Chapter 16
Zoonotic Transmission of Antimicrobial Resistant Enterococci: A Threat to Public Health or an Overemphasised Risk?

Valeria Bortolaia and Luca Guardabassi

Abstract Enterococci are intrinsically resistant to various antimicrobial classes and able to acquire resistance to clinically relevant drugs via horizontal transfer. Consequently, limited therapeutic options are available for treatment of enterococcal infections. Zoonotic transfer of antimicrobial resistance in enterococci has been studied for two decades. The first studies hypothesizing possible animal-to-human transmission of resistant strains and mobile genetic elements are dated 1993. Since then a considerable amount of papers has been published on this subject, providing the groundwork for important decisions limiting antimicrobial use in animal husbandry. In this chapter, the relative contribution by animal enterococci to antimicrobial resistance in human infections was reviewed taking into consideration the potential impact associated with different enterococcal species, animal hosts, epidemiological routes and mechanisms of transfer. The authors conclude that potential zoonotic risks mainly concern horizontal transfer of resistance genes and clonal transmission of multidrug-resistant *Enterococcus faecalis* sequence type ST16. The impact of clonal transmission from food animals to people appears to be negligible for other multidrug-resistant *E. faecalis* and *E. faecium* lineages responsible for hospital infections. Although it has been demonstrated experimentally that antimicrobial resistant enterococci of animal origin can transiently colonise the human digestive tract and transfer their resistance genes to the indigenous microflora, the actual risks associated with foodborne transmission are controversial, mainly limited to poultry meat products and possibly differ between geographical areas. Research is warranted to explore the ecology of enterococcal mobile genetic elements carrying resistance genes of clinical relevance and to develop suitable technologies to perform this type of studies.

Enterococci are commensal bacteria in the intestinal microbiota of humans and animals (Gilmore et al. 2013). *Enterococcus faecalis* and *E. faecium* are the most frequent species in humans and domestic animals, although species distribution has

V. Bortolaia (✉) · L. Guardabassi
Faculty of Health and Medical Sciences, Department of Veterinary Disease Biology, University of Copenhagen, Stigbøjlen 4, 1870 Frederiksberg C, Denmark
e-mail: vbo@sund.ku.dk

L. Guardabassi
e-mail: lg@sund.ku.dk

A. Sing (ed.), *Zoonoses–Infections Affecting Humans and Animals,*
DOI 10.1007/978-94-017-9457-2_16

a certain degree of host-specificity and is influenced by diet and environmental conditions (Aarestrup et al. 2002; Kühn et al. 2003). Both species are opportunistic pathogens that can cause a variety of infections, especially hospital-acquired infections, including endocarditis, bacteraemia, meningitis, wound and urinary tract infections, and peritonitis (Arias and Murray 2012). *E. faecalis* is the species most frequently isolated from human clinical specimens, followed by *E. faecium* (Hidron et al. 2008; Oteo et al. 2007). Together these two species are ranked as the third cause of bacteraemia in European and in American hospitals accounting for approximately 11–13 % of all bacteraemia cases (Ammerlaan et al. 2013; de Kraker et al. 2013). *E. faecalis* and *E. faecium* are also among the most common pathogens recovered from catheter-associated, skin and soft tissue infections (Arias and Murray 2012).

Enterococci are intrinsically resistant to important antimicrobial classes in clinical practice. Consequently, therapeutic options against enterococci are limited. Treatment of life-threatening infections usually consists of a combination of penicillin (ampicillin or penicillin) and aminoglycoside (gentamicin or streptomycin) (Arias and Murray 2008). Glycopeptides such as vancomycin are the best alternative, if the causative strain is resistant to one or both these drugs or in cases where aminoglycoside use is contraindicated due to nephrotoxicity. Other second tier agents include quinupristin-dalfopristin (for *E. faecium* only) and newer drugs such as linezolid, daptomycin, tigecycline and fifth-generation cephalosporins. Older antibiotics such as chloramphenicol, doxycycline, minocycline and nitrofurantoin may be used for specific indications (Arias and Murray 2008).

Over the last two decades many authors have hypothesized that animal enterococci may serve as a reservoir of resistant strains and resistance genes to humans. Antimicrobial resistant enterococci in food of animal origin gained attention from researchers and public health authorities in the early 1990s. At that time, vancomycin-resistant enterococci (VRE) were emerging as nosocomial pathogens worldwide (Leclercq et al. 1988; Uttley et al. 1988; Sundsfjord et al. 2001). Although initially it was believed that nosocomial use of vancomycin was the only factor selecting for VRE, this assumption was partly revisited after a considerable reservoir of VRE was reported in the community and in production animals in Europe (Bates et al. 1993; Klare et al. 1993; Torres et al. 1994; Goossens 1998; Martone 1998). In 1995, two independent studies established a correlation between usage of the vancomycin-analogue avoparcin as a growth promoter in livestock and occurrence of VRE in chickens and pigs (Klare et al. 1995; Aarestrup et al. 1995). Subsequent studies confirmed that i) there is cross-resistance between avoparcin and vancomycin (van den Bogaard et al. 1997a); ii) avoparcin use was associated with occurrence of VRE in animal faeces and meat products (Bager et al. 1997; Aarestrup et al. 2000a); and iii) occurrence of VRE in food animals and meat products was correlated to occurrence of VRE in faecal samples of community-dwelling humans (Pantosti et al. 1999; Klare et al. 1999; van den Bogaard 2000). The risk that animal VRE could be transmitted through the food chain was regarded as high, since enterococci are particularly resistant to heat, disinfectants and other decontamination procedures used at slaughterhouses (Giraffa 2002). All these evidences provided the basis for establishing and maintaining the ban of avoparcin use enforced in the EU since 1997

