Antibiotics in animal feed and their role in resistance development

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Animals and humans constitute overlapping reservoirs of resistance, and consequently use of antimicrobials in animals can impact on public health. For example, the occurrence of vancomycin-resistant enterococci in food-animals is associated with the use of avoparcin, a glycopeptide antibiotic used as a feed additive for the growth promotion of animals. Vancomycin-resistant enterococci and vancomycin resistance determinants can therefore spread from animals to humans. The bans on avoparcin and other antibiotics as growth promoters in the EU have provided scientists with a unique opportunity to investigate the effects of the withdrawal of a major antimicrobial selective pressure on the occurrence and spread of antimicrobial resistance. The data shows that although the levels of resistance in animals and food, and consequently in humans, has been markedly reduced after the termination of use, the effects on animal health and productivity have been very minor.

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Abbreviations
AGP antimicrobial growth promoter
GRE glycopeptide-resistant enterococci
WHO World Health Organization

Introduction

Food-animals are kept in large groups and often housed with high animal densities. Meat-producing animals are raised rapidly to slaughter weight, which is often before they reach physical maturity. The youth of the animals, their housing in large groups and frequent movement and mingling of animals facilitate the introduction and spread of disease. Treatment of entire groups of animals as soon as clinical symptoms appear in a few members of the group is common practice (therapeutic usage). Furthermore, in many production systems the treatment of groups of animals in advance of clinical symptoms is practiced routinely (prophylactic use); for example, in connection with weaning, movement of animals, mingling of animals from different litters or other factors that predispose outbreaks of disease. Finally, antibiotics are used to enhance growth rates and increase feed efficiency in food-animals (growth promotion). The majority of antibiotics are administered to animals with their feed. From the farmers' perspective, this is a practical way of administering therapeutic antibiotics to large groups of animals, but it has the obvious disadvantage in that the sick, weaker animals with appetite loss consume smaller amounts of antibiotics than healthy animals. Nearly all antibiotics used for prophylaxis are administered through feed or water and all antibiotics used for growth promotion are administered as feed additives. The concentrations of antibiotics in the feed for therapy and prophylaxis are usually higher than the concentrations of antibiotics for growth promotion. The latter concentrations, often referred to as 'sub-therapeutic concentrations', should not be mistaken for sub-inhibitory concentrations from a microbial perspective.

Recommendations of the 'Swann Report' led to changes in the European Unions' feed additives regulation in the early seventies. Antibiotics used for human or animal therapy should not be used at the same time for growth promotion. The regulation, however, did not include a provision to withdraw approvals of antimicrobial growth promoters (AGPs) should members of the same class of antimicrobials at a later time come into use for humans. When glycopeptides and streptogramins that were being used for growth promotion (avoparcin and virginiamycin respectively) became important drugs (vancomycin and Quinupristin-Dalfopristin) for the treatment of multi-resistant nosocomial infections, this initially had no impact on their use as AGPs.

In 1994, the first wake-up call was sounded. It was shown that glycopeptide-resistant enterococci (GRE) could be isolated from food-animals in Great Britain and subsequently in Germany and Denmark [1–3], and it was suggested that food-animals could serve as a reservoir for the GRE infections that were rapidly spreading in hospitals in Europe and the US, causing considerable alarm because they were resistant to all commonly used drugs and therefore virtually untreatable. An association with the use of avoparcin as a growth promoter in food-animals was suggested.

Occurrence of resistant bacteria in food and food-animals and association with growth promoter use

The association between the use of avoparcin as a growth promoter and the occurrence of GRE in food-animals was documented in an epidemiological study of exposed and non-exposed flocks of chickens and pigs [4]. This study showed a strong statistical association between prior use of avoparcin as a growth promoter on the farm and detection of GRE in animals raised on the same farm. Furthermore, it ruled out several other potential contributory factors. Thus, an association between the use of an AGP and occurrence of bacteria resistant to the AGP from food-animals was documented.

Subsequent investigations of enterococci isolated from animal faeces and from food of animal origin, conducted on several continents and in multiple countries, have confirmed the close association between the use of antimicrobials as growth promoters and high levels of resistance to medically important antibiotics, primarily, in enterococci. This association has been most thoroughly investigated for the avoparcin–GRE association [5–14], but it has also been shown for AGPs belonging to other classes of antimicrobials, notably macrolides (tylosin and spiramycin) [7], evernimicins (avilamycin) [7], streptogramins (virginiamycin) [7,9,10] and bacitracin [7].

