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Antimicrobial Resistance in *Enterococcus* spp. of animal origin

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ABSTRACT Enterococci are natural inhabitants of the intestinal tract in humans and many animals, including food-producing and companion animals. They can easily contaminate the food and the environment, entering the food chain. Moreover, *Enterococcus* is an important opportunistic pathogen, especially the species *E. faecalis* and *E. faecium*, causing a wide variety of infections. This microorganism not only contains intrinsic resistance mechanisms to several antimicrobial agents, but also has the capacity to acquire new mechanisms of antimicrobial resistance. In this review we analyze the diversity of enterococcal species and their distribution in the intestinal tract of animals. Moreover, resistance mechanisms for different classes of antimicrobials of clinical relevance are reviewed, as well as the epidemiology of multidrug-resistant enterococci of animal origin, with special attention given to beta-lactams, glycopeptides, and linezolid. The emergence of new antimicrobial resistance genes in enterococci of animal origin, such as *optrA* and *cfr*, is highlighted. The molecular epidemiology and the population structure of *E. faecalis* and *E. faecium* isolates in farm and companion animals is presented. Moreover, the types of plasmids that carry the antimicrobial resistance genes in enterococci of animal origin are reviewed.

INTRODUCTION

Enterococcus species are natural inhabitants of the intestinal tract in humans and animals, and due to their ubiquity in human and animal feces and their persistence in the environment, enterococci are considered indicators of fecal contamination in water (1). Moreover, enterococci serve as important key indicator bacteria for several human and veterinary resistance surveillance systems.

During the evisceration process at slaughterhouses, fecal enterococci can contaminate food products of animal origin. Some studies reported that over 90% of food samples of animal origin are contaminated with enterococci at the slaughterhouse, mostly with *Enterococcus faecalis*, followed by *Enterococcus faecium* (1, 2). In addition, enterococci are opportunistic pathogens which have become one of the main causes of nosocomial and community-acquired human infections, including septicemia, endocarditis, and urinary tract infections, among others (3).

The genus *Enterococcus* presently contains over 50 species, and *E. faecalis* and *E. faecium* are the predominant isolated species, accounting for more than 80% of isolates. In addition, these two species are considered the third- and fourth-most prevalent nosocomial pathogens worldwide (4). Other *Enterococcus* species, such as *E. hirae*, *E. avium*, *E. durans*, *E. gallinarum*,

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E. casseliflavus, and *E. raffinosus*, are rare causes of human clinical infections and are thought to be more opportunistic in nature than *E. faecium* and *E. faecalis* (5–10). *E. faecalis* and *E. faecium* are also the most representative enterococcal species detected in the human intestine, whereas other species, such as *E. durans* and *E. avium*, are occasionally detected (11). The most commonly encountered enterococcal species in the guts of animals are *E. faecalis*, *E. faecium*, *E. hirae*, and *E. durans*; other species are also detected sporadically or in particular age groups (such as *E. cecorum* in older poultry) (11, 12). Several members of the genus *Enterococcus* can cause bovine mastitis, endocarditis, septicemia and amyloid encephalopathy with sudden death in chickens (13), and diarrhea in dogs, cats, pigs, and rats (12). In the past decade, *E. cecorum* has emerged as an important poultry pathogen, associated with arthritis and osteomyelitis (14–15).

The intrinsic resistance of these bacteria to several antimicrobial agents has compromised the choice of therapeutic options to treat enterococcal infections. Those intrinsic resistances confer resistance to semisynthetic penicillins (low-level resistance), aminoglycosides (low-level resistance), vancomycin (*E. gallinarum*, *E. casseliflavus*, and *E. flavescens*), and polymyxins and streptogramins (*E. faecalis*) (11). Moreover, enterococci frequently acquire antimicrobial resistance genes through plasmids and/or transposons. The antibiotic resistances in *Enterococcus* species have been reviewed previously (3, 16–18), with focuses on specific agents (such as vancomycin [19–22] or aminoglycosides [23]) or sources (livestock/food [24–26]). The zoonotic transmission potential of antimicrobial-resistant enterococci has also been reviewed (27). In this review, we update the available knowledge on the prevalence and molecular mechanisms of antimicrobial resistance in enterococcal isolates from a wide range of animals (livestock, pets, and wildlife) and animal-derived food, with particular emphasis on beta-lactams, vancomycin, and linezolid. Furthermore, we outline the major clonal lineages and plasmids responsible for antimicrobial resistance in *Enterococcus* from farm and companion animals.

DIVERSITY OF ENTEROCOCCAL SPECIES IN THE ANIMAL INTESTINAL TRACT

Enterococci are ubiquitous bacteria in the gastrointestinal tract of humans and a wide range of animals (mammals, reptiles, birds, and some invertebrates). In addition, they are commonly found in vegetables, water, soil, and food derived from animals (including fermented and dairy products) (11). Enterococci are classified as lactic acid bacteria and are highly adaptable to different environmental conditions. They survive over a wide range of temperature (10 to 45°C), and pH (4.8 to 9.6) and are able to grow at high salt concentrations (up 6.5% NaCl). Most of them can hydrolyze esculin in the presence of 40% bile salts, a characteristic used for phenotypic identification processes (11). These and other properties explain the utilization of enterococci in diverse roles; for instance, they have been used as probiotics, starter cultures, bio-preservatives, and indicators of fecal contamination of water and sanitary quality of food (28–30).

Genomic analysis revealed that members of the genus *Enterococcus* have a low G+C content, ranging from 34.29 to 44.75% (31). For a long time, *Enterococcus* species were considered streptococci of Lancefield group D. In 1984, application of nucleic hybridization and 16S rRNA sequencing led to a reclassification of *Streptococcus faecium* and *Streptococcus faecalis* in the genus *Enterococcus* (32). Currently, this genus includes around 50 species (33). Many of them were discovered in this century, mostly recovered from nonhuman sources, such as plants (*E. plantarum*, *E. ureilyticus*), water (*E. quebecensis*, *E. rivorum*, *E. ureasiticus*), animals (*E. canis*, *E. phoeniculicola*, *E. devriesei*), and food products (*E. thailandicus*, *E. italicus*) (34–42).

A recent genomic study which compared the concatenated nucleotide sequences of the core genes of 37 enterococci belonging to a variety of species divided these strains into 6 branches: (i) the *E. faecium* branch (containing *E. faecium*, *E. mundtii*, *E. durans*, *E. hirae*, *E. ratti*, *E. villorum*, *E. thailandicus*, *E. phoeniculicola*), (ii) the *E. faecalis* branch (*E. faecalis*, *E. termitis*, *E. quebecensis*, *E. moraviensis*, *E. caccae*, *E. haemoperoxidus*, *E. silesiacus*), (iii) the *E. dispar* branch (*E. dispar*, *E. canintestini*, *E. asini*), (iv) the *E. casseliflavus* branch (*E. casseliflavus*, *E. gallinarum*, *E. aquimarinus*, *E. saccharolyticus*, *E. italicus*, *E. sulfureus*, *E. cecorum*, *E. columbae*), (v) the *E. pallens* branch (*E. pallens*, *E. hermanniensis*, *E. devriesei*, *E. gilvus*, *E. malodoratus*, *E. avium*, *E. raffinosus*), and (vi) the *E. canis* branch, which contained only one strain (31). Results showed that most strains from human and other mammals were clustered into the *E. faecium*, *E. faecalis*, *E. dispar*, and *E. pallens* branches, whereas the majority of the bird isolates belonged to the *E. casseliflavus* branch.

In 1963, Mundt and colleagues carried out a survey of the occurrence of enterococci among animals living in the wild environment (43). They obtained enterococci from the feces of 71% of the studied mammals, 86% of

the reptiles, and 32% of the birds. In addition, patterns of food and animal species dependence were observed. In general, enterococci were only isolated sporadically in samples recovered from herbivorous mammals. However, they were abundant in rodents, bats, and larger animals with omnivorous or carnivorous diets (43), but as demonstrated in several other reports, the differences in the proportions of enterococci in each niche, as well as the species distributions, varied not only according to the diet, but also according to seasonal changes, individual characteristics (gender, age), and geographic location (11, 44).

In general, *E. faecium*, *E. faecalis*, *E. hirae*, and *E. durans* are the most prevalent enterococcal species in the gastrointestinal tract of humans and other mammals (11). *E. cecorum* is also a relevant member of the normal enterococcal microbiota in the gut of farm and pet animals (cattle, pigs, dogs, cats) and birds (poultry and pigeons) (45–47). However, in chickens, a significant age-dependent increase in gut colonization has been reported for this species. *E. cecorum* has been found to be a dominant part of the enterococcal gastrointestinal microbiota in mature chickens (48). Some other species, such as *E. gallinarum* and *E. avium*, which were first described in chickens, have not been frequently detected among enterococcal gut populations in poultry (49, 50).

In cattle and swine, the proportions of the enterococcal species vary across studies. *E. faecium*, *E. durans*, *E. hirae*, and *E. faecalis* were unanimously found in different surveys (46, 50–52). In some works, *E. faecalis* was the predominant enterococcal species in the gut of bovines and swines (46, 53). In others, *E. hirae* and *E. faecium* were described as the more abundant bacteria in both livestock species (44, 51, 52). As observed, variations between geographical regions might explain these differences in the composition of the enterococcal populations (44). *E. casseliflavus*, *E. gallinarum*, *E. avium*, and *E. cecorum* have also been reported as part of the bovine and swine microbiota, but they were present in lower proportions (46, 50, 51). Additionally, some minoritary species, such as *E. villorum* and *E. thailandicus*, have been sporadically detected in feces from cattle and pigs (52, 54, 55).

The enterococcal microbiota of the intestinal tract of dogs and cats showed a predominance of *E. faecalis* and *E. faecium*, followed by *E. hirae* (56–59). *E. avium* has been commonly isolated in canines and also, although in smaller proportions, in feline feces (56, 57). Other species, such as *E. durans*, *E. gallinarum*, *E. casseliflavus*, *E. cecorum*, and *E. raffinosus*, have been occasionally reported (56, 58, 59). In addition, some newly characterized species were isolated from anal swabs and chronic otitis externa (*E. canis*) and fecal samples (*E. canintestini*) of dogs (34, 60).

Enterococci are also normal residents of the gut of a wide range of free-living animals. In pigeons, the predominant species is *E. columbae* and, to a lesser extent, *E. cecorum*. However, *E. faecium* and *E. faecalis* are rare in these birds (61). Another study reported a high prevalence of enterococci among three species of coraciiform birds (74%), with a dominance of *E. faecalis*, followed by *E. casseliflavus* (62). In Portugal, *E. faecium* was the most frequently encountered species in buzzard fecal samples (63), and *E. faecium*, *E. durans*, and *E. gallinarum* were found in the feces of a variety of wild birds (64). The enterococcal gut microbiota has also been analyzed in wild marine species. *E. faecium* was identified as the most abundant species in echinoderms collected from Azorean waters. Minor species, such as *E. hirae*, *E. faecalis*, and *E. gallinarum*, were also detected (65). In a recent study in southern Brazil, different wild marine animals were analyzed using real-time quantitative PCR to identify and quantify enterococci in feces. These bacteria were found in all the studied animal species, with a dominance of *E. faecalis* and *E. mundtii* in most of the marine mammals; *E. faecalis* in green turtles, Magellanic penguins, and albatross; and *E. hirae* and *E. gallinarum* in white-backed stilts (66). Enterococci are also a relevant part of the facultative anaerobic microbiota of the gastrointestinal tract of large wild mammals (wolf, wild-boar, deer, etc.) and rodents (67–69).

Administration of antibiotics in both human and animal medicine may shift the gut microbial community, allowing drug-resistant strains (e.g., vancomycin-resistant enterococci) to proliferate dramatically. Because many enterococcal infections are caused by normal inhabitants of the gastrointestinal tract that become opportunistic pathogens, the selection of antibiotic-resistant strains raises the risk of developing difficult-to-treat infections. The following sections give an overview of the mechanisms and prevalence of antimicrobial resistance in enterococci in the animal setting.

ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI OF ANIMALS AND FOODS OF ANIMAL ORIGIN

Beta-Lactam Resistance

Enterococci are intrinsically resistant to cephalosporins and present a natural reduced susceptibility to penicillins, due to the expression of low-affinity penicillin

binding proteins (PBPs) that bind weakly to beta-lactam antibiotics. For this reason, the MICs for penicillins are higher in enterococci than in streptococci or other Gram-positive organisms, which do not produce chromosomally encoded low-affinity PBPs (17). *E. faecalis* isolates normally exhibit lower MIC values for penicillins than *E. faecium* (18).

All enterococci have at least five PBPs, and six putative PBP genes have been detected by genomic analysis in *E. faecalis* and *E. faecium* (class A: *ponA*, *pbpF*, *pbpZ*; class B: *pbp5*, *pbpA*, *pbpB*) (18). The expression of the species-specific chromosomally located *pbp5* gene, which encodes PBP5, with low affinity binding for penicillins and cephalosporins, is associated with intrinsic resistance to beta-lactams. In *E. faecium*, the *pbp5* gene is included within an operon, together with two other genes that are also implicated in cell wall synthesis (*psr* and *ftsW*) (18).

Acquired (enhanced) resistance for penicillins (penicillin or ampicillin) has been frequently detected among clinical *E. faecium* isolates, being rare in *E. faecalis*. High-level ampicillin resistance in *E. faecium* (MIC, ≥128 μg/ml) has been associated with increased production of PBP5 (requiring a higher concentration of the agent to saturate the active site) or with specific amino acid changes in its sequence, which make the low-affinity PBP5 even less susceptible to inhibition by penicillins (70, 71). The amino acid substitutions near the Ser-Thr-Phe-Lys, Ser-Asp-Ala, and Lys-Thr-Gly motifs, which are part of the active-site cavity, seem to be the most significant ones (16).

Combinations of specific amino acid changes in the C-terminal transpeptidase domain of PBP5 (especially the substitution Met-485-Ala/Thr, but also the changes Ala-499-Ile/Thr, Glu-629-Val, and Pro-667-Ser), and the insertion of serine or aspartic acid after position 466, have been associated with ampicillin resistance in *E. faecium* isolates (72–76). It has been found that single substitutions at positions 485, 499, 629, and 466-insertion have only slight influence on ampicillin MIC, but when combined, the effect increases. Mutations in genes encoding other species-specific proteins that participate in cell wall synthesis may also slightly increase the MIC value (76).