in accordance with the precautionary principle (Anonymous 1997). Additional links between occurrence of antimicrobial resistant enterococci in production animals and in healthy humans were hypothesized on the basis of similarities in the patterns of resistance to quinupristin/dalfopristin, erythromycin, tetracyclines, gentamicin and chloramphenicol, as well as in the distribution of genes conferring resistance to these antimicrobials (Welton et al. 1998; Werner et al. 1998; Aarestrup et al. 2000a; Aarestrup et al. 2000b; Del Campo et al. 2003; Klare et al. 2003; Kieke et al. 2006). These findings contributed to support the ban on use of all antimicrobials as growth promoters in the EU, which was ratified through different EU Regulations enforced in 1999 and in 2006 (Anonymous 1998a; Anonymous 1998b; Anonymous 2003). Use of growth promoters is still allowed in the USA, even though avoparcin has never been used in this country.

This chapter is a review of the literature regarding the possible contribution of enterococci of animal origin to antimicrobial resistance problems encountered in human medicine. The topic is reviewed taking into consideration the roles played by different enterococcal species (*E. faecium* vs. *E. faecalis*), animal hosts (food vs. companion animals), epidemiological routes (foodborne transmission vs. transmission by contact with animals) and mechanisms of transfer (clonal transmission vs. horizontal gene transfer). The chapter is organised into five sections addressing genetic basis of antimicrobial resistance in enterococci (16.1), prevalence of resistance in human, animal and food isolates (16.2), evidence of transmission between animals and humans (16.3), genetic links between clinical and animal strains (16.4) and concluding remarks by the authors (16.5).

16.1 Genetic Basis of Antimicrobial Resistance in Enterococci

Enterococci tolerate low concentrations of β-lactams, quinolones, aminoglycosides and lincosamides, and are able to metabolise preformed folic acid, thereby bypassing inhibition of folate synthesis by trimethoprim and sulphonamides (Murray 1990; Hollenbeck and Rice 2012). Moreover, they have a particular ability to acquire exogenous resistance genes via conjugative transposons and plasmids (Werner et al. 2013). This section summarises the genetic basis of antimicrobial resistance in enterococci with focus on acquired resistance to antimicrobials of clinical relevance and potential animal reservoirs.

16.1.1 β-lactam Resistance

Enterococci display intrinsic resistance to cephalosporins and decreased susceptibility to penicillins (Fontana et al. 1990; Zhang et al. 2012). In *E. faecium,* high-level penicillin resistance is mainly associated with mutations or overproduction of *pbp5*, which encodes a penicillin binding protein (PBP5) with low affinity for

penicillins (Arias and Murray 2012). An additional gene (*ddcY*) which mediates ampicillin resistance by altering the pathway of peptidoglycan synthesis was identified in ampicillin-resistant *E. faecium* (AREF) mutants selected *in vitro* (Mainardi et al. 2000). The clinical importance of this resistance mechanism appears to be limited, since this gene is present in a relatively small proportion (17 % out of 29 strains and 25 % out of 8 strains according to different studies) of *E. faecium* strains (Mainardi et al. 2000; Sacco et al. 2010; Zhang et al. 2012). Finally, a further, uncommon mechanism of ampicillin resistance in *E. faecium* is represented by enzymatic drug inactivation. Chromosomal beta-lactamase-encoding genes conferring ampicillin resistance were recently reported in different *E. faecium* strains (Sarti et al. 2012).

Transferability of ampicillin resistance determinants was shown *in vitro*, but the frequency and importance of this event in nature is unknown. Rice et al. demonstrated that *pbp5* is transferable horizontally and, recently, Novais et al. showed that five (28 %) out of 18 *E. faecium* strains isolated from the pig farm environment could transfer ampicillin resistance by conjugation, though the underlying genetic basis of resistance was not investigated (Rice et al. 2005; Novais et al. 2013).

Ampicillin-resistant *E. faecium* (AREF) have been detected in production animals worldwide with considerable differences between countries (see Sect 2). For example, in 2011 occurrence of ampicillin resistance in *E. faecium* isolates from chickens was 3 and 75 % in Denmark and Ireland, respectively (EFSA 2013). In addition, significant temporal variations in occurrence of AREF in production animals could be observed within the same country. In Denmark, prevalence of ampicillin resistance in *E. faecium* was 3 and 23 % among pig isolates in 2010 and 2011, respectively (DANMAP 2011). Notably, AREF are commonly detected in dogs and, to a lesser extent, in cats (Butaye et al. 2001; Simjee et al. 2002; Rodrigues et al. 2002; Moyaert et al. 2006; Damborg et al. 2009; Ghosh et al. 2011, 2012; de Regt et al. 2012).

In *E. faecalis*, penicillin resistance is mainly mediated by mutations in *pbp4* (Ono et al. 2005). Plasmid-mediated beta-lactamases were first described in *E. faecalis* in 1983, but their occurrence remains rare (Murray 1992; Hollenbeck and Rice 2012). Occurrence of acquired penicillin resistance is rare in *E. faecalis* of both human and animal origin.