Since the early seventies, the majority of antimicrobials used for growth promotion in Europe have been active against Gram-positive bacteria. Thus, the focus of these studies has been on Gram-positive organisms, notably enterococci. Only two substances with Gram-negative activity have been approved and widely used, carbadox and olaquindox. These substances belong to the chinoxalines, a class of drugs not currently used for therapy in humans or animals. Carbadox and olaquindox were banned in Europe in 1999 because of their toxicological properties (cytotoxic) and the potential occupational hazards relating to their use. Recently a conjugative plasmid conferring resistance to olaquindox was described in *Escherichia coli* isolated from pig manure [15].

In the US and several other countries, classes of antibiotics active against Gram-negative bacteria, such as tetracyclines, have been used for growth promotion for decades and are still being used today. It is difficult to determine the impact of this use on bacterial resistance in food-animals because the drugs are simultaneously being used for therapy and prophylaxis. When tetracycline was banned as a growth promoter in Europe in the early seventies, some studies showed reduced levels of resistance [16], whereas other studies showed no change in the levels of resistance to tetracycline, probably because of co-selection [17].

The selection of resistant bacteria in food-animals by the AGPs and subsequent spread between animals in the farm environment are important factors in the propagation of resistant bacteria in the animal reservoir. The rate of spread of resistant bacteria from animals to the environment, and, more importantly, in the food production chain, are key determinants for the spread to humans.

Spread of bacteria resistant to growth promoters beyond the farm

Bacteria from animals spread to the food products during slaughter and processing. This has been extensively documented for conventional foodborne pathogens, such as *Salmonella*, *Campylobacter* and *E. coli*. The detection of enterococci resistant to AGPs in food products derived from animals where AGPs have been used therefore comes as no surprise [6,8–11].

Resistant bacteria can also spread from the farm to the environment. Resistant bacteria and active antibiotics, or active metabolites of antibiotics, are spread on farmland with manure. In a recent study, a transient increase in the prevalence of tetracycline-resistant bacteria from soils was detected following the treatment of farmland with pig manure slurry [18]. Also, the examination of bivalve shellfish was used to assess the occurrence of GRE entering the environment. A total of 2.7% of shellfish contained GRE, which can probably be attributed to human and, more likely, agricultural sources [19].

Direct transmission of resistant enterococci between animals and farm workers has been demonstrated in several studies [20–22]. More importantly, despite much heterogeneity of molecular subtypes of enterococci from humans and food-animals, several studies have found identical or closely related subtypes in animals, food and humans, supporting that the foodborne route of transmission is of significance [23–26,27••].

Transmission of resistant bacteria from food-animals to humans results in more healthy humans in the society carrying resistant bacteria. This has been clearly documented for GRE. Several studies from Europe have found relatively high carrier rates of GRE in healthy humans in the community and in humans at the point of hospital admission [28–31]. Similarly, high levels have not been shown in the US where avoparcin has not been used as a growth promoter. The carriage of Quinupristin-Dalfopristin-resistant enterococci by healthy humans in the community have likewise been linked to the use of virginiamycin as a growth promoter [32,33].

Willems and colleagues have studied the association between GRE in food-animals and humans in the community and in hospitals by amplified-fragment length polymorphism [26,27••]. The genetic relationship among fecal vancomycin-resistant *Enterococcus faecium* isolates from populations of pigs, poultry, human healthy volunteers and hospitalised patients were investigated in two studies. In the first study, the majority (74%) of GRE isolates from hospitalised patients were not grouped together and when compensating for double counting of outbreak related isolates only 26% grouped together with the food-animal isolates. In the second study, the majority (86%) of GRE isolates from pigs, healthy volunteers and hospitalised patients grouped together. The first study included clinical human isolates, whereas the second study included non-clinical fecal isolates from hospitalised patients. This might explain the difference between the two studies. Taken together, the two studies show that a substantial proportion of GRE isolates in hospitals (26–86%) are indistinguishable from isolates from food-animals by molecular subtyping.

Several groups have performed studies on the molecular diversity and evolutionary relationships of the transposon that carries the vancomycin-resistance gene cluster (Tn1546) in GRE from humans and animals [34–38]. Differences between the Tn1546 types have included point mutations in Tn*1546*, orf1, *vanA*, *vanX*, *vanY* IS*1251*, *vanS–vanH*, IS*1216*V and *vanX–vanY*. Moreover, insertions of an IS1216V-IS3-like element in orf1, of IS1251 in the *vanS–vanH* intergenic region, and of IS1216V in the *vanX–vanY* intergenic region have been detected. Identical Tn1546 types were found among isolates from humans and farm animals in all of these studies. This strongly suggests that animals and humans share from a common vancomycin-resistance gene pool.