Two distinct allelic forms have been identified when the whole sequence of the *pbp5* gene is considered, which differ in 5% of the sequence, yielding two types of PBP5 (PBP5-S and PBP5-R) with changes in 21 amino acid residues. PBP5-S is usually detected in community-associated ampicillin-susceptible *E. faecium* isolates (MIC of usually ≤2 μg/ml), and PBP5-R is usually detected in hospital-associated ampicillin-resistant isolates (MIC of usually ≥16 μg/ml) (77, 78). A hybrid-like type of PBP5 (PBP5-S/R), with a sequence between the other two types, has been observed in some isolates, with a MIC for ampicillin of around 4 μg/ml (77, 78).

Considering the population structure of *E. faecium*, two main lineages have been postulated in humans: (i) subclade A1, hospital-associated, enriched in mobile genetic elements, usually implicated in human infections, and in most cases, ampicillin-resistant (MIC, ≥16 μg/ml) with the consensus allele *pbp5*-R, and (ii) clade B: community-associated, detected in isolates from healthy humans (not implicated in infections), generally ampicillin-susceptible (MIC, ≤2 μg/ml), and harboring the consensus allele *pbp5*-S. The subclade A2 includes *E. faecium* isolates mostly from animal settings, exhibits a wide range of ampicillin MIC values (0.5 to 128 μg/ml), and generally carries the hybrid-like *pbp5* allele (*pbp5*-S/R) (72, 78, 79). In addition to amino acid sequence alteration in PBP5, elevated levels of this protein are also observed in highly ampicillin-resistant isolates of clade A (subclade A1 and part of A2), but not in the ampicillin-susceptible isolates of subclade A2 and clade B, suggesting a differential regulation process in each clade. The upstream region of *pbp5* seems to have a role in the level of expression of the gene (72).

In *E. faecalis*, acquired ampicillin resistance is unusual but is generally mediated by mutations in *pbp*4 (27, 80). Selected strains of *E. faecalis* produce a plasmid-mediated beta-lactamase that is similar to the enzyme produced by *Staphylococcus aureus* (17, 81), encoded by the *blaZ* gene, although some polymorphisms in this gene have also been detected in some isolates. This beta-lactamase is expressed in a constitutive way in *E. faecalis*, in contrast to the inducible production in *S. aureus*. The enzyme is produced in low amounts in *E. faecalis*, and for this reason, the strain can appear as ampicillin susceptible when the MIC is tested *in vitro*. In any case, this mechanism of resistance is very infrequently seen in *E. faecalis*. Very unusual beta-lactamase-producing *E. faecium* strains have also been reported (82). Chromosomal beta-lactamase-encoding genes conferring ampicillin resistance have also been detected in *E. faecium* isolates (83).

The *in vitro* transferability of *pbp5* in *E. faecium* isolates (84), which suggests a mechanism by which high-level ampicillin resistance conferred by mutated *pbp5* alleles could be disseminated among clinical isolates, has been reported. Moreover, Novais *et al.* (85) demonstrated *in vitro* ampicillin-resistance transference

by conjugation in 28% of the *E. faecium* isolates from a pig farm environment, although the genetic basis of this transference was not determined. Codiversification of the *E. faecium* core genome and *pbp5* has been recently analyzed, showing evidence of *pbp5* horizontal transfer (86).

Various studies have evaluated the prevalence of penicillin or ampicillin resistance in enterococci from food-producing animals, pets, or wild animals, as well as in those from food of animal origin. For *E. faecium*, the prevalence of resistance is variable depending on the country and the type of animal. Reflecting this, no resistant *E. faecium* isolates were detected in a surveillance study performed in a cattle population at slaughter in Australia (87), but a rate of 30% resistance was detected in isolates of poultry in Portugal (88). For pets, the following ampicillin resistance rates were reported among *E. faecium* isolates: 63% and 37% in dogs and cats, respectively, in the United States and 3% in pets in Portugal (58, 88). Moreover, ampicillin-resistant *E. faecium* isolates were detected in 23% of the dogs screened in a cross-sectional study in the United Kingdom and in 76% of the dogs analyzed in a longitudinal study in Denmark (89). Most of these resistant isolates belonged to the hospital-adapted clonal complex CC17. Frequencies of ampicillin resistance in the range of 4.5 to 7.7% have been detected in *E. faecium* isolates recovered from wild animals (wild boar, Iberian wolf, and gilt-head seabream) (74, 90, 91), but no resistant isolates were detected in Iberian lynx (92).

A surveillance study was performed analyzing the prevalence of antimicrobial resistance in 21,077 *Enterococcus* isolates obtained from retail meat samples in the United States between 2002 and 2014, through the National Antimicrobial Resistance Monitoring System (NARMS) (2). A low frequency of ampicillin resistance was detected among *E. faecium* isolates from ground beef and pork chops (4% and 2.7%, respectively), but higher percentages were detected in retail chicken (26%), and even higher in ground turkey (62.6%). Bortolaia *et al.* (25) reviewed ampicillin resistance data reported in European countries (Denmark, Sweden, The Netherlands, Slovenia) and the United States for *E. faecium* isolates recovered from poultry meat, comparing them to human isolates in the same countries (93–95). Human isolates showed very high rates of ampicillin resistance in all countries (>80%), but resistance in food isolates was significantly lower than in those of humans. Of note is the detection of 10% ampicillin resistance in *E. faecium* of (imported) broiler meat in Denmark and >50% resistance in isolates of turkey meat in the United States. Almost no ampicillin-resistant *E. faecalis* isolates (with very few exceptions) have been reported in animals or food of animal origin.

Glycopeptide Resistance

Mechanism of resistance

Vancomycin and teicoplanin are important members of the glycopeptide family and are used for the treatment of severe human infections. Avoparcin, another member of this family, has been extensively used in the past as a growth-promoter in food-producing animals in many countries.

The mechanism of action of glycopeptides is the inhibition of the synthesis of the bacterial cell wall, by the link to the D-Ala-D-Ala terminus of the pentapeptide precursor of the peptidoglycan, preventing cross-linking of the peptidoglycan chain and inhibiting cell wall synthesis. The main mechanism of glycopeptide resistance in enterococci implicates the alteration of the peptidoglycan synthesis pathway. In this sense, the terminus D-Ala-D-Ala of the pentapeptide to which vancomycin binds is modified to D-Ala-D-Lac (causing high-level vancomycin resistance; MIC, >64 μg/ml) or to D-Ala-D-Ser (low-level vancomycin resistance; MIC, 4 to 32 μg/ml). These modified cell-wall precursors bind glycopeptides with reduced affinity (about 1,000-fold and 7-fold for D-Lac and D-Ser substitutions, respectively) (18, 22).

The first vancomycin-resistant enterococci (VRE) with an acquired mechanism of resistance were detected three decades ago in clinical *E. faecium* isolates in France and the United Kingdom (96, 97). Since then, VRE have been extensively described in hospitals worldwide; they have been seen especially frequently in the United States since the 1990, mostly in patients in intensive care units, and at lower levels in Europe since the 2000s (21). According to surveillance data from the European Centre for Disease Prevention and Control (EARS-Net), the European Union/European Economic Area population-weighted mean percentage of vancomycin resistance in *E. faecium* was 11.8% in 2016, and national percentages ranged from 0 to 46.3%; the prevalence of vancomycin resistance for *E. faecalis* was lower (98).

Vancomycin resistance is mediated by *van* operons, which encode the modified peptidoglycan precursors. To date, eight *van* operons have been identified in enterococci mediating acquired vancomycin resistance (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*), and one additional operon in intrinsic vancomycin resistance (*vanC*) (18, 19, 99–102). Three variants of the gene *vanC* have been described (*vanC1*, *vanC2*, and *vanC3*), intrinsic to *E. gallinarum*, *E. casseliflavus*, and *E. flave-*

scens, respectively. Moreover, different subtypes have been identified for *vanB* (*vanB1*, *vanB2*, and *vanB3*), *vanD* (*vanD1* to *vanD5*) and *vanG* (*vanG1*, *vanG2*) (100, 103, 104). An additional variant, *vanF*, has also been described, but until now only in the environmental microorganism *Paenibacillus popilliae* (105).

vanA and *vanB* are the most frequent genotypes among VRE with acquired resistance mechanisms of humans and animals, mostly among *E. faecalis* and *E. faecium*. The genotypes *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN* are very unusual in VRE isolates, and *E. faecalis* (*vanE/G/L*) and *E. faecium* (*vanD/M/N*) are the most common carriers (22).

The *vanA* operon is associated with the transposon Tn*1546* and includes seven open reading frames transcribed under two different promoters (106). Regulation is mediated by a *vanS-vanR* (sensor-kinase-response regulator) two-component system, transcribed with a common promoter (107). The remaining genes are transcribed from a second promoter (22). The proteins encoded by *vanH* (dehydrogenase that converts pyruvate into lactate) and *vanA* (ligase that forms a D-Ala-D-Lac dipeptide) modify the synthesis of peptidoglycan precursors; moreover, the proteins encoded by both *vanX* (dipeptidase that cleaves D-Ala-D-Ala) and *vanY* (D,D-carboxipeptidase), interrupt the formation of the D-Ala-D-Ala end of the pentapeptide, and the *vanZ* gene is related to teicoplanin resistance (22, 108). Different insertion elements (ISs) can be included in the *vanA* operon, rendering different variants (109).

The *vanB* operon has been associated with different transposons (Tn*1547*, Tn*1549*, and Tn*5382*). Tn*1549* is widely prevalent among *vanB*-type enterococci, usually located in the chromosome and less frequently on plasmids (22). The structure of the *vanB* operon is similar to that of *vanA*, with two promoters and seven open reading frames, but with important differences, mostly in the two-component signaling regulatory system (encoded by $vanR_B$ and $vanS_B$) and in the absence of a homolog of *vanZ* (substituted by *vanW*, of unknown function); consequently, *vanB* enterococci show vancomycin resistance (high or low level) but teicoplanin susceptibility (22, 108). The structure of the different *van* operons and their mechanisms of action have been extensively reviewed in previous studies (17–19, 21, 22, 108, 110).

Origin of vancomycin resistance

Partially preassembled glycopeptide resistance-associated gene clusters present in environmental organisms are suggested as the source of the vancomycin resistance genes in VRE (105, 111). The environmental organism *P. popilliae*, carrier of a *vanF* variant with high similarity at the amino acid level to *vanA*, has been suggested as the potential origin of vancomycin resistance in enterococci. To a lesser extent, this role could also be attributed to glycopeptide-producing organisms (e.g., the vancomycin-producing organism *Amycolatopsis orientalis*), which require these genes to inhibit the action of produced glycopeptides (111). Nevertheless, the genes in these organisms are probably not the direct source of the enterococcal vancomycin resistance genes since they are similar but not identical; in this sense, transference could have occurred from a common ancestral bacterium or via one or more bacterial intermediaries. In addition, considering the differences in G+C content, as well as the sequence homology among different organisms, it is possible that the genes of the *van* cluster could have more than one origin (111).

Historical aspects related to glycopeptide resistance

During the 1990s, VRE with the *vanA* genotype emerged in food-producing animals, healthy humans, food products, and environmental samples throughout Europe and other countries; this emergence was linked to the use of the glycopeptide avoparcin since the mid-1970s, in subtherapeutic concentrations, as an animal growth promoter (22, 26, 112, 113). This hypothesis was tested in poultry flocks and pig herds receiving or not receiving avoparcin, confirming the significant role of avoparcin in VRE selection in the animals (112, 113). This association was also corroborated in an animal model with young chickens receiving avoparcin supplementation (114). Avoparcin was banned as a growth promoter in the European Union in 1997, and a clear decrease in VRE fecal carriage in food-producing animals and healthy humans was observed (115), as well as in food-derived products. Nevertheless, VRE persisted in the animal setting many years after the avoparcin ban (116, 117). A similar situation happened in Taiwan after the ban of avoparcin in 2000 that resulted in a clear decrease of VRE prevalence in chickens, although it still persisted in this animal population (118). In dogs, high rates of fecal VRE carriage were reported before the avoparcin ban in the European Union (119), although no VRE was detected in dogs in Spain a decade after the ban (120). The frequency of human infection by VRE in the European Union was low during the period of high prevalence in animals, but an increase in the frequency of VRE-related human infections was evidenced since 1999 (22).

The situation in the United States and Canada was completely different than that in the European Union. Avoparcin use has never been approved in animal production in those countries, and VRE was not reported in animals until the end of the 2000s (20, 76, 121, 122). Nevertheless, in North America, VRE was a frequent cause of human infections, especially in patients in intensive care units, which was attributed to the high use of vancomycin in humans (22, 123). The differences in VRE prevalence in humans and animals in the European Union and the United States before and after the avoparcin ban in the European Union introduce some doubts about the possible routes of transmission of VRE determinants between animals and humans (22, 124).

Different theories have been postulated to explain the persistence of VRE in food-producing animals after the avoparcin ban in the European Union and in other countries, such as coselection by the use of other antimicrobials (e.g., erythromycin and tetracycline). It has been shown that *vanA* and *erm*(B) genes (the latter implicated in erythromycin resistance) are frequently located in the same transferable plasmids (113). Moreover, the gene *tcrB*, implicated in copper resistance, has been detected in pig *E. faecium* isolates in the same plasmid as *vanA* and *erm*(B) (125). However, the presence of plasmid addition systems in the same plasmid that carries the *vanA* gene could force bacteria to retain the resistance (125).

VRE in food-producing animals and food of animal origin

Tables 1 and 2 summarize the papers that have been published related to the prevalence and mechanisms of vancomycin resistance in enterococci isolated from food-producing animals and food of animal origin, respectively, as well as the genetic lineages of the isolates (when available). The data are organized by animal species (poultry, pigs, and cattle, among others) and by the year the isolates were recovered. Many of the studies were performed in European countries, but studies in the American, African and Asian countries, as well as Australia and New Zealand are also included.