16.1.2 Aminoglycoside Resistance

Enterococci display intrinsic low-level resistance to aminoglycosides (Galimand et al. 2011; Arias and Murray 2012). High-level aminoglycoside resistance is caused by ribosomal mutations and/or acquisition of genes encoding enzymes that modify the drug (Chow et al. 2000; Arias et al. 2010). The most commonly detected gene is the transposon-associated *aac(6')-Ie-aph(2'')-Ia*, which mediates resistance to gentamicin, but not to streptomycin (Chow et al. 2000; Arias and Murray 2012). High-level streptomycin resistance may result from ribosomal mutations or acquisition of *ant(6')-Ia* (Arias et al. 2010). These mobilisable genes conferring high-level gentamicin and streptomycin resistance have been detected in enterococcal isolates

from production and companion animals worldwide (Torres et al. 2003; Harada et al. 2005; Novais et al. 2005; Jackson et al. 2010; Larsen et al. 2011; Frye and Jackson 2013; Novais et al. 2013). There are no peculiar differences between *E. faecium* and *E. faecalis* in the occurrence of aminoglycoside resistance.

16.1.3 Glycopeptide Resistance

Resistance to glycopeptides such as vancomycin and teicoplanin is mainly mediated by *van* operons which encode modified peptidoglycan precursors terminating in D-Ala-D-Lac or D-Ala-D-Ser and retain lower affinity to glycopeptides compared to the wild-type peptidoglycan precursor terminating in D-Ala-D-Ala (Courvalin 2006). Nine *van* operons have been described to date in *E. faecium* and *E. faecalis*, with *vanA* and *vanB* being the most frequently detected among clinical isolates, with notable country-based differences (Lebreton et al. 2011; de Garnica et al. 2013). Indeed, VanA is the predominant type of glycopeptide resistance reported among clinical isolates in Europe and North America, whereas VanB is more common among isolates in Australia (Cetinkaya et al. 2000; Christiansen et al. 2007; Deshpande et al. 2007). Interestingly, an increased occurrence of *vanB* was recently observed in hospital-associated *E. faecium* isolates in France, Germany and Spain (Bourdon et al. 2011; Klare et al. 2012; Lopez et al. 2012). Vancomycin resistance operons have been described both on chromosome and on plasmids, mainly in association with conjugative transposons Tn*1546* and Tn*1546*-variants (*van A*), and Tn*1547* and Tn*1549* (*vanB*) (Hegstad et al. 2010). Interestingly, glycopeptide resistance operons displaying 79–94 % nucleotide identity to *vanA* operon in enterococci have been described in *Paenibacillus* spp. isolated from soil, indicating that this glycopeptide resistance determinant may have originated in the environment a long time before emerging in clinical settings (Guardabassi et al. 2005). A novel glycopeptide resistance mechanism mediated by mutations in *ddcY* (see paragraph 1.1) was described in *E. faecium* strains obtained *in vitro* through serial exposure to increasing concentrations of glycopeptides (Cremniter et al. 2006). This mechanism of resistance, which leads to cross-resistance to ampicillin and glycopeptides (both vancomycin and teicoplanin) through bypass of PBPs-mediated peptidoglycan cross-linking, has not been described in clinical isolates.

VRE, generally harbouring *vanA,* are still detected in production animals from countries that used avoparcin, although at low prevalence (Lim et al. 2006; EFSA 2013). Noteworthy, avoparcin use was discontinued in Sweden already in 1984 and no VRE were detected in production animals until 2000 (Nilsson 2012). Then, from 2000 to 2005 the proportion of broiler flocks positive for VRE strikingly increased from less than 1>40 %, mainly due to spread of a single *E. faecium* clone for reasons which are still unknown (Nilsson 2012). VRE have not been reported to date in production animals from most countries where avoparcin has never been used (e.g. Canada and Australia), with the exception of the USA where vancomycin-resistant *E. faecium* (VREFm) was reported once in swine (Coque et al. 1996; Diarra et al. 2010; Donabedian et al. 2010; Fard et al. 2011; Tremblay et al. 2011; Tremblay et al. 2012).

VRE have also been isolated from companion animals in various countries (Devriese et al. 1996; Simjee et al. 2002; Manson et al. 2003; Torres et al. 2003; Herrero et al. 2004; Poeta et al. 2005). Independent of host species, occurrence of vancomycin resistance is generally more frequent in *E. faecium* than in *E. faecalis*.

16.1.4 Daptomycin Resistance

Daptomycin resistance is mediated by mutations in two groups of chromosomal genes (Arias et al. 2011; Tran et al. 2013). The first group include genes encoding a three-component regulatory system (LiaFSR) involved in homeostasis of the cell envelope, while the second group of genes encode enzymes (GdpD and Cls) involved in cell membrane phospholipid metabolism (Arias et al. 2011; Humphries et al. 2012; Tran et al. 2013). In addition, mutations in the mannose-specific phosphotransferase system (PTS) were also suggested to be involved in daptomycin resistance in *E. faecium* (Humphries et al. 2012). Resistance to this lipopeptide antibiotic is rare in humans (and it is mainly developed during therapy) and has never been reported in animals (Novais et al. 2004; Hollenbeck and Rice 2012). Nevertheless, a zoonotic potential of daptomycin-resistant *E. faecium* was hypothesized in a recent study showing that proximity of the residence of patients to animal or crop operations was associated with occurrence of daptomycin resistance (Kelesidis and Chow 2013).

