Human Health Hazard from Antimicrobial-Resistant Enterococci in Animals and Food

Ole E. Heuer, Anette M. Hammerum, Peter Collignon, and Henrik C. Wegener

The use of antimicrobial agents in the modern farm industry has created a reservoir of resistant bacteria in food animals. Foods of animal origin are often contaminated with enterococci that are likely to contribute resistance genes, virulence factors, or other properties to enterococci in humans. The potential hazard to human health from antimicrobial-resistant enterococci in animals is questioned by some scientists because of evidence of host specificity of enterococci. Similarly, the occurrences of specific nosocomial clones of enterococci in hospitals have lead to the misconception that antimicrobial-resistant animal enterococci should be disregarded as a human health hazard. On the basis of review of the literature, we find that neither the results provided by molecular typing that classify enterococci as host-specific organisms nor the occurrence of specific nosocomial clones of enterococci provide reasons to change the current view that antimicrobial-resistant enterococci from animals pose a threat to human health. On the contrary, antimicrobial resistance genes appear to spread freely between enterococci from different reservoirs, irrespective of their apparent host association.

Enterococci cause many serious and life-threatening infections, including bloodstream infections. Enterococcal infections are often treated with a combination of an aminoglycoside (e.g., gentamicin) and a cell wall-active agent, such as penicillin (e.g., ampicillin) or a glycopeptide (vancomycin or teicoplanin). Enterococci are intrinsically resistant to many antimicrobial agents, and effective therapy has limited options. When ampicillin resistance is present, therapeutic options usually involve antibiotics that have more adverse reactions and are much more expensive (e.g., vancomycin, linezolid, and quinupristin/dalfopristin). Thus, when high-level gentamicin, ampicillin, or vancomycin resistance is present, successful therapy for infection may be impossible.

The use of large amounts of antimicrobial agents in the modern farm industry has created a reservoir of resistant bacteria in food animals [1]. Several antimicrobial agents that are frequently used in animals belong to the same class of antimicrobial agents as those that are important for use in therapy of some enterococcal infections in humans (e.g., ampicillin, gentamicin, and virginiamycin). Antimicrobial-resistant enterococci are prevalent in food animals and in food of animal origin [2–4], and thus may frequently be transferred to humans, either by ingestion of contaminated food or from the environment.

Resistant bacteria or resistance genes may, therefore, be frequently transmitted from an animal reservoir to humans [5]. Subsequent emergence of infections in humans caused by resistant bacteria that originate from the animal reservoir is of great concern. In the European Union, the use of antimicrobial agents (e.g., virginiamycin and avoparcin) as feed additives for growth promotion in food animals was banned because of cross-resistance to antimicrobial agents used in human therapy of enterococcal and other bacterial infections (e.g., quinupristin/dalfopristin and vancomycin). This is in contrast to the situation in the United States, where several antimicrobial agents that are regarded as important in human medicine are administered in large quantities to food animals, for purposes that include growth promotion. The consequences of this use of antimicrobial agents in food animals is illustrated by the evidence of an association between the use of gentamicin in food animals—particularly chickens and turkeys—in the United States and high-level gentamicin-resistant enterococci in humans [6].
Decreasing or stopping the use of antimicrobial agents for growth promotion in food animals in the European Union has been shown to reduce the carriage of resistant bacteria in the human population. After the European Union’s ban of the glycopeptide growth promoter avoparcin, the occurrence of vancomycin-resistant enterococci (VRE) in healthy humans in the community was reduced in Germany and in The Netherlands, as well as in hospitalized patients in Belgium [7–9].

The accumulating evidence of the beneficial consequences of the discontinuation of use of antimicrobial growth promoters in Europe has been dismissed by some because of the presumed host specificity of enterococci. The issue of host specificity of Enterococcus faecium has become important in this context because host specificity is seen by some scientists to preclude the likelihood of any adverse public health effects of the spread of antimicrobial-resistant E. faecium from animals to humans through the food chain or from the environment [10, 11]. Recently, the host specificity assumption was used in the US Food and Drug Administration’s risk assessment on streptogramin-resistant E. faecium (SREF) to assess the risk of virginiamycin use in food animals. In this risk assessment, the public health risk of zoonotic transmission of animal strains of enterococci was offered little weight, on the basis of indications of reluctance of animal enterococci to colonize the human intestinal tract [12] and of indications of host specificity of enterococci, as shown in studies of E. faecium isolates from populations of pigs, poultry, healthy human volunteers, and hospitalized humans, by amplified-frgment length polymorphism (AFLP) [13].