Most of the surveys of food-producing animals reported *E. faecium* as the major species of the genus *Enterococcus* exhibiting acquired resistance to vancomycin, in most cases with the *vanA* genotype. However, *vanA*-containing *E. faecalis* isolates, and to a lesser extent *E. durans* and *E. hirae* isolates, have also been frequently detected in food-producing animals (Table 1) (27, 85, 87, 114, 121, 122, 125–165). Other enterococcal species have occasionally been reported as *vanA* carriers, such as *E. mundtii* in poultry in Hungary (130) and *E. casseliflavus* in cattle in France (158) and in horses and swine in Italy (159). Available data indicate that *vanA* was, by far, the main gene responsible for acquired VRE in food-producing animals worldwide, regardless of the species. Nevertheless, *vanB* (and especially the *vanB2* variant) was occasionally detected. The first detection of *vanB2* in animals was in a vancomycin-resistant *E. hirae* isolate recovered from a pig in Spain in 2008 (145); later, *vanB*-positive *E. faecium* and *E. faecalis* isolates were detected in poultry in Czech Republic (132) and in *Enterococcus* species in pigs in South Africa (147). Moreover, *vanC1* was detected as an acquired gene in isolates of *E. faecium*, *E. faecalis*, and *E. mundtii* in poultry in Australia (140). In most of the studies, VRE were detected when a selective protocol with media supplemented with vancomycin was used (Table 1). Resistance frequencies varied depending on the type of animals tested (poultry, 0 to 77%; pigs, 0 to 25.3%; cattle, 0 to 0.5%), the year the study was performed, the country, and the protocol used for VRE recovery (see Table 1). *vanA*-containing enterococci have also been detected in ostriches and mullet fish in Portugal (prevalence of resistance of 7.4% and 3.9%, respectively) (164). In eight of the reviewed papers in which VRE were detected in food-producing animals, the multilocus sequence typing (MLST) data were provided for *vanA*-positive *E. faecium* (most isolates) or *E. faecalis* isolates. A wide variety of sequence types were identified among the *E. faecium* isolates from poultry and pigs (>30 sequence types) (27, 85, 121, 122, 127, 129, 144, 156). Also, the lineage sequence type 6 (ST6) (CC2) was identified in *E. faecalis* of pig origin (85).

The *E. faecium* species carrier of the *vanA* gene was the most frequent VRE detected in food of animal origin. Nevertheless, *vanA*-containing *E. faecalis*, *E. durans*, and *E. hirae* isolates were also frequently detected in these types of samples (Table 2) (2, 118, 128, 133, 162, 166–194). VRE with the *vanB* gene was found in *E. faecium* isolates from veal and chicken in Spain (ST17-*vanB2*) (188) and in different types of food in Greece (*vanB2/3*) and Spain (*vanB*) (181, 190). The identification of the unusual *vanN* gene in five *E. faecium* isolates from chicken meat in Japan is interesting, showing a low level of vancomycin resistance (MIC, 12 μg/ml) (177). Also notable is the unusual detection of *vanA*-containing *E. cecorum* isolates in chicken samples in Japan (168), *vanA*-positive *E. gallinarum* in fish in Egypt (193), and *vanC1*-positive *E. faecalis* isolates from sheep milk samples in Spain (192). The frequencies of detection of VRE

TABLE 1 Summary of reports about detection of VRE with acquired mechanisms of resistance in healthy food-producing animals

Animal species	Year of recovery of tested isolates	Country	% Prevalence[a]	Species ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Poultry	1996	Spain	11/15	*E. hirae*/*E. faecium* (*vanA*)	+	114
Poultry	1997	Netherlands	29 (poultry and farmers)	*E. faecium*/*E. durans*/*E. hirae* (*vanA*) *E. faecalis* (*vanA*)	+	126
Poultry	1997	Norway	0.8–4.6	*E. faecium* ST26/ST146/ST195/ ST242/ST248/ST9/ST241/ ST244/ST245 (*vanA*)	+	127
Poultry	1997–1998	Spain	16	*E. durans* (*vanA*)	+	128
Poultry	1998–1999	Norway	34	*E. faecium* (*vanA*)	+	125
Poultry	2000–2007	Sweden	<1 (2000), 40 (2005), 30 (2006–2007)	*E. faecium* ST13/ST370/ ST310 (*vanA*)	+	129
Poultry	2001–2004	Hungary	8.6 (2001), 23 (2002), 0 (2003–2007)	*E. faecium*/*E. durans*/ *E. mundtii* (*vanA*)	+	130
Poultry	2002–2003	New Zealand	5.8	*E. faecium*/*E. faecalis*/ *E. durans* (*vanA*)	+	131
Poultry	2002–2004	Czech Republic	2.1	*E. faecium* (*vanA*), *E. faecium*/ *E. faecalis* (*vanB*)	–	132
Poultry	2003	Korea	1.4	*E. faecium* (*vanA*)	+	133
Poultry	2003–2004	Canada	0		–	134
Poultry	2003–2004	Canada	0		–	135
Poultry	2004	Portugal	9.2	*E. faecium*/*E. durans*/*E. hirae* (*vanA*)	+	136
Poultry	2005	Hungary	1.1	*E. faecium*/*E. faecalis* (*vanA*)	+	137
Poultry	2005–2008	Greece	14.4	*E. faecium* (*vanA*)	+	138
Poultry	2009	Germany	17.6	*Enterococcus* spp. (*vanA*)	+	139
Poultry	2000	Australia	8.6	*E. faecium*/*E. mundtii*/ *E. faecalis* (*vanC1*)	+	140
	2008–2009		0	-	+	
Poultry	2010	Denmark	47	*E. faecium* ST10/ST12/ST22/ST26/ ST38/ST157/ST417/ST520/ST587/ ST784/ST785/ST839/ST840/ ST841/ST842 (*vanA*)	+	27
Poultry	2010–2011	Germany	0		–	141
Poultry	2013–2014	Poland	0.11	NS[c]	–	142
Poultry	NS	Italy	8.7	NS	–	143
Poultry	NS	Sweden	55–70 (few samples)	*E. faecium*- ST310- (*vanA*)	+	144
Pigs	1998	Spain	6.1	*E. faecium* (*vanA*), *E. hirae* (*vanB2*)	+	145
Pigs	1998–1999	Spain	8.0	*E. faecium* (NS), *E. hirae* (NS), *E. durans* (NS)	+	146
Pigs and farm facilities	2006–2007	Portugal	5	*E. faecium* ST132/ST185 (CC5) (*vanA*), *E. faecium* ST443 (*vanA*), *E. faecalis* ST6 (CC2) (*vanA*)	+	85
Pigs	2009	USA	10.9	*E. faecium* ST5/ST6/ ST185 (CC5) (*vanA*)	+	121
Pigs	2009–2010	USA	8.2	*E. faecium* ST5/ST6/ ST185 (CC5) (*vanA*)	+	122
Pigs	2014	South Africa	NS	*Enterococcus* spp. (*vanB*)	–	147
Pigs	NS	Australia	0		NS	148
Poultry and pigs	1998–1999	Costa Rica	14.8 (poultry), 10.9 (pigs)	*E. faecium*/*E. faecalis*/ *E. durans*/*E. hirae* (*vanA*)	+	149
Poultry and pigs	1998–2000	European countries	8.2	*E. faecium*/*E. faecalis*/ *E. hirae* (*vanA*)	+	150

(continued)

TABLE 1 Summary of reports about detection of VRE with acquired mechanisms of resistance in healthy food-producing animals *(continued)*

Animal species	Year of recovery of tested isolates	Country	% Prevalence[a]	Species ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Poultry and pigs	2002	UK and Wales	24 (poultry), 5 (pigs)	*E. faecium* (*vanA*)	+	151
Poultry and pigs	2005	France	1.6 (poultry), 6.2 (pigs)	*E. faecium*/*E. faecalis*/ *Enterococcus* spp. (*vanA*)	+	152
Poultry and pigs	2009	China	0		–	153
Poultry and cattle	2014	Nigeria	0		–	154
Poultry and cattle	NS	Ethiopia	30–54	NS	–	155
Poultry, pigs, cattle	2008–2013	China	0.2 (pigs), 0 (poultry), 0 (cattle)	*E. faecium* ST6 (CC5) (*vanA*)	+	156
Poultry, pigs, cattle	NS[a]	Austria	47.8 (poultry), 0.5 (cattle), 0 (pigs)	*E. faecium*/*E. durans* (*vanA*)	+	157
Cattle	2003–2004 (healthy cattle)–2006 (sick cattle)	France	0.1 (healthy cattle), 0.4 (sick cattle)	*E. faecium*/*E. faecalis*/ *E. casseliflavus* (*vanA*)	+	158
Cattle	NS[a]	Australia	0		–	87
Equines and swine	2005	Italy	6.7 (equine), 16.1 (swine),	*E. faecium*/*E. faecalis*/ *E. casseliflavus* (*vanA*)	+	159
Sheep, pigs, cattle	2008–2009	Portugal	25.3 (pigs), 2.7 (sheep), 0 (cattle)	*E. faecium*/*E. hirae* (*vanA*)	+	160
Farm animals	1998–2003	USA	0		+	161
Farm animals	2000–2001	Korea	0.67	*E. faecium* (*vanA*)	+	162
Farm animals	2001	Korea	16.7 (chicken), 1.9 (pigs), 0 (cattle)	*E. faecium* (*vanA*)	+	163
Ostriches	2009–2010	Portugal	7.4	*E. durans* (*vanA*)	+	164
Mullet fish	2006–2007	Portugal	3.9	*E. faecium* (*vanA*)	+	165

[a]Some characteristics (year of isolation, or type of samples) are included in parenthesis.
[b]ST (CC), sequence type (clonal complex), if data are available.
[c]NS, not specified.

with acquired resistance in food samples were variable (Table 1). In chicken and pork food samples analyzed from 1996 to 1999, the prevalence was in the range of 4.2 to 34% (Table 2), with a few exceptions (1.3%) (167). Very high frequencies were detected in different types of food in Korea (44%) (133), but no VRE were found in the studies performed in the United States (2, 171, 185). In some cases, isolates showing a phenotype usually associated with the *vanB* genotype (high-level resistance to vancomycin, susceptibility to teicoplanin) were detected in *Enterococcus* strains harboring the *vanA* gene (118, 168, 173).

VRE in companion animals

Table 3 shows the detection of VRE with acquired mechanisms of resistance in companion animals. *vanA*-containing *E. faecium* is a unique type of VRE with acquired resistance reported in dogs and cats (136, 145, 195–202). These isolates, recovered from fecal samples from 1996 to 2003, were found in the United States, Spain, and Portugal, with variable frequencies of detection (ranging from 2.8 to 22.7%) (136, 145, 195, 196). No VRE were detected in studies performed in the following years (Table 3), not even in sick dogs (197, 200). Vancomycin-resistant *E. faecium* and *E. durans* isolates were detected in fecal samples of equids obtained in 2007 to 2008 (prevalence 4.4%) in a study performed in Portugal (202).

VRE in free-living animals

Table 3 also shows the detection of VRE with acquired mechanisms of resistance in free-living animals, including different species of mammals and birds (136, 165, 203–226). Many studies have been performed with this

TABLE 2 Summary of reports about detection of VRE with acquired mechanisms of resistance in food samples of animal origin

Origin of the food sample	Year of recovery of tested isolates	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Chicken	1995–1996	Japan	3.0	*E. faecium*/*E. faecalis* (*vanA*)	–	166
Chicken	1997–1998	Spain	27.2	*E. faecium*/*E. faecalis*/ *E. durans*/*E. hirae* (*vanA*)	+	128
Chicken	1997–1998	Belgium	1.3	*E. faecium* (NS[c])	–	167
Chicken	2000–2001	Korea	60 (15 samples)	*E. faecium* (*vanA*)	+	162
Chicken	2000–2003	Taiwan	13.7 (2000), 3.7 (2003) (*E. faecalis*), 3.4 (2000)–0 (2003) (*E. faecium*)	*E. faecium*/*E. faecalis* (*vanA*)	+	118
Chicken	2009	Japan	4.5 (22 samples)	*E. cecorum* (*vanA*)	+	168
Chicken	2009	Colombia	3.7	NS[c]	–	169
Chicken	2009–2010	Turkey	0	–	–	170
Chicken	NS	USA	0	–	–	171
Chicken	NS	UK	18.5	NS	+	172
Food and poultry	1999–2001	Portugal	34.0	*E. faecium*/*E. faecalis*/ *Enterococcus* spp. (*vanA*)	+	173
Chicken and pork	1996–1997	Germany	12.3	*E. faecium*/*E. durans*/ *E. faecalis*/*E. hirae* (*vanA*)	+	174
Chicken and pork	1997–1998	Italy	15.9 (1997), 8.1 (1998) (poultry product) 4.2 (1997), 6.9 (1998) (pork products)	*E. faecium*/*E. faecalis*/ *E. durans*/*E. hirae* (*vanA*)	+	175
Chicken and pork	1999	France	10.2	*E. faecium*/*E. durans* (*vanA*)	+	176
Chicken and pork	2011	Japan	0.6 (chicken meat)	*E. faecium* (*vanN*)	+	177
Various types of food	2000–2002	Germany	0		–	178
Various types of food	2010	Argentina	7.5	*E. faecium* (*vanA*)	–	179
Various types of food	2010	Iran	NS	*E. faecium* ST669 (*vanA*)	+	180
Various types of food	2010–2012	Greece	21.9	*E. faecium* (*van*A), *E. faecium* (*vanA* + *van*B2/3), *E. faecium* (*vanB2/3*)	+	181
Various types of food	2012	Italy	3.53	NS	–	182
Various types of food	NS	Spain	5.4	NS	–	183
Various meats	1997–1998	Italy	14.6 (1997), 8.0 (1998)	*E. faecium*/*E. faecalis*/ *E. durans*/*E. hirae* (*vanA*)	+	184
Various meats	2001–2002	USA	0		–	185
Various meats	2003	Korea	44	*E. faecium* (*vanA*)	+	133
Various meats and feces	2004–2006	Japan	0		+	186
Various meats	2007–2008	Canada	0		–	187
Various meats	2007–2009	Spain	3.9	*E. faecium* ST78 (CC17)/ST425 (*vanA*), *E. durans*/*E. hirae* (*vanA*), *E. faecium* ST17 (CC17) (*vanB2*)	+	188
Various meats	NS	Canada	0		–	189
Various meats	NS	Spain	19.4	*E. faecium*/*E. durans*/*E. hirae* (*vanA*), *E. faecium* (*vanB*)	+	190
Retail meats	2002–2014	USA	0		–	2
Cheese	2005	France	0		+	191
Milk (sheep)	NS	Spain	1.7	*E. faecalis* ST168 (*vanC1*)	–	192
Shellfish	NS	UK	2.7	NS	–	172
Fish (Tilapia)	NS	Egypt	37.5 (8 samples)	*E. faecalis*/*E. gallinarum* (*vanA*)	–	193
Frozen food	NS	Thailand	9.7	NS	–	194

[a]Some characteristics (year of isolation or type of samples) are included in parenthesis.
[b]ST (CC), Sequence type (clonal complex), if data are available.
[c]NS, not specified.

type of animal, including in various countries in Europe, the Americas (United States, Canada, and Brazil), and Africa (Tunisia and Tanzania). The most frequently detected mechanism of resistance was *vanA*, mainly among *E. faecium* isolates, followed by *E. faecalis* (*E. durans* and *E. hirae* were infrequently detected). Occasionally, enterococci were found to be *vanB* carriers: two small mammals (*Rattus rattus*) harbored *vanB2*-containing *E. faecalis* ST6 isolates in Spain (204), and *E. faecium vanB* was detected in wild game meat, also in Spain (226). The frequencies of detection of *vanA*-containing enterococci in wild animals ranged from 0 to 13.5%, with the highest values detected in red foxes, seagulls, and buzzards in Portugal (9 to 13.5%) (216, 220, 222). Interestingly, *vanA*-containing *E. faecium* isolates detected were ascribed to different sequence types included in the high-risk clonal complex CC17 (ST18, ST262, ST273, ST280, ST313, ST362, ST412, ST448, and ST555). These isolates were detected in corvids in the United States and in mullet fish, gilt-head seabream, seagulls, buzzards, partridges, red foxes, and Iberian wolves in Portugal (Table 3).