16.1.5 Linezolid Resistance

The most frequently reported mechanism of linezolid resistance is associated with mutations in domain V of 23S rRNA described both in *E. faecium* and in *E. faecalis* (Arias and Murray 2012). In addition, transferable *cfr* has been recently reported in *E. faecalis* from cattle, swine and farm sewage in China and *E. faecalis* and *E. faecium* in humans in Spain and Thailand (Cercenado et al 2010; Diaz et al. 2012; Liu et al. 2012; Liu et al. 2013). This gene has been found both on conjugative and on non-conjugative plasmids, often in association with IS*Enfa4*, IS*1216* and IS*256*-like elements, thus showing a high propensity to disseminate by conjugation and recombination events (Diaz et al. 2012; Liu et al. 2012; Liu et al. 2013; Shen et al. 2013).

16.1.6 Streptogramin Resistance

E. faecalis is intrinsically resistant to streptogramins such as quinupristin/dalfopristin, whereas *E. faecium* displays innate low-level resistance to streptogramin B (quinupristin) (Singh and Murray 2005). Several plasmid-mediated streptogramin resistance genes, with *vat*(D) and *vat*(E) being the most frequently detected, have been described in animal and human *E. faecium* isolates worldwide, and yet

unknown resistance mechanisms are likely to exist (Jensen et al. 1998; Soltani et al. 2000; Hammerum et al. 2001; Hershberger et al. 2004; Simjee et al. 2006; De Graef et al. 2007; Jung et al. 2010; Frye and Jackson 2013).

16.1.7 Tigecycline Resistance

The mechanisms of tigecycline resistance are currently unknown. Occurrence of resistance to this tetracycline analogue is sporadic in clinical and animal isolates (Waites et al. 2006; Dowzicky and Chmelarová 2011). Tigecycline-resistant *E. faecalis* isolates were reported in samples from chicken meat and swine in Portugal (Freitas et al. 2011b).

16.2 Prevalence of Antimicrobial Resistance

Prevalence of antimicrobial resistance and distribution of antimicrobial resistance genes vary significantly depending on host species and geographical regions. Local data on prevalence of antimicrobial resistance often reflect the specific patterns of antimicrobial usage within each host species and marked differences between hosts may provide useful epidemiological indications on potential reservoirs of antimicrobial resistance within defined geographical areas. The two following paragraphs summarise available national data on prevalence of antimicrobial resistance in *E. faecium* and *E. faecalis* from human patients and production animals. Most data derive from studies in Europe and the USA, as in these regions occurrence of antimicrobial resistance in animal and human isolates has been monitored for longer time and in a more systematic way compared to other regions of the world.

16.2.1 Patterns of Antimicrobial Resistance in Animal and Human E. faecium

Clinically-relevant antimicrobial resistance phenotypes in *E. faecium* that may be linked to animal reservoirs include resistance to ampicillin, gentamicin, vancomycin and quinupristin-dalfopristin. Resistance to linezolid and daptomycin is rare or even absent in animal populations (see Sect 1.4 and 1.5).

Recent data from Europe and North America show that ampicillin resistance is frequent (≥ 89 %) among human clinical isolates, irrespective of geographical origin (Table 16.1). Ampicillin resistance occurs less frequently among isolates from animal sources, with the exception of turkey meat in the USA in which ampicillin resistance occurred in 75 % of isolates (Table 16.1). Thus it seems that animal sources, mainly poultry, could contribute to occurrence of ampicillin resistance in clinical settings only to a limited extent. A marked difference in the prevalence of

Table 16.1 Prevalence of antimicrobial resistance in *Enterococcus faecium* isolated from humans (clinical isolates) and animals (indicator isolates) within the same country. (Pen, penicillin; Gen, gentamicin; Van, vancomycin; Ery, erythromycin; Qui/Dal, quinupristin/dalfopristin; Tet, tetracycline; Lin, linezolid.)

Country	Isolation year	Origin (no. of isolates)	Prevalence of antimicrobial resistance (%)							Reference
			Pen	Gen	Van	Ery	Qui/Dal	Tet	Lin	
Denmark	2011	Human (619)	93	74	1.3	–	–	–	–	DANMAP2011
	2011	Swine (116)	23	1	1	33	2	62	0	DANMAP2011
	2011	Pork, DK (27)	0	0	0	15	0	7	0	DANMAP2011
	2011	Broilers (107)	3	0	0	15	1	5	0	DANMAP2011
	2011	Broiler meat, DK (83)	2	0	0	19	1	10	0	DANMAP2011
	2011	Broiler meat, imported (64)	28	0	0	61	12	34	0	DANMAP2011
	2011	Cattle	–	–	–	–	–	–	–	DANMAP2011
	2011	Beef (16)	0	0	0	0	0	0	0	DANMAP2011
USA	2009	Humans ([a])	–	19	75	–	–	–	–	The surveillance network USA, 2013
	2004–2005	Humans (280)	89	–	68	–	–	–	0	Waites et al. 2006
	2011	Chicken meat (221)	19	9	0	22	32	43	0	NARMS 2011
	2011	Turkey meat (40)	75	10	0	32	55	82	0	NARMS 2011
	2011	Pork (37)	3	0	0	11	13	49	0	NARMS 2011
	2011	Beef (82)	4	0	0	6	11	22	0	NARMS 2011

[a] 8283 isolates tested for vancomycin resistance and 4772 isolates tested for gentamicin resistance

ampicillin resistance has been observed between isolates from pig (23 %) and pork (3 %) in Denmark, indicating that hygienic measures at slaughter effectively reduce human foodborne exposure to ampicillin-resistant strains of pig origin.