The esp gene in enterococci was initially found only in hospital-derived human isolates of enterococci [14–16]. This finding has been claimed to support the view that animal and human populations of enterococci do not overlap [10]. However, the gene was later observed in animal isolates and in isolates from humans in community settings [17–]. More recently a successful nosocomial genetic lineage of E. faecium (complex-17) was identified [16, 21]. Complex-17 has not been observed among animal enterococci, which may lead to the assumption that it evolved independently of the animal reservoirs of antimicrobial-resistant enterococci.

However, the existence of successful clones in particular reservoirs does not preclude the possibility that such clones were introduced from other reservoirs. More importantly, resistance genes may be exchanged between enterococci from different reservoirs. Recent studies indicate that, although animal enterococci may be relatively reluctant to colonize the human intestinal tract [12, 22], a transfer of resistance genes from animal to human enterococci does occur in vivo in the human gut [23]. Depending on the frequency of horizontal gene transfer and the availability of donor strains in the food supply, colonization does not seem to be essential for transfer of resistance genes; rather, transfer can occur as resistant bacteria are passing through the intestinal tract.

On the basis of recent scientific evidence, this article examines whether the apparent host specificity of enterococci and the occurrence of successful clones of nosocomial enterococci carrying unique features rarely found in animal enterococci are reasons to change the current view that antimicrobial-resistant enterococci in the animal reservoir pose a potential hazard to human health.

**DOES HOST SPECIFICITY PRECLUDE ANIMAL-TO-HUMAN GENE TRANSFER IN ENTEROCOCCI?**

Molecular typing of bacterial isolates obtained from different sources has been used to investigate potential host associations. Using highly discriminatory methods such as PFGE, it is difficult to establish the degree of genetic relatedness between epidemiologically unrelated strains because the banding patterns of such isolates are often diverse. Not surprisingly, the likelihood of finding genetically related isolates from different sources increases substantially when investigations are focused on isolates with temporal and spatial epidemiological relationships [24–29].

AFLP has been suggested as an alternative to PFGE because of its ability to establish genetic relatedness between strains that, through the use of PFGE, would show no similarity [30]. In a frequently quoted Dutch study, the genetic relationship among VRE strains isolated from hospitalized patients, nonhospitalized persons, and various animal sources was investigated by AFLP. Four AFLP genotypes were discriminated. Most isolates from hospitalized patients were grouped with isolates from pigs in one genogroup. Isolates from hospitalized patients were grouped with a subset of isolates from cattle and with isolates from cats and dogs in a second genogroup. Isolates from chickens and turkeys were clustered in a third genogroup, and most cattle isolates were clustered in a fourth genogroup. The authors concluded that their results supported a hypothesis of host specificity of enterococci [13]. However, this conclusion implies that hospitalized patients and persons in the community should be regarded as different hosts. In a later AFLP-based study conducted by the same group, VRE isolates from pigs and hospitalized patients as well as from nonhospitalized humans were clustered in the same genogroup [30]. Taken together, the 2 studies indicate that VRE from humans have a huge overlap with VRE from pigs and some overlap with VRE from poultry and cattle.

Irrespective of the degree of overlap between groups of enterococci from different hosts, resistance genes spread freely between enterococci in different reservoirs, as shown by the common observation of identical resistance genes in enterococci from animals and from humans [28, 29, 31–33]. Anti-
microbial-resistant enterococci in animals are likely to serve as a reservoir from which resistance genes are transferred to enterococci in humans, either through human consumption of food of animal origin, by direct contact between animals and humans, or via the environment. Apparent host specificity of enterococci, therefore, does not preclude animal to human transfer of antimicrobial resistance in enterococci.