It is difficult to establish the direction of bacterial transmission by comparing isolates from animals and humans. However, sequencing of the VanA glycopeptide-resistance gene cluster has led to the identification of, what appears to be, a stable base-pair variation in VanA at position 8234. In this position, either a G (G-type) or a T (T-type) was found. The T-type appears almost exclusively in isolates of GRE from pigs, whereas the G-type appears to be exclusive in isolates from broilers. Both types occur in humans. This distribution has been found in isolates from several countries and continents. Although the reason for this inhomogeneous distribution between animals remains obscure, it does provide additional strong support for the hypothesis that GRE primarily transmits from animals to humans [39].

Termination of the use of antibiotic growth promoters and impact on resistance

The documentation which states that the use of antibiotics for growth promotion in food-animals has led to the creation of a major food-animal reservoir of bacteria resistant to AGPs and also to medically important last-resort antibiotics, such as vancomycin and Quinupristin-Dalfopristin, and furthermore, that these bacteria could spread to humans by animal contact, food or the environment, led the European Union to impose a ban on all AGPs that belonged to classes also used in human medicine. In some countries, for instance Denmark, the farmers, in response to consumers concerns, took a step further and voluntarily discontinued the use of all AGPs.

The termination of the use of AGPs represents a major reduction in antibiotic usage in food-animals and consequently a reduction in the selective pressure favouring the occurrence of resistant bacteria in food-animals, food and humans. In Denmark, the total usage of antimicrobials in food-animals has been reduced by 54%.

The reduction (or deseletion) of resistance has been most extensively studied for enterococci in food animals. Studies have shown reduced carrier rates of enterococci resistant to glycopeptides, Quinupristin-Dalfopristin, macrolides and evernimicin after the ban on avoparcin, virginiamycin, tylosin, spiramycin and avilamycin (Figure 1) [40,41,42••,43]. A noticeable example of co-selection was documented in Denmark. When avoparcin was banned, initially GRE levels in pig herds did not decline. Only after the ban on macrolide growth-promoters did the levels decline substantially. Investigations showed a genetic linkage between VanA and the macrolide resistance gene *ermB* in GRE of porcine origin. Thus, the persistence of GRE in pig herds after the ban of glycopeptides could be explained by the genetic link between *ermB* and *vanA* and co-selection by use of macrolides for treatment and growth promotion [43,44].

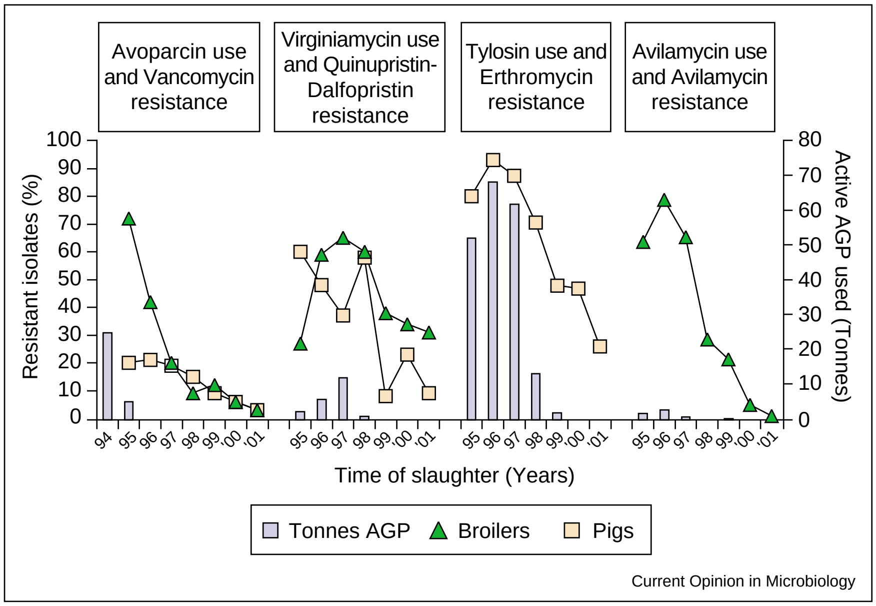
Reductions in occurrence of AGP-resistant bacteria in food have also been documented after the bans [8,45••]. The reduction in food-animals and food has been paralleled by reductions in human carriage of GRE in the community in at least three countries (Germany, the Netherlands and Belgium) where this has been investigated (Figure 2) ([46–49], Goosens H, personal communication). Thus, the association between AGP-resistant enterococci in the food production and human carriage has been further confirmed.

Although the termination of AGP usage has resulted in marked reductions in occurrence of resistant bacteria in food-animals, these reductions have not led to complete disappearance of the strains. Several investigations have shown that, although the probability of randomly picking an AGP-resistant enterococcus from an animal or a food product has been reduced, the resistant strains are still present in the farm environment, food-animals and even in the foodstuffs in low levels [50–52]. Thus, removing the selective pressure has reduced the number of viable resistant bacteria that can transfer from animals to humans via food, but the seeds of resistance remain firmly planted in the farm environment many years after termination. If we were to readmit the use of these antibiotics for growth promotion the resistant strains would very quickly reach pre-termination levels.