Occurrence of gentamicin resistance greatly varies between countries and host species. In Denmark, gentamicin resistance is high (74 %) among clinical isolates, low (1 %) in pig isolates and absent in meat isolates, indicating that there is no significant animal reservoir of this resistance in this country. Differently, in the USA gentamicin resistance occurs less frequently (19 %) than in Denmark among clinical isolates, but is more frequent in poultry meat isolates (9 and 10 % in broiler and turkey meat, respectively). These data suggest that poultry meat is a potential reservoir of gentamicin resistance for *E. faecium* isolated from human infections in the USA.

Geographical patterns of vancomycin resistance are also useful to infer potential epidemiological links between animals and humans. In Denmark, prevalence of vancomycin resistance is extremely low (ca. 1 %) among clinical and swine isolates, and was not detected among poultry isolates by the conventional monitoring program in 2011 (Table 16.1). On the contrary, vancomycin resistance is widespread in American hospitals, being detected in up to 75 % of clinical isolates (Table 16.1).

In this country, vancomyicin resistance is virtually absent among isolates from production animals, which strongly indicates absence of animal reservoirs. Altogether, it appears that occurrence of vancomycin resistance among human clinical isolates is primarily driven by hospital use of glycopeptides and only marginally influenced by zoonotic transmission from animals.

Different considerations can be made with regard to the occurrence of quinuprisitin/dalfopristin resistance. This phenotype occurs at variable frequency (1–55 %) among isolates from poultry meat both in Europe and in the USA (Table 16.1; EFSA 2013). The frequent recovery of resistant strains from poultry products in Europe is surprising, since the quinuprisitin/dalfopristin analogue virginiamycin, which is likely to select for quinuprisitin/dalfopristin resistance, has not been used since 1999, while it is still used in the USA for growth promotion. Genetic linkage of quinopristin/dalfopristin resistance genes to genes conferring resistance to antimicrobials used in animal production (e.g. macrolides) may explain the persistence of these genes in food animals in Europe (Hammerum et al. 2001). Data on occurrence of quinopristin/dalfopristin resistance in human isolates are not readily available. However, it seems that prevalence of this resistance phenotype is lower in human isolates compared to animal isolates, suggesting that existence of an animal reservoir is possible for quinupristin/dalfopristin resistance (Donabedian et al. 2006; Kieke et al. 2006; Hammerum et al. 2009).

Finally, it is important to note that occurrence of resistance to antimicrobials used in animals like erythromycin and tetracyclines is generally high among animal and meat isolates, with the exception of cattle and beef (Table 16.1). Although veterinary use of these antimicrobials does not constitute a direct risk to public health since they generally are not used for treatment of human enterococcal infections, it may favour co-selection of genes conferring resistance to clinically relevant antimicrobials, as hypothesized for quinupristin/dalfopristin and glycopeptides (Hammerum et al. 2001; Novais et al. 2005).

16.2.2 Patterns of Antimicrobial Resistance in Animal and Human E. faecalis

From a contemporary clinical perspective, the only antimicrobial resistance phenotype in *E. faecalis* that could be significantly linked to animal reservoirs is gentamicin resistance, since resistance to ampicillin, vancomycin and linezolid is rare or even not detected in animal isolates, (Ghosh et al 2012; Liu et al. 2012; 2013). According to recent data, gentamicin resistance is relatively high among clinical isolates in Europe (43 %) and in the USA (29 %) (Table 16.2; Kuch et al. 2012). In Denmark, gentamicin resistance was reported at comparable prevalence in clinical (31 %) and in swine (21 %) isolates (Table 16.1). Similarly, in the USA occurrence of gentamicin resistance in poultry meat isolates was 30–34 %, which is comparable to values observed in clinical isolates (Table 16.2). These data indicate the possible existence of an animal reservoir of gentamicin-resistant *E. faecalis*, though linked to different animal sources in different geographical areas. Interestingly, as observed for *E. faecium*, occurrence of gentamicin-resistant strains is significantly higher in Danish pigs (21 %) than in pork (2 %) (Table 16.2). These data indicate low risk of carcass contamination in pig slaughtering and consequent low human exposure to gentamicin-resistant through consumption of pork.

As observed for *E. faecium*, resistance to erythromycin and tetracycline is widespread among *E. faecalis* isolates from animals and meat (Table 16.2), possibly as a consequence of the massive use of these antibiotics in livestock production.

16.3 Transmission of Antimicrobial Resistance Between Animals and Humans

Transmission of antimicrobial resistance between animal and human enterococci may happen through different epidemiological routes and mechanisms. Humans are exposed to animal enterococci by direct contact with animals and animal-contaminated environments or indirectly, through consumption of contaminated food of animal origin and vegetables from crops treated with animal manure. Once acquired, strains of animal origin may transiently colonise the human digestive tract and transfer mobile genetic elements (MGE) containing antimicrobial resistance genes to the indigenous microflora, including bacteria other than enterococci.

16.3.1 Foodborne Transmission

Foodborne transmission may result from consumption of contaminated animal food products and cross-contamination in the kitchen (Wegener et al. 1997). *E. faecium* and *E. faecalis* generally contaminate raw meat and cheese at concentrations of 10^2–10^4 and 10^5–10^7 colony forming units (CFU) per gram, respectively

Table 16.2 Prevalence of antimicrobial resistance in *Enterococcus faecalis* isolated from humans (clinical isolates) and animals (indicator isolates) within the same country. (Pen, penicillin; Gen, gentamicin; Van, vancomycin; Ery, erythromycin; Tet, tetracycline; Lin, linezolid)