Resistance to Linezolid

The widespread occurrence of VRE in many countries makes it necessary to look for other therapeutic options, and linezolid is an important one. This oxazolidinone, introduced in 2000 in the United States and in 2001 in the United Kingdom, is an important agent for the treatment not only of VRE, but also of other Gram-positive bacteria, such as methicillin-resistant *S. aureus*.

Linezolid resistance is still unusual among enterococci but has emerged in recent years in human and animal isolates (227). Mutations in the central loop of domain V of the 23S rDNA is the most common mechanism of resistance in enterococci, the amino acid change G2576T being the predominant one, although other changes have also been described (G2505A, U2500A, G2447U, C2534U, and G2603U) (18). *E. faecalis* and *E. faecium* possess four and six 23S rDNA alleles per genome, respectively, and depending on the number of mutated versus wild-type alleles per genome, these correlate with the level of resistance of the isolates (227). In some cases, this mechanism appears during the course of treatment with oxazolidinones, and nosocomial transmission of linezolid-resistant enterococci has been reported (228). Linezolid-resistant *E. faecalis* and *E. gallinarum* isolates of swine origin were detected in China (MIC, 8 to 16 μg/ml), and the nucleic acid change G2576T was identified in the 23S rDNA of these isolates (229). Mutations in the ribosomal proteins L3, L4, and L22 can confer decreased susceptibility to linezolid in enterococci and staphylococci (230).

In recent years, there has been concern about the emergence of transferable resistance to linezolid, associated with the acquisition of the *cfr* gene or with the recently described *optr*A gene. The *cfr* gene has been detected in enterococci of both human and animal origin (231) and encodes an rRNA methyltransferase that modifies the adenine residue at position 2503 in domain V of the 23S rRNA; it confers resistance to oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A (the phenotype named $PhLOPS_A$) (18). Among oxazolidinones, linezolid is mostly affected by *cfr*; tedizolid, a new compound of this family, showed increased activity in *cfr*-positive enterococci, so these isolates are susceptible to this agent. Table 4 summarizes the data published until now in relation to linezolid resistance mechanisms in enterococci of animal and food origin, as well as in enterococci of environmental origin (229, 232–241).

The *cfr* gene was identified for the first time in enterococci in 2011, specifically in an *E. faecalis* isolate recovered on a dairy farm in China (232). Since then, *cfr* has been detected in human clinical *E. faecalis* isolates (242), as well as in swine *E. casseliflavus*, *E. gallinarum*, and *E. faecalis* isolates in China and Brazil (233–235) and in a cattle *E. faecalis* isolate in China (234). A second variant of the *cfr* gene, named *cfr*(B), has been described in *E. faecium* isolates of human origin. This new plasmid-located variant is more similar to a *cfr*-like gene of *Clostridium difficile* than to the *cfr* genes of staphylococci or other enterococcal species (243, 244), and it has so far not been detected in enterococci of animal origin.

The novel *optr*A gene confers transferable resistance to oxazolidinones (both linezolid and telizolid) and phenicoles (chloramphenicol and florfenicol) and has been detected in *E. faecalis* and *E. faecium* isolates of both human and animal origin (236). This gene encodes an ABC transporter and has been detected more frequently in *E. faecalis* than in *E. faecium* isolates and more frequently in isolates from food-producing animals (pigs and chickens) than in those of human origin (236). The *optr*A gene has been detected both in chromosomal and in plasmidic locations in animal and human *E. faecalis* and *E. faecium* isolates. As shown in Table 4, *optr*A-positive enterococci have been detected in food-producing animals (poultry, pigs, and occasionally, cattle) in Asiatic countries, mostly in *E. faecalis* and *E. faecium* belonging to many different sequence types, and sporadically in *E. gallinarum*. The prevalence

TABLE 3 Summary of reports about detection of VRE with acquired mechanisms of resistance in companion and free-living animals

Animal species	Isolation year	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Companion animals						
Dogs (sick)	1996–1998	USA	2.8	*E. faecium* (*vanA*)	–	195
Dogs/cats	1998	Spain	22.7 (22 samples)	*E. faecium* (*vanA*)	+	145
Dogs	1998–2003	Spain	12.6	*E. faecium* (*vanA*)	+	196
Dogs/cats	2003	Portugal	2.6 (dogs) 0 (cats)	*E. faecium* (*vanA*)	+	136
Dogs (sick)	2008–2009	USA	0		–	197
Dogs	2009	Spain	0		+	120
Dogs/cats (antibiotic-treated)	2011–2012	Japan	0		–	198
Dogs/cats	2014	Egypt	0		–	199
Dogs (sick)	NS[c]	Portugal	0		–	200
Dogs/cats	NS	Turkey	3.8 (dogs) 0 (cats)	NS[c]	–	201
Equids	2007–2008	Portugal	4.4	*E. faecium*/*E. durans* (*vanA*)	+	202
Free-living animals						
Wild mammals	1997–2000	England	1.2 (badgers) 4.6 (woodmice)	*E. faecium* (*vanA*)	–	203
Wild animals	2004	Portugal	0		+	136
Wild animals	2008–2013	Spain	2.0	*E. faecium*-ST915 (*vanA*), *E. faecalis* ST6 (CC2) (*vanB2*)	+	204
Wild animals	2012	Poland	14.9	NS	–	205
Wild animals	2014–2015	Spain	0.3	*E. faecium* ST993 (*vanA*)	+	206
Wild birds	2006–2010	Portugal	2.7	*E. faecium*/*E. durans* (*vanA*)	+	207
Wild birds	2009–2010	Portugal	1.3	*E. faecium*/*E. durans*/*E. hirae* (*vanA*)	+	208
Wild birds	2010	Portugal	0.7	*E. faecalis* (*vanA*)		209
Wild birds	2012	Tunisia	3.6	*E. faecium* (*vanA*)	+	210
Corvids (American crows)	2012	USA	2.5	*E. faecium* ST18 (CC17)/ST555 (CC17)/ST749/ ST750/ST751/ST752–(*vanA*), *E. faecalis* ST179 (CC16)/ST16 (CC16)/ST6 (CC2) (*vanA*)	+	211
Corvids (American crows)	2012–2015	USA	2.7	*E. faecium*-ST362/ST412 (CC17)-(*vanA*)	+	212
Corvids	2012–2013	Canada	0.7	*E. faecium* ST448 (*vanA*)	+	213
Corvids	2013	Slovakia	1.3	*E. faecium* ST917/ST6 (CC5) (*vanA*)	+	214
Wild boars	2005–2006	Portugal	3	*E. faecium* (*vanA*)	+	215
Mullets fish	2006–2007	Portugal	3.8	*E. faecium* ST273 (CC17)/ ST280 (CC17) (*vanA*)	+	165
Seagulls	2007	Portugal	10.5	*E. faecium*-ST5 (CC17)-(*vanA*), *E. durans*-(*vanA*)	+	216
Gilt-head seabream	2007	Portugal	5.9	*E. faecium* ST273 (CC17)/ST313 (CC17)/ ST76 (*vanA*), *E. faecalis* ST6 (CC2) (*vanA*), *E. durans* (*vanA*)	+	217
Wild rabbits	2007–2008	Portugal	3.9	*E. faecium*-(*vanA*)	+	218
Pigeons	2007–2008	Brazil	0		–	219
Buzzards	2007–2009	Portugal	9	*E. faecium* ST273 (CC17)/ST5 (*vanA*), *E. durans* (*vanA*)	+	220
Partridges	2015–2016	Portugal	2	*E. faecium*-ST18(CC17)/ST448(CC17)/ ST139(CC5)-(*vanA*)	+	221
Red foxes	2008–2009	Portugal	13.5	*E. faecium* ST262 (CC17)/ST273 (CC17) (*vanA*), *E. durans* (*vanA*)	+	222
Iberian wolf, Iberian lynx	2008–2010	Portugal	0.5 (Iberian wolf) 0 (Iberian lynx)	*E. faecium* ST18 (CC17)/ST573 (*vanA*)	+	223

(continued)

TABLE 3 Summary of reports about detection of VRE with acquired mechanisms of resistance in companion and free-living animals *(continued)*

Animal species	Isolation year	Country	% Prevalence[a]	Species-ST (CC)[b] (genotype)	Vancomycin selection method	Reference
Buffalo, wildebeest, zebra	2010–2011	Tanzania	2.8 (buffalo) 20 (zebra) 2.5 (wildebeest)	NS	+	224
Camels	NS	Spain	0		–	225
Wild game meat	NS	Spain	30.9	*E. faecium*/*E. faecalis*/*E. durans* (*vanA*), *E. faecium* (*vanB*)	–	226

[a]Some characteristics (year of isolation, type of samples) are included in parenthesis.
[b]ST (CC), sequence type (clonal complex), if data are available.
[c]NS, not specified.

of *optr*A-positive enterococci represents 10% and 5.7% of total *E. faecalis* and *E. faecium* isolates, respectively, obtained from fecal samples of poultry and pigs in a study performed in China (236). In a recent study carried out in Korea, 11,659 *E. faecalis* and *E. faecium* isolates obtained from fecal and carcass samples of healthy cattle, pigs, and chickens from farms and slaughter houses from 2003 to 2014 were tested for linezolid resistance, detecting a rate of resistance of 0.33%, mainly attributed to *optr*A carriage (238). The *optr*A gene has also been detected in sporadic isolates of *E. faecalis* and *E. faecium* ($n = 3$) obtained in meat samples in Denmark (imported poultry and veal), which represented <0.1% of total enterococci recovered from these samples (239). In Colombia, *optr*A has been detected in three *E. faecalis* isolates from poultry meat, coharboring the *fexA*, *tet*(L), and *Isa*(A) resistance genes (240). Both *cfr* and *optr*A have been detected in VRE isolates of human origin (245), but not in animal isolates so far.

The *optr*A gene has also been detected in two *E. faecalis* isolates of the lineage ST86 recovered from urban wastewater in Tunisia, accounting for 1% of all chloramphenicol-resistant enterococci tested (241); *optr*A was located within a transferable mosaic plasmid, which also contained the *fexA* and *erm*(A) genes.

At least 12 and 5 polymorphic variants of the *optr*A gene have been detected among human and animal enterococci, respectively (237, 246–248). The wild OptrA type ($OptrA_{E349}$) and the variants Tyr176Asp + Lys3Glu-Gly393Asp or Thr481Pro or Thr112Lys or Gly393Asp have been found in animal isolates (237, 246). Functional *cfr* and *optrA* genes have been identified in both enterococci and *S. aureus*. In most of the animal isolates, *optr*A is located close to other genes, as is the case of *fexA* (implicated in phenicol resistance) and a novel *erm*(A)-like gene. This *erm*(A)-like gene encodes an rRNA methylase, which shows 85.2% amino acid identity to the Erm(A) protein of transposon Tn*554* of *S. aureus* (237).

Most of the *cfr*-positive enterococci of food-producing animals (>90%) showed a MIC for linezolid of ≥8 μg/ml, but two *E. faecalis* isolates presented a MIC of 4 μg/ml. *optr*A-positive isolates of food-producing animal and food origin showed a linezolid MIC in the range of 2 to >8 μg/ml, presenting 19% of the isolates' MICs in the range of 2 to 4 μg/ml (categorized as susceptible according to EUCAST breakpoints and susceptible-intermediate according to CLSI) (Table 4). It is interesting to note that *cfr*- and *optr*A-positive enterococci could appear as linezolid-susceptible, probably leading to an underestimation of their actual incidence.

Oxazolidinones are not used in food-producing animals. Nevertheless, the emergent detection in these animals of linezolid-resistant enterococci carrying the *optr*A gene in transferable plasmids, linked to resistance genes for antibiotics commonly used in animals (phenicols, tetracyclines, lincosamides, and aminoglycosides), suggests its role in the coselection of multiresistant bacteria, which poses a risk for public health.

To summarize, transferable linezolid resistance genes, mostly *optr*A, have been detected in enterococci of food-producing animals and food of animal origin in various European, South American, and Asian countries, but so far not in Africa. These mechanisms of resistance have not been detected so far, to our knowledge, in pets or in wild animals.