Effect of AGP termination on food-animal health and productivity

So far, only a few studies have investigated the effects of terminating AGP on animal health and productivity. In broilers in Denmark, necrotic enteritis was at most a

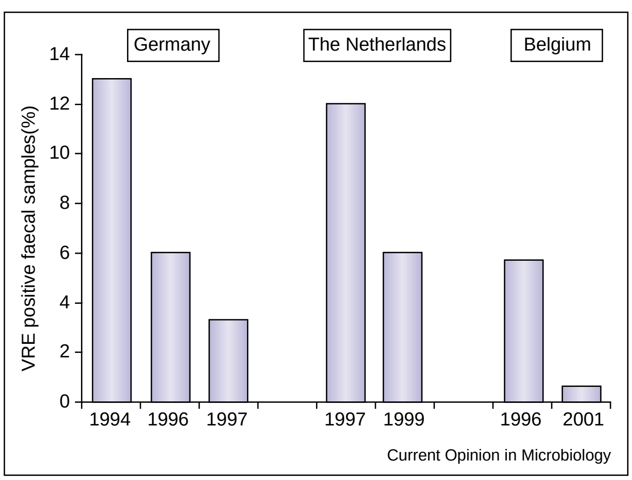
Figure 1



Volumes of active AGP used in food-animals in Denmark and prevalence of *Enterococcus faecium* resistant to medically important, or potentially important drugs in stool samples from healthy animals at slaughter 1995-2001. Avoparcin (glycopeptide) and Virginiamycin (streptogramin) was used in both pigs and broilers, while Tylosin (macrolide) and Avilamycin (evernimicin) primarily was used in pigs and broilers respectively. Data taken from DANMAP (2002). DANMAP 2001 – Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. http://www.vetinst.dk.

minor broiler health problem following the termination of AGPs, probably because producers continued to use ionophores for the prophylaxis of coccidiosis. There were no changes in weight gain or mortality in broilers. The effects of AGP termination on poultry production are small and limited to decreased feed efficiency that is offset completely by savings in the cost of AGPs [53••]. In Danish swine, there was a significant increase in antimicrobial treatments for diarrhea in the post-weaning period after the complete termination of antimicrobial growth promoters, and the termination of AGPs resulted in some loss of productivity in the weaners. There has been no major effect of the AGP termination on productivity or feed efficiency in finishers [54].

Figure 2



Vancomycin-resistant *Enterococcus faecium* in stool culture samples from healthy humans in the community (The Netherlands and Germany) and in hospitalised patients (Belgium) following the European Union prohibition of the glycopeptide avoparcin as a growth promoter. The German data presented in this figure was taken from [58]. The Netherlands data was taken from [59]. The Belgian data was obtained from H Goosens (personal communication).

The results from Danish swine agree with previous results from other Nordic countries [55,56]. When the intensity and the export-driven nature of the Danish animal production are considered, the Danish experience strongly suggests that AGP use could be terminated in all high-producing industrialised countries with only very minor impact on productivity. By contrast, the benefits in terms of reduced levels of resistance in animals, food and humans would be major.

An international board of experts recently evaluated the effects of the discontinuation of AGPs in Denmark on behalf of the World Health Organization (WHO) [57••]. The evaluation report can be downloaded from the homepage of the WHO (www.who.int) or from the Danish Veterinary Institute (www.vetinst.dk).

Conclusions

Routine use of antimicrobials in food-animals for growth promotion constitutes a serious public health problem, especially in the case where the same classes of antimicrobials are being used in humans. Growth promoter use creates a major food animal reservoir of resistant bacteria, with a potential for spread to humans by food intake or by animal contact. Recent experience from a number of European countries shows that the use of antimicrobials for growth promotion provides insignificant benefits to agriculture and that it can be terminated. Ending the use of antimicrobial growth promoters has led to reductions in the prevalence of resistant bacteria in food and food animals, as well as in humans, in the countries where this has happened.

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Changes in the occurrence of antimicrobial growth promoter (AGP) resistance among *Enterococcus faecium* from broilers and broiler meat from 1995 to 2001 and relations between the occurrence of AGP resistance among *E. faecium* isolates from Danish broilers and AGP resistance among *E. faecium* isolates from the broiler meat in the same quarter within the year was investigated by logistic regression analyses. After the AGP withdrawal a significant decline in resistance to avilamycin, erythromycin, vancomycin and virginiamycin was observed among *E. faecium* from broilers and broiler meat. In addition, a decline in the occurrence of AGP resistance among *E. faecium* from Danish broilers was associated with a decrease in the predicted probability of isolating an AGP-resistant *E. faecium* isolate from a randomly selected broiler meat product.

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