HOSPITAL-ADAPTED CLONES OF ENTEROCOCCI

The presence of dominant strains of enterococci in hospitals may indicate adaptation to the hospital environment rather than strict host specificity. Adaptation may be associated with factors in the environment, in the patient, or in the strain itself (e.g., selective pressure from antimicrobial use, immune status of the patient, or virulence of the strain). The mechanism by which some clones of enterococci become successful clones in the hospital environment is poorly understood, but the occurrence of particular genes in isolates from the hospital environment—and not in isolates from other reservoirs—may indicate that these genes are involved in the process.

In the context of human pathogenic enterococci, the putative virulence gene esp has been investigated, and the presence of this gene [15] has been used as an argument to support the hypothesis of segregation of animal and human enterococci [10]. The esp gene in enterococci is significantly associated with infection-derived isolates of Enterococcus faecalis [34] and E. faecium [14], and it has been shown to be transferable [35]. However, the esp gene, which was initially detected only in isolates of human origin, was later also observed in enterococcal isolates from animals [19, 20, 36]. The esp gene has been shown to be part of a large pathogenicity island that harbors multiple virulence factors in E. faecalis [34]. A recent study demonstrated an E. faecalis isolate of pig origin with a pathogenicity island very similar to a pathogenicity island in an E. faecalis isolate from a human patient [37]. The identification of this pathogenicity island among enterococci isolated from farm animals indicates the occurrence of nonhuman reservoirs of infective enterococci. Thus, this gene, which seemed initially to be a unique marker of successful nosocomial clones of enterococci, may originate from enterococci in other reservoirs.

One recently identified genetic lineage of E. faecium, designated complex-17, is ampicillin resistant, pathogenicity island positive, and well adapted to the hospital environment. Complex-17 is associated with hospital outbreaks and global hospital spread [16, 21]. The fact that this lineage has not been isolated from animals could be used to argue that it evolved independently of other reservoirs of enterococci. However, historical evidence from the emergence of VRE suggests that successful nosocomial clones of enterococci acquired vancomycin resistance after they were already well established in the hospital environment [38, 39]. As a result of selective pressure in the hospital environment, successful clones may obtain virulence genes or resistance determinants from enterococci from other reservoirs when these new clones are introduced to the hospital environment.

Given the enormous heterogeneity of enterococci in the animal reservoir, finding a strain in this reservoir that is identical to particular strains from the hospital environment is unlikely. However, the introduction into the hospital environment of enterococci from animal reservoirs is likely to occur frequently (e.g., with new patients or via foods). Such strains may not survive for long in an adverse and highly selective hospital environment, but they eventually may complement strains that have already established by contributing resistance genes or other properties that enhance their survival in the hospital environment.

WHAT HAPPENS WHEN HUMANS INGEST ANIMAL ENTEROCOCCI?

Results from experiments that have investigated colonization of the human gut by enterococcal strains of animal origin indicate that animal enterococci only transiently colonize the human gut. Animal enterococci are readily available in food of animal origin [40], which is, therefore, presumably frequently ingested by humans. Studies by Sørensen et al. [12] and by Berceri [22] revealed transient intestinal carriage of quinupristin/dalfopristin- and vancomycin-resistant E. faecium of animal origin when these strains were ingested by healthy human volunteers. Interestingly, when E. faecium strains of human origin are fed to human volunteers, the same duration of colonization is observed [41]. This suggests that host specificity is not the main issue that determines prolonged colonization of the gut. Some evidence of how reluctant enterococci are to long-term colonization has come from a study of the adhesive capacity to intestinal mucus of enterococci isolates from different animal species and humans, as performed by Laukova et al. [42]. They found that various Enterococcus strains (isolated from pigs, dogs, goats, chicken, and cattle) did not preferentially bind to mucus from the same host species that the isolates originated from, and were found not to exhibit host specificity in their adhesion—although some sources appeared to contain more adhesive strains than others.

Furthermore, typing of the most common enterococci in the intestine of human volunteers has revealed that the enterococcal flora of the human intestine often changes, which indicates that enterococci in general do not colonize for extended periods of time [43].