Resistance to Aminoglycosides

Enterococci are intrinsically resistant to clinically achievable concentrations of aminoglycosides due to their low cell wall permeability. In addition, some species, such as *E. faecium* [*aac*(6′)-*Ii*], *E. durans* [*aac*(6′)-*Id*], and *E. hirae* [*aac*(6′)-*Ih*], intrinsically express a chromosomal-encoded acetyltransferase that confers resistance to

TABLE 4 Mechanisms implicated in linezolid resistance in enterococci of animals, food of animal origin, and the environment

Origin	Species	Sample	Year of isolation	Country	Number of linezolid resistant isolates (%)	Linezolid MIC (μg/ml)	Mechanism of linezolid resistance (genetic location, number of isolates)[a]	ST (number of isolates)[b]	Characteristics (number of isolates)	Reference
Food-producing animals										
Cattle	*E. faecalis*	Feces	2009	China	1	4	*cfr* (P)		*fexB*-linked	232
Pigs	*E. faecalis*	Feces	2009	China	1 (0.3% of *E. faecalis*)	4	*cfr* (P)	ST21	*fexA* (in a different plasmid)	233
Pigs	*E. faecalis*	Rectal swabs	2013	Brazil	5 (2% of *E. faecalis*)	8–>8	*cfr* (5) Mutation in ribosomal L3 protein (V149I) (1)	ST591 (2); ST29, ST590, ST592 (1 each)		234
Pigs	*E. gallinarum* (*n* = 1), *E. casseliflavus* (*n* = 24)	Rectal swabs	2012	China	25 (31.5% of florfenicol-resistant enterococci)	8–16	*cfr* (25) (C,1; P, 8)		*fexA*-linked (5), *fexA-fexB*-linked (1)	235
Poultry and pigs	*E. faecalis*	Feces	2012–2013	China	20 (10% of *E. faecalis* of animal origin)	2[c]–8	*optrA* (C,8; P, 9; P/C, 3)	ST21 (2); ST27, ST59, ST74, ST93, ST116, ST256, ST330, ST403, ST475, ST476, ST480, ST593, ST618, ST619, ST620, ST621, ST622, ST623 (1 each)	*fexA*-linked (14), *fexB*-linked (2), *fexA-fexB*-linked (1) *optrA* amino acid changes: Y176D, T481P (2); K3E, Y176D, G393D (1); T112K, Y176D (2); Y176D, G393D (1)	236, 237
Poultry and pigs	*E. faecium*	Feces	2005, 2009, 2012, 2014	China	5 (5.7% of *E. faecium* of animal origin)	4[d]–8	*optrA* (C, 1; P/C, 4)	ST957 (2); ST32, ST184, ST29 (1 each)	*fexB*-linked (4)	236, 237
Poultry and pigs	*E. faecalis*	Feces and carcass	2008, 2012, 2014	Korea	12 (0.2% of *E. faecalis*)	8–>16	*optrA* (12)	ST21 (4); ST49 (2); ST16, ST32, ST256, ST403, ST728, ST729 (1 each)	*fexA*-linked (11)	238

Poultry and cattle	*E. faecium*	Feces and carcass	2008, 2010, 2012–2014	Korea	27 (0.7% of *E. faecium*)	8–>16	*optrA* (23) Mutation in ribosomal L4 protein (N130K) (7)	ST195 (6); ST32 (2); ST157 (2); ST1171 (2); ST1168 (2); ND (4); ST8, ST120, ST121, ST236, ST241, ST1166, ST1167, ST1169, ST1170 (1 each)	*fexA*-linked (19)	238
Pigs	*E. faecalis* (*n* = 5), *E. gallinarum* (*n* = 1)			China	6 (17.14% of enterococci)	8–16	23S rRNA mutation: G2576T	ST29, ST146, ST220, ST283, ST535		229
Meat										
Cattle	*E. faecalis*	Danish veal	2015	Denmark	1 (<0.1% of *E. faecalis*)	8	*optrA* (P)	ST22	*fexA*-linked	239
Poultry	*E. faecium*	Imported turkey and broiler meat	2012, 2013	Denmark	2 (<0.1% of *E. faecium*)	8	*optrA* (P, 1)	ST22, ST873		239
Poultry	*E. faecalis*	Meat	2010–2011	Colombia	3 (0.5% of enterococci)	8	*optrA* (P, 3)	ST59 (2), ST489	*fexA*-linked	240
Environment										
Wastewater	*E. faecalis*	Urban wastewater treatment plant	2014	Tunisia	2 (1% of chloramphenicol-resistant enterococci)	4	*optrA* (P)	ST86 (2)	*fexA*-linked (2), *optrA* amino acid changes:M1L (2), K3E (1) and I622M (1)	241

[a]P, plasmid; C, chromosome.
[b]ND, not determined.
[c]Five *optr*A-positive *E. faecalis* isolates showed a linezolid MIC of 2 μg/ml, and five isolates showed an MIC of 4 μg/ml.
[d]Two *optr*A-positive *E. faecium* isolates showed a linezolid MIC of 4 mcg/ml.

TABLE 5 Summary of articles on the antimicrobial resistance in *Enterococcus* isolated from animals from 2013 to 2017

Animal	Country	Enterococcal specie	Percentage of resistance to[a]				Reference
			TET	ERY	HLR-GEN	CIP	
Food-producing and farm animals							
Camels	Tunisia	*Enterococcus* spp.	4	11	0	ND	254
Chickens and pigs	China	*Enterococcus* spp.	92.5	72.8	30	ND	153
Aquaculture	Spain	*E. faecalis* *E. faecium*	0 5	5 37	ND ND	62 26	255
Rainbow trout	Spain	*Enterococcus* spp.	27.1	41	7	0	256
Marine aquaculture	Italy	*Enterococcus* spp.	66	25	0	ND	257
Tibetan pigs	Tibet	*E. faecalis* *E. faecium*	93.5 44.4	93.5 20	0 0	0 0	258
Farm animals	Argentina	*E. faecalis*	100	50	0	ND	259
Chickens	Southeast Asian countries	*E. faecalis* *E. faecium*	69.2 92.2	70.9 79.4	11.1 13.9	17.9 82.8	260
Poultry	Italy	*Enterococcus* spp.	65.2	87.8	60	70.4	143
Beef and sheep	Tunisia	*E. faecalis*	15	30	2.5	22.5	261
Pigs	South Africa	*E. faecalis* *E. faecium*	ND ND	100 98.3	ND ND	45 94.1	147
Poultry	Germany	*E. faecalis* *E. faecium*	82 67	70 89	98 89	14 72	141
Poultry	Nigeria	*Enterococcus* spp.	81.6	100	20	ND	262
Chicken and turkey	Canada	*Enterococcus* spp.	94	64–74	8–15	2–8	135
Fish and seafood	Switzerland	*E. faecalis*	16	ND	4	0	263
Infection in poultry	Poland	*E. cecorum*	29	51	1	ND	15
Poultry and cattle	Nigeria	*Enterococcus* spp.	61	61	32.7	9.7	154
Mastitis in cows	China	*Enterococcus* spp.	6.7	ND	50	25	264
Cattle	Australia	*E. faecalis* *E. faecium*	7.3 11.7	10.4 8.3	0 0	ND ND	87
Pigs	Nigeria	*E. faecalis* *E. faecium*	60 20	68.7 31.5	85 40	0.5 7.4	265
Donkey	Spain	*E. faecium*	68	44	0	60	266
Pets and companion animals							
Healthy dogs and cats	Japan	*Enterococcus* spp.	ND	40	44	25	267
Hospitalized dogs	Korea	*E. faecalis* *E. faecium*	64.3 66.7	50 44.4	7.1 33.3	0 55.6	268
Antibiotic-treated dogs and cats	Japan	*E. faecalis* *E. faecium*	58 15	52 28	38 21	30 8	198
Various animals	Poland	*E. faecalis* *E. faecium*	36 7	48 60	ND ND	38 46	205
Healthy cats and dogs	Italy	*Enterococcus* spp.	97.4	81.7	40.8	7.8	269
Wild animals							
Iberian lynx	Spain	*Enterococcus* spp.	33	30	4	7	92
Iberian wolf	Portugal	*Enterococcus* spp.	55	22	1	15	90
Echinoderms	Portugal	*E. faecium*	31.7	33	0.8	30.8	65
Red foxes	Portugal	*E. faecalis* *E. faecium*	81 96	36 56	18 12	ND ND	270
Wild birds	Portugal	*E. faecalis* *E. faecium*	32.3 45	15.3 15	0 0	15.3 32.5	209
Eurasian otter	Portugal	*E. faecalis* *E. faecium*	32 78	ND ND	52 11	ND ND	271
House flies	USA	*E. faecalis*	60	18	5	0	272
Wild birds	Tunisia	*E. faecalis* *E. faecium*	33 16	33 53	50 11	66 38	261
Wild fur seals	Brazil	*E. faecalis*	0	25	0	10	273

(continued)

TABLE 5 Summary of articles on the antimicrobial resistance in *Enterococcus* isolated from animals from 2013 to 2017 *(continued)*

			Percentage of resistance to[a]				
Animal	Country	Enterococcal specie	TET	ERY	HLR-GEN	CIP	Reference
Wild marine animals	Brazil	*Enterococcus* spp.	14.5	32.2	0	20.9	274
Seafood	Tunisia	*E. faecalis* *E. faecium*	42.9 18.2	38.1 27.3	14.3 0	52.4 63.6	275
House flies	Thailand	*Enterococcus* spp.	75	42.5	ND	ND	276

[a]TET, tetracycline; ERY, erythromycin; HLR-GEN, high-level resistance to gentamicin; CIP, ciprofloxacin.

tobramycin, kanamycin, and amikacin (249). The chromosomally encoded methyltransferase EfmM has been exceptionally described in an *E. faecium* isolate (250) codifying resistance to kanamycin and tobramycin. Acquired resistances to aminoglycosides are detected in strains from both animals and humans and usually confer a high level of resistance to gentamicin, kanamycin, and streptomycin.

High-level resistance to gentamicin in enterococcal isolates of animal origin was first described in 1998 in Denmark (251) and in 2001 in the United States (252). The acquired genetic mechanisms identified in animal isolates are identical to those described in human isolates. The most frequent ones are the bifunctional enzyme encoded by *aac(6')-Ie-aph(2")-Ia* (conferring resistance to gentamicin, kanamycin, amikacin, netilmicin, and tobramycin) and *aph(3)-III*a (conferring resistance to kanamycin and amikacin) (23, 253). High-level gentamicin resistance can also be due to the expression of the unusual *aph(2")-Ic*, *aph(2")-Id*, *aph(2")-Ie*, and *aph (2")-Ib* genes (17, 23); *aph(2"")-Ic* seems to be more frequent in enterococci of animal origin, and some farm animals could be a reservoir of this gene (252). High-level resistance to streptomycin is commonly caused by punctual ribosomal mutations, although acquisition of some modifying enzymes has been also described [*ant(3")-Ia* and *ant(6')-Ia*]. Table 5 summarizes papers (from 2013 to 2017) that analyzed the rates of antimicrobial resistance (high level to gentamicin and others such as tetracycline, erythromycin, and ciprofloxacin) in enterococcal isolates from animals (65, 15, 87, 90, 92, 135, 141, 143, 147, 153, 154, 198, 205, 209, 254–276).

Resistance to Tetracycline

This family of antimicrobials integrates several antibacterial active compounds (277), although tetracycline, chlortetracycline, oxytetracycline, and doxycycline are the most used in veterinary. Despite Roberts' extensive 1996 review about tetracycline resistance mechanisms (278), a more recent update was published in 2005 (279). Almost 60 tetracycline resistance genes have been described, although the most frequent ones in *Enterococcus* are those implicated in ribosomal protection [*tet*(M), *tet*(O), *tet*(S)], efflux, or enzymatic inactivation [*tet*(K), *tet*(L)]. In *Enterococcus*, as occurs in other Gram-positive microorganisms, the ribosomal protection protein mechanism encoded by the *tet*(M) gene is the most frequent, independent of the origin of the strains. The transferability of the tetracycline resistance determinants in the absence of plasmids has been described (280); the Tn*916*/Tn*1545* conjugative transposon family carrying the *tet*(M) gene is responsible, usually in combination with *erm*(B).

Resistance to Macrolides/ Lincosamines/Streptogramins

Numerous chemically diverse compounds are integrated into the macrolide family, with erythromycin being the most representative. Resistance to this antibiotic was immediately reported after its introduction in human clinical use in 1952; moreover, enterococci are intrinsically resistant to clindamycin and lincomycin. Tylosin, spiramycin, and virginiamycin were widely used in pigs and other animals before the European Union limited their use. After the ban, erythromycin resistance in *Enterococcus* strains from animals decreased spectacularly (281), demonstrating the link between consumption of the antibiotic and the increase in resistance rates, even in different environments.

Chromosomal intrinsic resistance to macrolides by *msr*(A) and to lincosamides by *linB* in *E. faecium* has been described (282, 283). Acquired resistance to macrolides can be codified by various genetic determinants (up to 92 have been described) (284), although the most common worldwide is *erm*(B), usually carried by Tn*917*, which is widespread in human and animal isolates. Other relevant genes in the genus *Enterococcus* are the efflux genes *mef*(A), conferring resistance to macrolides, *vgb*(A), conferring resistance to virginiamycin, *lnu*(B), conferring resistance to lincosamide, and *vat*(D) and *vat*(E), conferring resistance to streptogramins.

Resistance to Quinolones

Fluoroquinolones have reduced antimicrobial activity against enterococci, with levofloxacin and moxifloxacin being the most active compounds. Acquired resistance is the consequence of mutations in the *gyrA* and *parC* genes (285, 286, 287) or the acquisition of the *qnr* genes (287). Efflux pumps such as EmeA for *E. faecalis* (288) and NorA-like for *E. faecium* (289) have also been described, although their frequency is low. Resistance to ciprofloxacin is a conserved feature among the high-risk *E. faecium* CC17 clone linked to nosocomial outbreaks (290) and among almost all isolates with resistance to glycopeptides. Fluoroquinolones have never been used as growth promoters, although their use for veterinary therapy is common.

MOLECULAR EPIDEMIOLOGY AND POPULATION STRUCTURE OF ENTEROCOCCI IN FARM AND COMPANION ANIMALS

Epidemiological studies of farm and companion animals were originally driven by the interest in establishing a relationship between antibiotic-resistant isolates from human and nonhuman hosts. At present, the resistance phenotypes of clinical relevance that may be linked to animals mainly comprise resistance to ampicillin, gentamicin, quinupristin-dalfopristin, vancomycin, and linezolid.