Country	Isolation year	Origin (no. of isolates)	Prevalence of antimicrobial resistance (%)						Reference
			Pen	Gen	Van	Ery	Tet	Lin	
Denmark	2011	Human (513)	-	31	0	–	–	–	DANMAP 2011
	2011	Pigs (117)	0	21	0	54	85	0	DANMAP 2011
	2011	Pork, DK (133)	0	2	0	8	17	0	DANMAP 2011
	2011	Pork, imported (45)	0	4	0	11	36	0	DANMAP 2011
	2011	Broilers (110)	0	0	0	15	17	0	DANMAP 2011
	2011	Broiler meat, DK (34)	0	0	0	18	26	0	DANMAP 2011
	2011	Broiler meat, imported (69)	0	1	0	49	67	0	DANMAP 2011
	2011	Cattle	–	–	–	–	–	–	DANMAP 2011
	2011	Beef, DK (20)	0	0	0	5	20	0	DANMAP 2011
	2011	Beef, imported (30)	0	3	0	7	17	0	DANMAP 2011
USA	2009	Humans[a]	–	29	4	–	–	–	The surveillance network, USA, 2013
	2005	Humans (740)	3	–	5	–	–	0.5	Waites et al., 2006
	2011	Chicken meat (186)	0	30	0	35	63	0	NARMS 2011
	2011	Turkey meat (392)	0	34	0	47	92	0	NARMS 2011
	2011	Pork (334)	0	1	0	4	79	0	NARMS 2011
	2011	Beef (269)	0	0	0	3	18	0	NARMS 2011

[a] 23,826 isolates tested for vancomycin resistance and 14,990 isolates tested for gentamicin resistance

(Giraffa 2002). The hypothesis that antimicrobial resistant enterococci of animal origin could be transferred to the intestine of healthy humans via food is indirectly supported by studies describing clonally related strains in meat products and in the faeces of meat consumers. Donabedian et al. described closely related gentamicin-resistant *E. faecalis* strains in multiple pork samples and one human sample, and indistinguishable strains in a chicken meat sample and a human sample in the USA (Donabedian et al. 2003). Agersø et al. demonstrated clonal relatedness between five vancomycin-resistant *E. faecalis* (VREFs) from turkey meat and from the intestine of healthy humans in Denmark (Agersø et al. 2008). Finally, in an additional study from Denmark, Hammerum et al. described the detection of highly related vancomycin-resistant *E. faecium* (VREFm) isolates in pig samples and in the intestine of a healthy human who reported no contact with pigs but had eaten pork (Hammerum et al. 2004).

Evidence that animal enterococcal strains occurring in food have the ability to colonise the human intestine for a variable time period has been shown by experiments conducted on healthy human volunteers. In an experiment performed on himself, Berchieri established that a minimum concentration of 10^7 CFU of VREFm of poultry and pig origin was necessary to be able to isolate the same strain from faeces for a period of 20 days (Berchieri 1999). Sørensen et al. demonstrated that VREFm from poultry meat and quinupristin/dalfopristin-resistant *E. faecium* from pork ingested at 10^7 CFU could be detected in the faeces of 8 out of 12 volunteers 6 days after ingestions, at different concentrations (Sørensen et al. 2001). One out of 12 volunteers excreted the strain also 14 days after ingestion (Sørensen et al. 2001). In a similar experiment, Lester et al. demonstrated that animal VREFm transiently colonising the human gut could transfer *vanA* to resident commensal *E. faecium* strains in three out of six volunteers, indicating that occurrence of VREFm in food may result in transfer of vancomycin resistance to consumers (Lester et al. 2006). Recently, Al-Ahmad et al. showed that foodborne *E. faecalis* could integrate into dental oral biofilm in 5 out of 6 volunteers for at least 5 days, indicating a potential risk for endodontic infections that may evolve into bacteraemia (Al-Ahmad et al. 2010).

In conclusion, based on the current knowledge, ingestion of antimicrobial resistant enterococci of animal origin can result in colonisation of the human digestive tract for a variable time, likely depending on the numbers of enterococci ingested as well as on host factors, and exchange of MGEs containing antimicrobial resistance genes with the indigenous microflora.

16.3.2 Transmission via Direct Contact with Food Animals

Farm and slaughterhouse workers and veterinarians are the main categories at risk for this transmission route, since they are daily exposed to high numbers of animals. High density of animals and animal excreta implies also a high load of faecal bacteria in farm environments. Various studies showed that genetically related antimicrobial resistant enterococci can be isolated from animal faeces, insects, dust

and air inside and in proximity of farms, which indicates the existence of multiple sources of human exposure to animal enterococci (Graham et al. 2009; Ahmad et al. 2011; Braga et al. 2013; Novais et al. 2013). Evidence of human infections caused by antimicrobial-resistant enterococci transmitted by direct contact with production animals is limited. Das et al. reported a VREFs-infected wound in a worker who was injured while working at a factory packaging chickens (Das et al. 1997). The strain isolated from the wound had the same resistance profile as isolates from the factory and the patient had no risk factors for a VREFs infection, strongly supporting animal origin of the infection (Das et al. 1997).

Different studies reported occurrence of genetically related enterococci strains displaying specific resistance phenotypes in the faeces of animals and healthy farm workers. VREFm clones shared by turkey, turkey farmers and turkey slaughterers and by broiler and broiler farmers were detected in The Netherlands and in Norway (van den Bogaard et al. 1997b; Simonsen et al. 1998; Stobberingh et al. 1999; Jensen et al. 2003). Clonally related quinupristin/dalfopristin-resistant *E. faecium* were isolated from a poultry farmer and his animals in The Netherlands (Jensen et al. 1998). Closely related plasmids and indistinguishable Tn*1546* variants harbouring *vanA* have been reported in genetically unrelated VREFm isolated from poultry and workers within farms (Stobberingh et al. 1999; van den Bogaard et al. 2002; Sletvold et al. 2007), suggesting that *vanA* of animal origin may be horizontally transferred to the intestinal microbiota of farm workers.

