examples of the spread of resistance genes from man to animals include the following. (i) Rifampicin and fusidic acid resistance genes were detected in an enterococcal strain recovered from porcine faeces in Germany, although these compounds had not been used in animals but only in humans in Germany (Klare *et al.* 1995). (ii) The enzyme aminoglycoside acetyltransferase AAC(3)II, conferring gentamicin resistance, was demonstrated in bacteria from calves in France in 1986. *Escherichia coli* resistant to gentamicin (and not apramycin) emerged in French hospitals in the mid 1970s, possessed the enzyme AAC(3)II and became widely distributed. From 1986–1990, this strain appeared in calves in a limited area of Western France. Observations suggested that the emergence of this strain was hospital-related and that spread to animals occurred later (Chaslus-Dancla *et al.* 2000).

4.1. Amplification

Although transfer of bacteria and resistance genes from man to animals might occur on a rather limited scale, it can be extremely important because of the potential for amplification of organisms within the animal population. The conditions in which animals are kept can allow faecal–oral recycling because animals, even when kept extensively, are not entirely separated from their faecal material, and there is potential for contamination of food with such faecal material to varying degrees in almost all animal husbandry systems. Thus, organisms that are introduced at a low rate to the animal population can be amplified by faecal–oral recycling and returned to man via the food chain or other means (direct contact, environmental contamination). This transfer from man to animals, although possibly limited, probably deserves detailed attention as a critical control point in the control of certain zoonotic organisms and resistant bacteria. It is interesting to note that *E. coli* O157 could also exploit a similar means of spread, with a low level of human sewage contamination 'seeding' the environment, being amplified in livestock and then returning to man.

4.2. Host specificity of strains

The situation with regard to *Salm. typhimurium* DT104 has already been discussed, with clonal spread of this organism to both man and animals occurring in many countries of the world. The situation is different for many other organisms, however. For example, *Streptococcus agalactiae* and *Staph. aureus* can both cause bovine mastitis and human infections,

though the strains affecting man and animals can be separated by various techniques. Even though different subtypes of a bacterial 'species' may affect man and animals, the ability of bacterial strains to transfer genes between each other means that either population may act as a reservoir of resistance genes. However, considering bovine *Staph. aureus* strains, the resistance picture is quite different from that occurring in human strains, and resistance has actually declined in several European countries over the last few years (Aarestrup and Jensen 1998). This is very different from the situation in man, where methicillin-resistant *Staph. aureus* is an increasing problem in hospitals and, more recently, in the wider community. There have been very few reported instances of resistance in bovine *Staph. aureus* to methicillin, and these have been considered as mainly the result of transfer from man to cattle (Devriese and Hommez 1975). Thus, there is no evidence that bovine strains of *Staph. aureus* contribute to the problems observed in human staphylococci. In other cases, the clonal structure of bacterial populations can be very complex (for example, the enterococci and *Campylobacter*), and this can make molecular epidemiology difficult. Since enterococci are emerging as human pathogens, it is desirable that levels of resistance in animal enterococcal strains are minimized, as these may provide a reservoir of resistant enterococci or resistance genes that could be transferred to human enterococcal strains. Experimental work in Denmark has demonstrated transfer of a streptogramin resistance gene between non-isogenic strains of *Enterococcus faecium* (DANMAP 2000). Differences in the host specificity of bacterial subtypes, and the propensity of different bacterial species to exchange genetic information, are therefore important factors to consider when trying to assess the significance to man of the development of resistance in bacteria from animals.

4.3. Avoparcin–vancomycin resistance

The commensal flora of animals may be affected by the administration of antimicrobials both as growth promoters and for therapeutic purposes. Avoparcin is a glycopeptide antimicrobial formerly licensed for use in animals in the EU as a growth promoter, but its use is no longer permitted. Enterococci are common commensals in the enteric flora of some domestic animals, and some strains of enterococci have emerged in recent years as important causes of nosocomial infections in man. Resistance to avoparcin in enterococci can be mediated by *vanA* genes, which also confer resistance to vancomycin, an important antimicrobial for treatment of human Gram-positive infections, including enterococcal infections. In Europe, problems with vancomycin-resistant enterococci have not, in general, been as serious as those reported in the USA, where problems have occurred in some hospitals even though avoparcin has never been used as an animal growth promoter in the USA. The Van A phenotype mediates high-level glycopeptide resistance, and the *vanA* gene cluster is present on a transposon that is generally integrated into a conjugative plasmid which can be transferred both between enterococci and to other Gram-positive bacteria. Studies have shown that avoparcin selects for the emergence and spread of vancomycin-resistant enterococci (VRE) in animals, and VRE have also been detected on meat products. There has been a decline in the numbers of VRE in poultry (though not in pigs) since the banning of the use of avoparcin as a growth promoter in Denmark (Bager *et al*. 1999). A study comparing strains of VRE from poultry and humans revealed that there was little genetic overlap when the genome was compared by pulsed-field gel electrophoresis, although sections of the van gene cluster had a highly conserved sequence (Van den Braak *et al*. 1998). Molecular studies therefore suggest that the *vanA* gene cluster has spread by integration into a range of different conjugative plasmids and is frequently transferred between different *Ent. faecium* strains. Molecular typing of the *vanA* gene cluster has also shown polymorphisms of the insertions present in the non-coding regions of the cluster, and the same subtypes of VRE have been found in VRE of human and animal origin, suggesting that the resistance gene pools communicate (Witte *et al*. 2000). Possible factors influencing the emergence of VRE in countries such as the USA may include prolonged treatment with third generation cephalosporins, as well as the use of vancomycin in human medicine (Witte *et al*. 2000).

5. CONCLUSION

There is good evidence for the spread of resistance genes (or resistant bacteria) from animals to man; there is also evidence of resistance genes spilling back into the animal population. However, the relative importance and contribution of animals to the overall problem of antimicrobial resistance in man is more difficult to ascertain. Resistance genes may arise within the animal population, and the apramycin/gentamicin resistance plasmid provides a good example of this. Faecal–oral recycling can lead to spread of zoonotic or resistant bacteria within animal populations and amplification of the numbers of the animal population infected. Therefore, even though contamination of animal pastures (or surface waters to which animals have access) with human sewage may be intermittent and sporadic, it may be very important as the initial stage whereby animal herds are first infected with some organisms or resistance genes that have emerged outside the animal population. Further spread within the animal population is then likely as animals are transported between farms or to markets, etc. Different populations of some bacterial organisms affect animals and man, and the extent to which transfer of

resistance genes has occurred between these populations is variable. In the USA, even though avoparcin has not been used as a growth promoter, problems with vancomycin resistance have emerged in enterococci. This may have been the result of the selective pressure exerted on the organisms by the use of vancomycin and cephalosporins in hospitals within the USA. Alternatively, it may underline the global nature of the problem, since the strains (or resistance genes) may have emerged in countries where avoparcin had been used and spread by movement of animals, food or people to the USA.

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