Molecular typing of enterococci strains has been performed by different methods, including pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), MLST, cgMLST, Bayesian analysis of population structure (BAPS) and whole-genome sequencing (WGS) (revised in 291).

The emergence of VRE in European food-producing animals and food of animal origin in the early 1990s (128, 291, 292–296), as well as in the feces of healthy volunteers and food handlers (297–299), led to surveillance studies in the community setting that suggested a relationship between the extensive use of animal growth promoters in veterinary medicine (e.g., avoparcin and tylosin), the colonization pressure in animals, and the subsequent transmission to human hosts throughout the food chain (300, 301).

The first report of VRE in nonhuman hosts occurred in 1993 in the United Kingdom and documented the similarity between isolates of different origins (300). This study was followed by others, which confirmed the similarity of VRE strains from humans and farm animals exposed to avoparcin in different European countries (26, 292, 302–305). The potential selection of antibiotic-resistant enterococci by antibiotics led to the ban of avoparcin as an animal growth promoter in Sweden in 1986, Denmark and Switzerland in 1995, and in the rest of the European countries 2 years later (Commission Directive 97/6/EC). By 1999, other antibiotics (such as bacitracin, virginiamycin, and tylosin) were also banned as growth promoters for healthy animals in Europe, and this was followed in 2006 by a ban on all antibiotics as growth promoters. In this way, Europe led the first intervention against VRE at a global level. In contrast to western countries, the use of antimicrobials in livestock and poultry, as well as the standard policies on antimicrobial use, varies significantly among Asian countries (reviewed in 306). In Korea, avoparcin was used in the management of poultry and swine from 1983 to 1997 but was banned thereafter to reduce exposure of humans to VRE (133). After several years of avoparcin discontinuance in Korea, the prevalence of VRE in Korean livestock was investigated, and some studies reported that the VRE incidence rate in chicken samples was higher than that in pig samples (163, 307).

The ban led to a significant reduction of VRE colonization in animals, foods, and fecal samples of community-based people in different countries. However, VRE was recovered in feces of animals and humans years later, reflecting the important effects of previous livestock practices in the population structure of enterococci in animals.

Most information came from the species *E. faecium* and *E. faecalis*, the predominant species in the gastrointestinal tract of mammals, along with *E. hirae*, *E. durans*, and *E. cecorum* (11, 45, 46).

E. faecium

PFGE remained the "gold standard" for molecular typing of *E. faecium* until the recent introduction of WGS-based epidemiology (291, 308). By using PFGE, clonal dissemination of *E. faecium* strains with clinically relevant phenotypes (ampicillin, gentamicin, quinupristin-dalfopristin, and vancomycin) has been extensively documented between animals from the same or different farms and has also been suggested between animals and humans (309, 310). The data vary greatly among geographic areas and are normally associated with the use of antibiotics.

Ecological differentiation of *E. faecium* has been documented in epidemiological studies using AFLP, MLST, and/or BAPS (311–314). AFLP analysis originally revealed different subpopulations (or ecotypes)

corresponding to hospitalized patients, community-based people, and farm animals, including veal calves, poultry, and swine (311, 315). Later, MLST results using eBURST confirmed the split of *E. faecium* into host-specific subgroups, one from hospitalized patients (originally termed clonal complex 17 [CC17]) and others from domesticated animals (291, 316). More recently, BAPS analysis allowed the partitioning of 519 sequence types of 1,720 *E. faecium* isolates into 13 non-overlapping groups. Again, BAPS groups were significantly associated with isolates from hospitalized patients (BAPS 3-3) and farm animals (BAPS 2-1 and 2-4) (313). More recently, single-nucleotide polymorphism-based phylogenetic analysis of WGS data split *E. faecium* into isolates causing infections (clade A1), isolates from healthy humans (clade B), and isolates from healthy humans and animals (clade A2) (79). Clade A1 mostly comprises isolates from hospitalized humans associated with lineages 17 (including ST16 and ST17), 18 (ST18), and 78 (ST78 and ST192), although isolates from animals have been extensively reported (89, 304, 313). The ST78 isolates show putative evolutionary hallmarks with respect to pets (dogs and cats) and poultry isolates and diversified mainly through recombination and acquisition or loss of mobile genetic elements, which eventually led to adaptation to different ecological niches. Thus, ecological distinction is not absolute, and the main zoonotic risk linked to *E. faecium* isolates is represented by transfer of mobile genetic elements harboring antimicrobial resistance genes.

Poultry

E. faecium isolates resistant to macrolides, quinupristin-dalfoprisitin, or other streptogramins were extensively reported in poultry farms, revealing high heterogeneity of PFGE types and sequence types, although some similar patterns were eventually detected on farms in Europe, the United States, and Asia (317–319). Clonal dissemination of VRE of the *E. faecium* species (VREfm) in poultry farms exposed to antibiotics before and after the avoparcin ban (109, 302) were documented in European and Asian countries, with sequence types belonging to CC9 or CC96 being predominant in Europe and Malaysia, respectively (320). A dramatic increase of VREfm in Sweden from 2000 to 2009 was due to the clonal expansion of the clone ST310, despite the absence of selection by antibiotics in this country, where the use of antibiotics as animal growth promoters has been forbidden since 1986 (129). A Danish study showed a high rate of VREfm in Danish farms after the 15-year ban of avoparcin, with different sequence types and the presence of an ST842 clone in 36 flocks analyzed corresponding to eight farms broadly distributed across the country (85). Recently, clonally unrelated *E. faecium* isolates resistant to linezolid emerged on farms in China (236, 237). Common PFGE profiles or sequence types between humans and broilers have also been documented (321–323), but the human health risk associated with the presence of *E. faecium* in poultry meat is under debate (25).

Swine

VREfm has been extensively reported in pig farms in European countries before and after the avoparcin ban (113, 324, 325). Clonal spread of VREfm was documented in Denmark, Norway, Finland (113), Switzerland (326), Portugal (304), and Spain (327), with predominance of sequence types belonging to the CC5 lineage (ST5, ST6, ST185). The persistence of VREfm in pig farms after the avoparcin ban was associated later with the use of tylosin, which facilitated the coselection of strains resistant to both glycopeptides and macrolides due to the presence of both *vanA* and *erm*(B) genes in the same plasmid (113). VREfm was also detected in county fairs in Michigan from 2008 to 2010, which represents the first and only report of VREfm in livestock in the United States to date (121, 122). In Asia, the occurrence varies among countries and is sporadic in China (156). In all these studies, CC5 strains were predominantly identified. A particular ST6 (CC5) clone was identified on farms in different European Union countries and the United States, as well as in healthy volunteers and hospitalized patients, all carrying a Tn*1546* in *orf1* and a G-T point mutation in position 8234 at *vanX* (304, 328). In addition to tylosin, copper is frequently added to pig and cattle feeds, so colocation of heavy metal resistance determinants has also been demonstrated in Europe and the United States (329, 330). Copper resistance is often associated with resistance to macrolides [*erm*(B)], tetracyclines [*tet*(M)], and glycopeptides (*vanA*). Although clonal dissemination has been reported (330), a great diversity has been observed on farms (331). Major human clones CC9 and CC22 (previously classified as CC17) have also been documented in some studies (85, 332, 333).

Companion animals

A few studies have analyzed the fecal carriage of ampicillin-resistant *E. faecium* (AREfm) and VREfm in companion animals. High rates of AREfm were observed among fecal samples of dogs collected in the United Kingdom and Denmark in 2006 and 2008 (23% and

76%, respectively) (89, 334). Most of these isolates belonged to the major human clonal lineage originally called CC17, which suggested a possible transmission between hosts. Later, de Regt *et al.* demonstrated some unique metabolic features in these CC17 canine isolates that could have facilitated niche adaptation (335). A recent large Dutch country-wide population-based study reported a higher prevalence of fecal carriers of AREfm in dogs and cats than in a healthy human population (25.6%, 5.1%, and 1.5%, respectively). This study concluded that isolates from pets were genetically distinct from those of humans based on the lack of co-occurrence and the cgMLST results (336). Prior antibiotic use and eating raw meat were considered a risk factor for acquiring AREfm in all the available studies (197, 336). Clinical isolates from dogs and cats treated with amoxicillin belong to high clonal complex risks and were similar to those from humans (197, 337).

E. faecalis

A plethora of molecular methods have been used to type this species, including PFGE, AFLP, and MLST. In contrast to *E. faecium*, *E. faecalis* isolated from different sources/hosts cannot be grouped using MLST or AFLP. Studies using MLST data revealed the presence of many sequence types in different hosts, including farm animals, companion animals, and hospitalized patients (338, 339). Moreover, some sequence types are associated with a higher prevalence of antibiotic resistance, represented by ST2, ST8, ST9, ST16, ST40, and ST87 (303, 339, 340), all of them being overrepresented in humans. To date, ST16 has been recovered from humans and farm animals and is considered a zoonotic lineage (25), involved in the spread of resistance to all antibiotics used in animals, including bacitracin, phenicols, oxazolidinones (341). Clonal outbreaks of *E. faecalis* ST82, a common cause of amyloid arthropathy in poultry, have been reported on farms in Denmark, the United States, France, and Germany (342).

Although the detection of more-prevalent *E. faecalis* sequence types in distant geographical locations and different hosts suggests frequent horizontal gene transfer between different host populations (69, 211, 241, 339, 340, 343), some studies using comparative genomics discarded the idea of global transmission (344).

The incongruence in the topologies of the seven MLST gene trees revealed that this species is highly recombinogenic (291, 343). Subsequent analysis of the *E. faecalis* population structure based on MLST data using BAPS also yielded incongruent results and confirmed the lack of host-specific groups or ecotypes (313, 314). This issue was also demonstrated by studies that characterized the phylogenetic diversity of *E. faecalis* using whole genomes (phylogenomics and cgMLST) of clinical, human commensal, and animal isolates and that observed a lack of distinct clustering of isolates according to the source (291, 345).

Additional WGS studies are necessary to characterize and describe the role of animals in the evolution, genetic diversity, and population structure of *E. faecalis*.

PLASMIDS IN ENTEROCOCCI FROM FOODBORNE AND COMPANION ANIMALS

Horizontal gene transfer plays a relevant role in the dissemination of antibiotic resistance in nonhuman hosts, and plasmids play a central role in this dissemination. Classically, plasmid categorization is based on the presence and diversity of their replication machinery (346), which were established by replication-initiator protein (*rep*) schemes (347, 348) identified in Gram-positive species to date. In Fig. 1 we show the plasmid content (percentage and diversity of *rep* sequences) of the 67 *E. faecium* and 47 *E. faecalis* genomes of animal origin obtained from the WGS database of the NCBI. The enterococcal genomes from public databases were classified according to their origin (Table 6), information obtained from the Pathosystems Resource Integration Center (PATRIC) database (349). The *rep* genes obtained by the PlasmidFinder bioinformatics tool (350) belong to plasmid families with theta (RepaA_N, Inc18, Rep3_small tetha) or rolling-circle replication mechanisms (Fig. 1).

Plasmids conferring resistance in enterococci to vancomycin, macrolides, tetracycline, aminoglycosides, and heavy metals (copper, cadmium, bacitracin zinc) have been detected on farms that were exposed to antimicrobials used as growth promoters (avoparcin, virginiamycin, tylosin, or bacitracin zinc), therapeutically (tetracyclines, gentamicin, penicillins), or as dietary supplements (e.g., copper). Antibiotic-resistant plasmids have also been recovered from areas where selection was not apparent. Some emblematic examples are transferable *vanA* in commercial animal husbandry on farms in Michigan, where avoparcin has never been licensed for use in growth promotion (121, 122), and persistent *vanA*-Inc18 plasmids in Norwegian broiler flocks after the ban of some antibiotics. These studies suggest alternative routes of selection, introduction, and spread of *vanA*-type vancomycin resistance, plasmid fitness, and other phenomena (351).

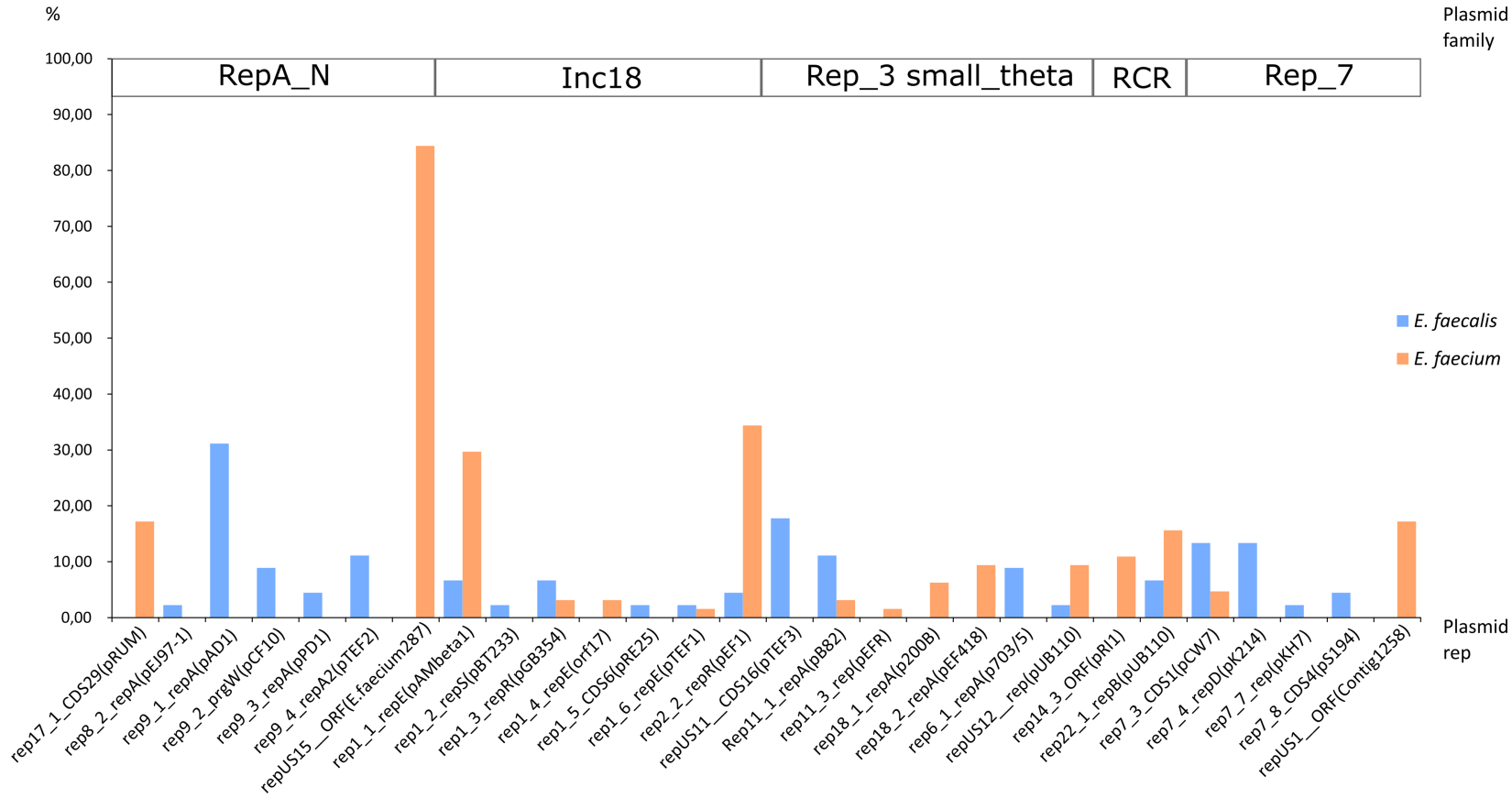

FIGURE 1 Plasmid gene content of 67 *E. faecium* and 47 *E. faecalis* genomes of animal origin from the NCBI whole-genome database. Plasmid data were obtained by the PlasmidFinder bioinformatics tool. The genomes from the database were classified by source, extracting the isolate information from the Pathosystems Resource Integration Center (PATRIC) database (344). Reps, replicases.