Transfer of the vanA gene cluster between E. faecium strains in the digestive tracts of mice that were colonized with a human microbiota has been demonstrated in the absence of selective pressure [44], and transfer of other resistance genes (encoding...
quinoypristin/dalfopristin or gentamicin resistance) between *E. faecium* strains has been demonstrated in the gastrointestinal tracts of streptomycin-treated mice [45] and gnotobiotic mice [46]. Furthermore, it has been shown that vanA can be transferred from *E. faecium* of animal origin to *E. faecalis* of human origin in vivo in the intestinal tracts of mice [47].

Results from a recent study show that transfer of resistance genes from enterococci of animal origin to enterococci of human origin does occur in healthy humans [23]. In this study, transconjugants were observed in 3 of 6 human volunteers after ingestion of vancomycin- and quinoypristin/dalfopristin-resistant *E. faecium* donor strains of animal origin and susceptible *E. faecium* recipient strains of human origin. In 1 volunteer, not only was vancomycin resistance transferred, but quinoypristin/dalfopristin resistance was transferred as well. The duration of colonization and the transfer frequency would probably increase in the presence of an antimicrobial, because many enterococci of animal origin and enterococci in the intestinal tracts of nonhospitalized persons are resistant to commonly used antimicrobials, such as penicillins, tetracyclines, and macrolides [2]; however, that hypothesis was not tested in the study.

These studies show that, although enterococci can be placed in an environment different from their origin where they may be reluctant to colonize, transfer of resistance genes does occur in vivo, in animals as well as in humans. The significance of this, however, will depend on the frequency of horizontal transfer and on the availability of donor strains in the food supply. Results from a Belgian study revealed a 64% prevalence of VRE carriage among nonhospitalized persons when healthy volunteers were given oral vancomycin or teicoplanin [48]. This suggests that a large proportion of nonhospitalized persons in Belgium carried VRE at the time of the study, and that the subsequent antibiotic exposure of the volunteers selected for VRE that was already present in their gut. A recent study has shown that the vanA gene is physically linked to a pRE25-related postsegregational killing system in *E. faecium* of animal origin [49]. If the bacterium loses its vanA plasmid during cell division, the postsegregational killing system leads to cell death. Only vanA-carrying cells would survive. The system could be significant to the persistence of vanA-carrying strains of *E. faecium* in the intestinal tracts of humans or animals.

The assumption used in the US Food and Drug Administration’s SREF risk assessment (that 10% of human SREF cases may be attributed to streptomycin resistance that originates in food animal isolates of *E. faecium*) relies on data that indicate that SREF carrier rates in healthy humans are low, that colonization is transient, and that clones associated with nosocomial infections are not commonly found in animals. We believe that the risk assessors’ interpretations have not considered inherent limitations of the studies, and thus, the assumption can be questioned. There is a need for further investigation to determine the extent of overlap between animal and human reservoirs of SREF. The benefits of the use of streptogramins in animals must be considered in light of the present and, more importantly, the potential future impact on human health.

**CONCLUSIONS**

Although results of molecular typing studies may suggest that enterococci are host specific or host adapted, this does not preclude animal-to-human transfer of antimicrobial resistance or other adaptive mechanisms. Because animal enterococci are obviously readily available in food of animal origin and, thus, are presumably frequently ingested by humans, enterococci from animals are likely to contribute resistance genes, virulence factors, or other properties that enhance survival to enterococci in humans. The existence of specific nosocomial clones of enterococci does not preclude that enterococci from the animal reservoir contribute to these clones. A frequent introduction to the hospital environment of enterococci from other reservoirs is likely to occur (e.g., with new patients or through foods).

Neither results provided by molecular typing studies that classify enterococci as host-specific organisms, nor the occurrence of specific nosocomial clones of enterococci, provide reasons to change the current view that antimicrobial-resistant enterococci from the animal reservoir constitute a hazard to human health. On the contrary, antimicrobial resistance genes appear to spread freely between enterococci from different reservoirs, irrespective of their apparent host association.

**Acknowledgments**

We thank Dr. Fred Angulo for thoughtful discussion at the initiation of this manuscript.

**Potential conflicts of interests.** All authors: no conflicts.

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