16.3.3 Transmission via Direct Contact with Companion Animals

A role of companion animals as reservoirs of antimicrobial-resistant enterococci was first hypothesized in 1996, when van Belkum et al. discovered that 17% of dogs and cats examined harboured VREFm while the incidence among people living in the same area was 2–3%, and that VREFm isolates from a dog, a cat and a human carrier were indistinguishable by pulsed-field gel electrophoresis (PFGE) (van Belkum et al. 1996). The authors concluded their article by raising the question, "which dog poses a greater risk to the postman: the one that barks or the one that wags its tail?" (van Belkum et al. 1996). Companion animals represent potential sources of antimicrobial-resistant bacteria, since they live in close contact with their owners and are often administered antimicrobials belonging to the same classes used for treatment in humans (Guardabassi et al. 2004; Jackson et al. 2009). Antimicrobial resistant enterococci can be isolated from different animal body sites and from faeces, which may represent a source of contamination of domestic and urban environment (Jackson et al. 2009; Ghosh et al. 2011). Ampicillin-resistant *E. faecium* (AREF) were detected in a considerable proportion of dogs and cats in different European countries, being present in 23% (of 183), 30% (of 79) and 76% (of 25) of dogs in the UK, The Netherlands and Denmark, respectively, and in 13% (of 85) of cats in The Netherlands (Damborg et al. 2009; de Regt et al. 2012). In addition, VREFm were detected in 1.4% (out of 71) and 13% (out of 87) of dog faeces samples examined in Portugal and Spain, respectively (Herrero et al. 2004;

Poeta et al. 2005). Occurrence of VRE in dogs was reported also outside Europe. The first VREFm reported in a dog in the USA was shown to harbour a mutated form of Tn*1546* described in human patients (Simjee et al. 2002). A VREFs from a dog with mastitis displayed a PFGE profile prevalent among human isolates in New Zealand (Manson et al. 2003). These studies suggest that VRE and *vanA* may be exchanged between humans and dogs, but the importance and prevalent direction of this transmission route is difficult to assess.

16.4 Genetic Links Between Clinical and Animal Strains

Even if it appears plausible that antimicrobial resistant enterococci of animal origin reach the digestive tract of humans and transfer resistance genes to human-adapted strains, the human health consequences associated to this biological phenomenon are controversial. This section reviews the current knowledge of the genetic similarities between clinical and animal strain populations of *E. faecium* and *E. faecalis*. This information is of paramount importance to assess the risk of zoonotic transmission.

16.4.1 Genetic Links Between Clinical and Animal E. faecium

Based on multilocus sequence typing (MLST), lineages 17 (which include, among others, sequence types ST16 and ST17), 18 (ST18) and 78 (ST78 and ST192) are the most important epidemic lineages associated with nosocomial infections worldwide (Willems et al. 2012). These hospital-associated lineages are generally characterised by ampicillin resistance and are particularly enriched in genes encoding colonisation and adhesion factors, which likely play a role in virulence (Somarajan and Murray 2013). Animal strains rarely overlap with the hospital-associated lineages, with the notable exception of canine *E. faecium* strains. Indeed, AREF belonging to the hospital-associated ST78 and ST192 are frequently detected in dogs, but they generally lack genes encoding putative virulence factors (Damborg et al. 2009). Thus it appears that animal and clinical *E. faecium* strains constitute two distinct subpopulations in relation to ampicillin resistance and occurrence of putative virulence factors. Similar conclusions have been drawn for VREFm. The population structures of *E. faecium* isolated from human patients and animals are generally diverse, and overlap only sporadically (Woodford et al. 1998; Jung et al. 2006; Biavasco et al. 2007; Donabedian et al. 2010; Freitas et al. 2011a; Hammerum 2012; Tzavaras et al. 2012; Getachew et al. 2013). Among lineages of clinical relevance, there are single reports of *vanA*-positive VREFm ST132 (related to ST18) in swine in Portugal, *vanA*-positive VREFm ST78 in rabbit meat, and *vanB*-positive VREFm ST17 in chicken meat and veal in Spain (Lopez et al. 2009). VREFm lineages grouped in clonal complex CC5, which are common among porcine strains of diverse geographical origin, have been sporadically reported as a cause of urinary

tract infections in hospitalised patients (Freitas et al. 2011a). These findings suggest that animal VREFm strains have a limited zoonotic potential, as further substantiated by recent evolutionary studies based on comparative genome analyses, which conclusively showed that animal and clinical strains, although evolutionary linked, constitute different subpopulations or clades that diversified mainly through recombination and acquisition or loss of MGEs and eventually adapted to different ecological niches (van Schaik et al. 2010; de Regt et al. 2012; Galloway-Peña et al. 2012; Willems et al. 2012; de Been et al. 2013; Lebreton et al. 2013).