TABLE 6 *Enterococcus* isolates with animal source from the Genbank database included in the plasmid *rep* genes *in silico* screening

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
Enterococcus faecalis	PF3		0	Adélie penguin (*Pygoscelis adeliae*)	2010	Antarctica	GCA_000505585.1
	DBH18		1	Mallard duck (*Anas platyrhynchos*)	2005	Spain	GCF_001563075.1
	ATCC 27959	40	0	Cattle	1975	United States	GCA_000395265.1
	ATCC 35038	59	2	Chicken (*Gallus gallus*)	1980		GCA_000392955.1
	ATCC 29212		0	Chicken (*Gallus gallus*)			GCA_900117325.1
	ATCC 29212		2	Chicken (*Gallus gallus*)			GCA_900117335.1
	F1	72	2	Cow	1900		GCA_000393095.1
	ATCC 6055	113	2	Cow	1937		GCA_000393375.1
	ARO1/DG	108	3	Dog (*Canis lupus familiaris*)			GCA_000157275.1
	UCD-PD3		1	*Felis catus*	2015	United States	GCF_001692955.1
	Fly1	101	0	Fruit fly (*Drosophila*)		United States	GCA_000157415.1
	Fly 2	102	1	Fruit fly (*Drosophila*)	2005		GCA_000393215.1
	P9-1		2	Magellanic penguin (*Spheniscus magellanicus*)	2013	Brazil	GCF_001400065.1
	P8-1		3	Magellanic penguin, (*Spheniscus magellanicus*)	2013	Brazil	GCF_001400055.1
	KB1		1	*Mus musculus*	2011	Germany	GCA_002221625.2
	KB1		0	*Mus musculus*	2015	Switzerland	GCF_001689055.1
	7330257-1	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390805.1
	7330259-5	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390825.1
	7330948-5	16	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390845.1
	7330112-3	16	6	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390765.1
	7430416-3	16	0	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390905.1
	19116	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390685.1
	7430275-3	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390865.1
	7430821-4	16	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390925.1
	D6	16	0	Pig (*Sus scrofa*)		Denmark	GCA_000157455.1
	7430315-3	35	3	Pig (*Sus scrofa*)	2002	Denmark	GCA_000390885.1
	D1	40	0	Pig (*Sus scrofa*)			GCA_000393575.1
	7330082-2	40	0	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390745.1
	D32	40	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000281195.1
	D3	47	2	Pig (*Sus scrofa*)			GCA_000392915.1
	7330245-2	47	2	Pig (*Sus scrofa*)	2001	Denmark	GCA_000390785.1
	L9		0	Pig (*Sus scrofa*)	2013	Brazil	GCF_001878735.1
	L12		0	Pig (*Sus scrofa*)	2013	Brazil	GCF_001886675.1
	IBUN9046YE		5	Pig (*Sus scrofa*)	2014	Colombia	GCA_002250145.1
	19		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788165.1
	17		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788185.1
	1		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788175.1
	32		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788255.1
	12		3	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788155.1
	18		3	Pig (*Sus scrofa*)	2011	Denmark	GCA_000788235.1
	Enfs51		2	Pig (*Sus scrofa*)	2012	Malaysia	GCF_001806515.1
	P.En250		3	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009545.1
	P. En090		3	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009565.1
	Enfs85		5	Pig (*Sus scrofa*)	2012	Malaysia	GCF_002009465.1
	XJ05		1	Sheep (*Ovis aries*)	2002	China	GCF_001263775.1
	OG1X	92	1	Tunicate (*Lissoclinum patella*)	2006	Solomon Islands	GCA_000320305.1
	2924	116	1	Turkey meat	2005	Denmark	GCA_000390725.1

(continued)

TABLE 6 *Enterococcus* isolates with animal source from the Genbank database included in the plasmid *rep* genes *in silico* screening *(continued)*

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
Enterococcus faecium	E1573	21	1	Bison (*Bison* sp.)	1994	Belgium	GCA_000321765.1
	825		4	Cattle	2004	United States	GCA_002360185.1
	E0045	9	3	Chicken (*Gallus gallus*)	1992	United Kingdom	GCA_000321465.1
	E2134	12	6	Chicken (*Gallus gallus*)	2004	Netherlands	GCA_000322185.1
	VAN 342	22	6	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395785.1
	VAN 332	38	4	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395745.1
	VAN 345	38	5	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395805.1
	E1575	158	4	Chicken (*Gallus gallus*)	1995	Belgium	GCA_000321805.1
	F9730129-1	245	4	Chicken (*Gallus gallus*)	1997	Denmark	GCA_000395965.1
	VAN 335	417	4	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395765.1
	VAN 219	784	3	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395705.1
	VAN 222	784	3	Chicken (*Gallus gallus*)	2010	Denmark	GCA_000395725.1
	7527		3	Chicken (*Gallus gallus*)	2003	United States	GCA_002360135.1
	6605		4	Chicken (*Gallus gallus*)	2003	United States	GCA_002360225.1
	5209		6	Chicken (*Gallus gallus*)	2003	United States	GCA_002360255.1
	E1574	27	3	Dog (*Canis lupus familiaris*)	1995	Belgium	GCA_000321785.1
	E4389	78	5	Dog (*Canis lupus familiaris*)		Denmark	GCA_000322425.1
	E4453	192	5	Dog (*Canis lupus familiaris*)			GCA_000239095.2
	E4452	266	5	Dog (*Canis lupus familiaris*)	2008	Netherlands	GCA_000239115.2
	GL3		0	Dromedary	2013	Algeria	GCF_001277795.1
	ICIS 96		5	*Equus caballus*	2014	Russia	GCA_002265255.1
	LS170308		2	*Moschus berezovskii*	2017	China	GCA_002831505.1
	E1622	104	0	Mouse	1959	Netherlands	GCA_000321945.1
	E1576	159	3	Ostrich (*Struthio camelus*)	2001	South Africa	GCA_000321825.1
	E0688	5	4	Pig (*Sus scrofa*)		Spain	GCA_000321605.1
	HF50104	5	2	Pig (*Sus scrofa*)	2008	United States	GCA_000396685.1
	HF50215	5	4	Pig (*Sus scrofa*)	2008	United States	GCA_000396785.1
	A17 Sv1	6	1	Pig (*Sus scrofa*)	1995	Denmark	GCA_000392125.1
	E8sv3	6	1	Pig (*Sus scrofa*)	1995	Denmark	GCA_000392145.1
	9730219-1	6	1	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391945.1
	9730357-1	6	1	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391965.1
	9731352-4	6	2	Pig (*Sus scrofa*)	1997	Denmark	GCA_000392005.1
	9830091-5	6	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392025.1
	9830512-2	6	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392045.1
	9931110-4	6	3	Pig (*Sus scrofa*)	1999	Denmark	GCA_000392105.1
	7330446-2	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391825.1
	7330519-3	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391845.1
	7330884-2	6	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391885.1
	7430166-3	6	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000391905.1
	S658-3	6	1	Pig (*Sus scrofa*)	2002	Denmark	GCA_000395425.1
	HF50204	6	1	Pig (*Sus scrofa*)	2008	United States	GCA_000396765.1
	HF50105	6	3	Pig (*Sus scrofa*)	2008	United States	GCA_000396705.1
	HF50106	6	3	Pig (*Sus scrofa*)	2008	United States	GCA_000396725.1
	9731349-1	82	4	Pig (*Sus scrofa*)	1997	Denmark	GCA_000391985.1
	9930238-2	133	1	Pig (*Sus scrofa*)	1999	Denmark	GCA_000392085.1
	7330381-1	133	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391805.1
	E0679	150	2	Pig (*Sus scrofa*)		Belgium	GCA_000321565.1
	E0680	151	3	Pig (*Sus scrofa*)		Germany	GCA_000321585.1
	E1578	160	1	Pig (*Sus scrofa*)	2001	Germany	GCA_000321845.1
	9830565-4	185	1	Pig (*Sus scrofa*)	1998	Denmark	GCA_000392065.1

(continued)

TABLE 6 *Enterococcus* isolates with animal source from the Genbank database included in the plasmid *rep* genes *in silico* screening *(continued)*

Species	Isolate	MLST	Plasmid rep	Isolation source	Year	Isolation country	Assembly accession
	7330614-1	185	1	Pig (*Sus scrofa*)	2001	Denmark	GCA_000391865.1
	HF50203	185	1	Pig (*Sus scrofa*)	2008	United States	GCA_000396745.1
	CICYT-205	437	3	Pig (*Sus scrofa*)		Spain	GCF_001622975.1
	7230532-1		1	Pig (*Sus scrofa*)	2000	Denmark	GCA_000391785.1
	2006-70-121		0	Pig (*Sus scrofa*)	2006	Denmark	GCA_000391765.1
	1970-07-08		0	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767345.1
	70-40-11		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804405.1
	70-61-3		1	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767355.1
	1970-08-02		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804415.1
	70-36-8		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000767365.1
	70-61-7		2	Pig (*Sus scrofa*)	2011	Denmark	GCA_000804385.1
	E2071	27	1	Poultry	2001	Denmark	GCA_000322165.1
	FDAARGOS_397		2	Tonsil crypt			GCA_002554355.1
	E0269	9	3	Turkey (*Meleagris gallopavo*)	1996	Netherlands	GCA_000321525.1
	E0164	26	3	Turkey (*Meleagris gallopavo*)	1996	Netherlands	GCA_000321505.1
	M3K31		3	Vulture	2010	Spain	GCF_001039515.1
	58M		1	Wooly mammoth (*Mammuthus primigenius*)	2014	Russia	GCF_001280775.1

Plasmids Conferring Resistance to Glycopeptides

Tn*1546* (*vanA*), the predominant mechanism of glycopeptide resistance in enterococci, has been successfully disseminated among poultry and swine through plasmids of the Inc18 and RepA_N families, respectively (352, 353). In poultry, an 18- to 25-kb fragment that includes the 10.85 kb of Tn*1546* (*vanA*), is conserved in Inc18 plasmids detected in Norwegian broiler flocks for more than 1 decade (from 1999 to years after the avoparcin ban) and in pIP186, the first Inc18 (*vanA*) plasmid described, in 1986, in an *E. faecium* clinical isolate (354, 355). The persistence of *vanA* plasmids on Norwegian poultry farms is attributed to the toxin-antitoxin system ω-ε-ζ originally described in pRE25, a plasmid of *E. faecalis* that carries resistance to different antibiotic families and is prevalent in animals and foods (127, 354). Analyses of the Tn*1546* insertion sites and plasmid backbones suggest spread of the *vanA* transposon across clonal lines in the broiler industry (125, 354–356). Bortolaia and Guardabassi recently associated the persistence of glycopeptide resistance in Danish poultry flocks after 15 years of the avoparcin ban with a nontransferable 54-kb plasmid in isolates that only confer resistance to glycopeptides (27). It is notable that broiler flocks raised in Denmark come from parent birds imported from Sweden, and the high occurrence of VREfm was also observed in Swedish broiler flocks until 2011 (129).

In swine, large plasmids belonging to the RepA_N family (150 to 190 kb, rep_{pLG1}), which carry a truncated variant of Tn*1546* and *tcrB* (coding for resistance to copper), have been detected in a pandemic CC5 *E. faecium* clone that has been circulating in swine farms in Spain, Portugal, Denmark, Switzerland, and the United States for decades and in other *E. faecium* lineages of pigs and humans, which suggests transmission (304). These plasmids used to carry the *erm*(B) gene (macrolide resistance) and, eventually, *trcB* (copper resistance) (see below).

Also, sporadic reports have documented the occurrence of strains carrying other *vanB* or *vanN* operons on plasmids in poultry meat (178, 188), farmed game meat and wild game meat (226). Finally, vancomycin-susceptible *E. faecalis* strains carrying *vanC1* on transferable elements (plasmids, transposons, and integrons) have also been reported in cloacal swabs of broilers (357) and feces of diseased pigs from different farms (358). Transmission of species-specific *vanC1* and *vanC2/C3* genes could be currently underestimated given the high prevalence of *E. gallinarum* and *E. casseliflavus*, respectively, in food-producing animals (159, 359–360) and the scarcity of studies that screen *vanC* genes in other species.

Plasmids Conferring Resistance to Macrolides, Streptogramins, and Lincosamides

These plasmids have been extensively recovered in enterococci from poultry and pig farms where macrolides

(spiramycin and tylosin) and streptogramins (virginiamycin) were used as growth promoters and pleuromutilins (tiamulin and valnemulin) were used to treat infections. Lincomycin, alone or in combination with spectinomycin, has been widely used to control respiratory and gastrointestinal bacterial pathogens in cattle, swine, poultry, dogs, and cats, and pirlimycin has been only used to treat bovine mastitis cases. Clindamycin is a common therapeutic option for topical infections in dogs and cats.

Macrolides

The most widespread gene that confers resistance to macrolides in enterococci is *erm*(B), which is located in different transposons and plasmids in species of the *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Clostridium* genera (346, 361). pRE25, a multidrug-resistant plasmid originally recovered from an *E. faecalis* isolate from a sausage sample, is the paradigm of the Inc18 family and has greatly contributed to the spread of *erm*(B) among animals and humans (346, 353, 362). This plasmid encodes resistance to 12 antimicrobials from 5 structural classes (macrolides, lincosamides, streptothricin, chloramphenicol, aminoglycosides) due to the presence of *erm*(B) (macrolide-lincosamide-streptogramin B), cat_{pIP501} (chloramphenicol), and Tn*5405*, which comprises the genes *aadE-sat4-aphA3* (aminoglycoside-streptothricin) (363, 364). The genes carried by pRE25 are present in several animal pathogens, namely, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *S. aureus*, *Bacillus subtilis*, *Campylobacter coli*, *Clostridium perfringens*, and *C. difficile*. The *erm*(B) gene has also been found in small plasmids in poultry samples (365) and in large plasmids in food samples in addition to other genes such as *msr*(C) and *lnu*(B), *tet*(L), and *tet*(W) (366). Its presence in chromosomes is also frequent.

The gene *erm*(A), associated with Tn*554* and commonly found in staphylococci from swine, has also been found in streptococci and sporadic isolates of *E. faecalis* and *E. faecium* from pigs, suggesting transfer events (282, 367). More recently, a novel *erm*(A)-like gene that confers high-level resistance to erythromycin (MIC, >128 μg/ml) has been detected in Inc18 plasmids with genes encoding resistance to phenicols and oxazolidinones (see below). This gene differs from the widespread *erm*(A) gene on Tn*554* and the *erm*(A) gene formerly called *ermTR*, predominant in staphylococci and streptococci, respectively (82 to 85% homology at the amino acid level). This *erm*(A) enterococcal variant has a 116-bp deletion in the translational attenuator (237).

Streptogramins

Genes conferring resistance to streptogramins (acetyltransferases encoded by *satG*/*vatE* and *satA*/*vatA* genes and ABC transporters encoded by *vgb*/*vgb*B) and macrolides [23S rRNA methylases encoded by *erm*(B), *erm*(A), *erm*(C) genes] are observed in a diversity of plasmids and clonal backgrounds. In addition, *vat* genes are often cotranscribed and cotransferred along with *vga*, *vgaB*, *vgb*, *vgbB*, or *erm*(B) genes through transposable elements, some of them previously observed in staphylococci (364, 368–373). Transferability of *vat* genes and streptogramin resistance in *E. faecium* strains through contaminated pork and chicken meat, raw manure, and surface/ground water has been extensively documented (374, 375).

Lincosamides

Resistance to this antibiotic family can be due to the presence of genes coding for ABC transporters or modifying enzymes, most of them located on plasmids and/or transposable elements. These elements have been extensively documented in staphylococci and to a lesser extent in streptococci, *Clostridium*, and other species of Gram-positive bacteria in animals.

ABC transporters that confer resistance to pleuromutilins, lincosamides, and streptogramin A antibiotics (PLS_A) include the genes *vga* and *vga*(A)v, *vga*(C), *vga*(E), *vga*(E)v, *eat*(A)v, *sal*(A), *lsa*(A), *lsa*(C), and *lsa*(E). They frequently appear within clusters in plasmids or transferable chromosomal regions previously reported in *S. aureus* (230). A 8,705-bp region flanked by IS*Efa8* and IS*1216*, and comprising genes coding for one or more antibiotics, namely *lnu*(B) (lincosamide), *lsa*(E) (PLS_A), *spw* (spectinomycin), *aadE* (streptomycin), and *erm*(B) (macrolide-lincosamide-streptogramin B), is common for plasmids of *S. aureus* (pV7037) and *E. faecium* (pY13, pXD4, pXD5) strains recovered from pigs (230, 376, 377). The pY13 plasmid also contains a copy of the genes *lnu*(B) (lincosamide) and *aphA3* (kanamycin/neomycin) and a second copy of *erm*(B), highlighting the redundancy of determinants in settings under high selective pressure.

Two genes coding for nucleotidyl transferases (*lnu*), which only confer resistance to lincosamides, have been described in *Enterococcus* from swine recovered on Chinese farms (229, 378). *lnu*(G) is part of a 4,738-bp functionally active transposon designated Tn*6260*, which was first detected in an *E. faecalis* isolate of swine origin; this element is similar to others of the Tn*554* family, which includes different antibiotic resistance genes (378). *lnu*(B) has been detected in porcine *E. fae-*

cium isolates, and it has been found in a nonconjugative plasmid linked to the *erm*(B), *lsa*(E), *spw*, *aadE*, and *aphA3* genes, which account for resistance to macrolides, lincosamides, streptogramins, pleuromutilins, streptomycin, spectinomycin, and kanamycin/neomycin (229).

Plasmids Conferring Resistance to Phenicols and Oxazolidinones

Genes coding for resistance to nonfluorinated phenicols (*cat*), nonfluorinated and fluorinated phenicols (*fexA*, *fexB*), and to both phenicols and oxazolidinones (*cfr*, *optrA*) have been detected in enterococcal species from animals, foods, and humans.

The production of chloramphenicol acetyltransferase (CAT) enzymes seems to be the main mechanism of resistance to chloramphenicol, although the number of studies addressing the diversity and the genetic context of *cat* genes in *Enterococcus* is still scarce. The predominant *cat* variants are *cat*(A-7), associated with pRE25-like plasmids of the Inc18 family, which are widely disseminated in food and farm animals, predominantly poultry (241), and *cat*(A-8), also known as cat_{pC223}, associated with pC223 plasmids originally detected in *S. aureus* that are now predominant in *E. faecalis* from swine. This gene eventually appears in tandem with *tet*(M) and *tet*(L) genes within the transposon Tn*6245*, and relics of this transposon have been observed in plasmids that also carry *fexA* and *optrA* (237). Although isolates positive for the *cat*(A-9) gene have been recently identified in *E. faecalis* from swine, their genetic context has not been characterized (A. Freitas, personal communication).

The florfenicol exporter gene *fexB* was initially detected in nonconjugative plasmids of *E. faecium*, *E. faecalis*, and *E. hirae* isolates collected on Chinese swine farms heavily exposed to florfenicol (379). These plasmids share common regions with the backbone of Inc18 plasmid derivatives (e.g., pVEF4), widely disseminated in Norwegian poultry farms (355). The *fexB* gene is bracketed by IS*1216* and would have been acquired by widespread pRE25-like plasmids, as occurred for other antimicrobial resistance genes flanked by this IS. The *fexB* gene has also been identified in enterococci from other farm animals (bovine) and aquaculture, although the plasmids have not been characterized (241, 380). A different epidemiological landscape occurs for the *fexA* gene, which is located on plasmids (241) and chromosomes (236) of enterococcal animal isolates, often in tandem with the *optrA* gene (237, 241) or the *cfr* gene (235). The *fexA* gene is inserted in the emblematic Tn*554* of staphylococci, although in enterococci traces of this transposon might be absent as a consequence of different events of horizontal gene transfer (237).

Enterococcal plasmids carrying *optrA* have been detected in poultry, swine, and humans. Despite differences in size (30 to 80 kb) and the backbone, all share similar regions upstream and downstream of the *optrA* gene (236, 237, 241). The presence of a novel *erm*(A)-like gene that confers a high level of resistance to erythromycin is notable (237). The genetic context of *optrA* is flanked by copies of IS*1216* in the same or opposite direction, which determine the mobility.

Conjugative and nonconjugative plasmids carrying the *cfr* gene flanked by different ISs (IS*1216*, IS*Enfa4*, IS*Enfa5*, IS*256*) have been described in animal isolates of various Gram-positive species, including enterococci. The nonconjugative pEF-01 (32.2 kb) plasmid represents the first description of a *cfr*-plasmid in this bacterial genus and was identified in a fecal *E. faecalis* isolate of bovine origin collected in 2009 on a Chinese farm (232). This plasmid has three Rep proteins of the Inc18 and Rep3 plasmid families and 9-kb and 6-kb regions which exhibit high similarity with the backbone of *vanA* Inc18 plasmids (pVEF1-2-3), which have been widely isolated on poultry farms (232). Moreover, the *cfr* gene was flanked by IS*1216*, which would facilitate recombination processes, and the plasmid also contains the *fexA* gene, which provides resistance to phenicols. Conjugative plasmids carrying the *cfr* gene bracketed by IS*Enfa4* copies were isolated from *E. thailandicus* and *E. faecalis* from Chinese swine farms. These are closely related to another emblematic Inc18 plasmid, pAMb1, and contained *erm*(B) and *erm*(A) genes, conferring the MLS_B phenotype and the ω-ε-ζ toxin-antitoxin module, which may promote the persistence of plasmids by encoding a system that kills or prevents the growth of plasmid-free cells (55). This genetic context has also been detected in streptococci and staphylococci and points to independent acquisition events for the *cfr* gene. The *cfr* gene bracketed by two copies of IS*Enfa5* has been documented in *E. gallinarum* and *E. casseliflavus* of swine origin (235).

Plasmids Conferring Resistance to Bacitracin

Bacitracin has been used as an animal growth promoter in China, and recent reports documented *E. faecalis* isolates with high-level resistance to this antibiotic (MIC, ≥256 μg/ml), due to the presence of the *bcrABDR* cluster, which is composed by the *bcrABD operon* and its regulatory gene, *bcrR*. The cluster bracketed by either two, one, or no IS*Enfa1* copies is located on transferable plasmids (341) or chromosomes. The structure IS*Enfa1*-

bcrABDR-IS*Enfa1* may be circulating and may have been transferred to other species by IS-mediated recombination. A multiresistant 79-kb pheromone-responsive plasmid carrying this IS*Enfa1-bcrABDR*-IS*Enfa1* platform as well as *optrA*, *fexA*, Tn*6425* (cat_{pC223}-*tetM-tetL*), Tn*5405* (*aph-sat-str*), and genes for resistance to copper and cadmium seems to be disseminated in Chinese farms (341), frequently associated with *E. faecalis* ST16. This *bcrABDR* cluster is also common in *E. cecorum*, a chicken commensal species (341).

Plasmids Conferring Resistance to Copper

Transferable resistance to copper (*tcrB*) in enterococci has been detected in piglets, calves, poultry, and humans in Europe, Asia, Australia, and North America (148, 331, 368, 381–383). Plasmids carrying *tcrB* are identified in intensively copper-supplemented livestock species, but plasmids with additional linkage with erythromycin [*erm*(B)] and/or vancomycin resistance (*vanA*) genes have only been observed in heavily copper-exposed swine (often with different copper compounds) in European countries where avoparcin was used as growth enhancer in the 1990s (148, 329, 381, 383, 384). The plasmids were detected in several enterococcal species (*E. faecium*, *E. faecalis*, *E. gallinarum*, *E. casseliflavus*, *E. mundtii*, *E. hirae*), and conjugation has been experimentally demonstrated from *E. faecalis* to *E. faecium* (381). Copper fed to feedlot cattle at a growth-promotion concentration (10 × basal requirement) was associated with increased frequencies of *tcrB*-positive, macrolide-resistant *erm*(B) and, eventually, tetracycline-resistant *tet*(M) enterococci; however, copper susceptibilities were not increased in piglets in which the effect of in-feed tylosin or chlortetracycline was evaluated (382, 385). Cotransmission of *tcrB* and *erm*(B) genes between *E. hirae* from a sediment-derived livestock isolate and *E. faecalis* has been experimentally demonstrated (386). A recent analysis of WGS of *E. faecalis* from copper-supplemented Danish pigs also documented the presence of a chromosomal cluster of genes involved in susceptibility to copper, including the *tcrYAZB* operon, in three of six isolates analyzed, all containing plasmids (387). A detailed characterization of this chromosomal region was not provided, although other authors, who also identified redundancy of copper genes in chromosomes, demonstrated its cotransferability with ampicillin resistance (331).

CONCLUDING REMARKS

This review summarizes the current knowledge concerning the epidemiology and population structure of antibiotic-resistant *Enterococcus* species from food-producing, wild, and companion animals. Members of this genus are normal components of the intestinal microbiota of animals, and some species may also be etiological agents of a wide variety of infections with *E. faecalis* ST16 (considered a zoonotic pathogen) or ST82 (an etiological agent of the amyloid encephalopathy in chickens).

Enterococcus species are frequent contaminants on foods (especially poultry meat), although the risk of transmission from animals to humans through the food chain is based on indirect evidence, and thus, the bacterial load necessary to colonize the human gut remains greatly unknown. The food and animal industries seem to have contributed to the spread of certain pathogenic lineages (*E. faecalis* ST82 and ST16 lineages) and multidrug-resistant strains. Other species adapted to animals seem to act as important reservoirs of adaptive traits (*E. cecorum*). However, transmission of antimicrobial resistance by horizontal gene transfer events represents the main risk of foods contaminated by enterococci. Genes encoding resistance to vancomycin, macrolides, phenicols, and linezolid have been extensively documented in animals, frequently in response to heavy selection by antimicrobials (antibiotics and heavy metals) used in prophylaxis or as growth promoters. Although the same genes and plasmids may be present in humans and animals, particular plasmid variants are often documented on farms, suggesting certain host specificity and transmission at a local level. Deep analysis of antimicrobial-resistant genes reveals a wide diversity of alleles [e.g., *erm*(A), *optrA, cfr*] and also the frequent presence of ISs (e.g., IS*1216*) that highlight the risk of frequent and independent acquisition and selection events of antimicrobial resistance on farms. More studies are necessary to establish the risks of the emergence and transmission of antibiotic-resistant enterococci from animals to humans.

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