16.4.2 Genetic Links Between Clinical and Animal E. faecalis

Also in *E. faecalis*, few genetic lineages, namely CC2, CC16 and CC87, are particularly enriched among nosocomial isolates and associated with multidrug resistance. However, differently from *E. faecium*, these hospital-associated clones are phylogenetically closely related to human commensal and animal strains indicating the absence of a clear boundary between clinical and non-clinical strains (McBride et al. 2007; Willems et al. 2011; Palmer et al 2012). CC2 strains including the epidemic VREFs clone ST6 are frequently associated with nosocomial infections and have sporadically been reported in animals such as pigs and natural gilthead seabream (*Sparus aurata*, a saltwater fish species) in Portugal and crows in the USA (McBride et al. 2007; Freitas et al. 2009; Freitas et al. 2011a; Barros et al. 2012; Kuch et al. 2012; Oravcova et al. 2013). Similarly, CC87 strains are associated with nosocomial infections (especially in Poland), but have not been described in animals to date (McBride et al. 2007; Kuch et al. 2012; Getachew et al. 2013). Therefore, the genetic link between animal and human *E. faecalis* seems to be weak for these two lineages. Interestingly, the ecology of CC16 is different and this lineage appears to have a broad host spectrum, since it is well represented both among clinical and among non-clinical isolates (Ruiz-Garbajosa et al. 2006; Willems et al. 2011). In a recent study examining 386 contemporary human *E. faecalis* from hospital and community sources in six European countries, ST16 (the presumed founder of CC16) strains represented 11% and 15% of the total hospital and community-associated strains, respectively (Kuch et al. 2012). Half of the ST16 strains from each source displayed gentamicin resistance, suggesting that gentamicin-resistant strains causing up to 6% of nosocomial infections may be acquired in the community (Kuch et al. 2012). Notably, ST16 was found to be highly predominant among gentamicin-resistant *E. faecalis* isolated from pigs and pork in Denmark in 2001–2002, and represented 9% of 22 *E. faecalis* isolates from endocarditis patients in the same country in 1996–2002 (Larsen et al. 2010). These porcine and human strains were shown to be closely related also by PFGE, strongly suggesting a link between gentamicin-resistant *E. faecalis* ST16 in pigs and human patients in Denmark (Larsen et al. 2010). Gentamicin-resistant ST16 has also been associated with nosocomial infections in Cuba, with urinary tract infections and poultry in Vietnam, and with swine and poultry in Portugal (Freitas et al. 2009; Quinones et al. 2009; Poulsen et al. 2012; Novais et al. 2013). To the best of the authors' knowledge, the

genetic background of gentamicin-resistant strains frequently isolated from poultry meat products in the USA (see Sect 2.2) has not been investigated. Multidrug-resistant ST16 displaying additional resistance to linezolid have been reported in patients in Greece and Thailand (Spiliopoulou et al. 2011; Diaz et al. 2012), and vancomycin-resistant strains have been isolated from American crows (Oravcova et al. 2013). Altogether, it appears that the genetic link between clinical and animal *E. faecalis* mainly concerns this clone.

16.4.3 Genetic Links Between Mobile Genetic Elements in Clinical and Animal Enterococci

Exchange of MGEs carrying antimicrobial resistance genes may represent an additional genetic link between animal and clinical strains. The possible existence of this genetic link is suggested by the fact that most genes conferring resistance to clinically relevant antibiotics are transferrable and indistinguishable MGEs have been detected in animal and human strains (Hegstad et al. 2010; Werner et al. 2013). Transferability of MGEs from animal to human enterococci strains has been demonstrated *in vitro* and/or in animal models, including transferability of gentamicin resistance in *E. faecalis* and of ampicillin, gentamicin and vancomycin resistance in *E. faecium* (Lester and Hammerum 2010; Ghosh et al 2011; Sparo et al. 2012; Novais et al. 2013). A recent study showed that at least half (18/36) of the VREFm strains isolated within the same hospital in the USA in the period 1998–2009 had acquired the *vanB*-containing Tn*1549* via independent insertion events, indicating *de novo* generation of VREFm rather than cross-transmission, presumably mediated by transfer of this resistance determinant from gut anaerobic bacteria (Howden et al. 2013). Analysis of polymorphisms in MGEs harbouring resistance genes can be a useful epidemiological marker, since specific mutations in transposons and resistance genes have been associated with different animal species. For example, a point mutation (G to T) in *vanX* in Tn*1546* has been consistently associated with *E. faecium* isolated from poultry (G) and pigs (T), while both types occur among human clinical isolates (Jensen 1998; Hammerum 2012). Similarly, different *erm*(B) alleles occur at different frequencies among macrolide-resistant *E. faecium* isolates from pigs and poultry, and all variants are present among isolates from healthy and diseased humans (De Leener et al. 2005). These data suggest that animal and clinical *E. faecium* exchange MGEs carrying antimicrobial resistance genes, but the extent of this mechanism of zoonotic transfer remains unknown. In this regard, it is of paramount importance to implement sequence-based methods for typing of enterococcal plasmids allowing reproducibility, transportability, and comparability of data via the Internet, such as those developed for Gram-negative bacteria (Jensen et al. 2010). The risk of horizontal transfer of resistance genes between animal and human strains has not been thoroughly investigated in *E. faecalis* and it would be particularly interesting to compare the MGEs associated with gentamicin resistance genes in animal and clinical isolates.

16.5 Concluding Remarks

Enterococci are among the leading causes of serious human infections like bacteraemia and endocarditis. These infections are generally treated empirically and the consequences of treatment failure may be fatal to the patient, if infection is caused by a strain resistant to first line agents. By this chapter, the authors made an attempt to evaluate to what extent resistance problems in human enterococcal infections are attributable to strains and MGEs of animal origin. The authors' conclusion is that in general clinical *E. faecium* strains are not directly linked to animal sources, whereas the boundary between animal and clinical *E. faecalis* strains is not well defined. A clear overlap is evident for multidrug-resistant *E. faecalis* ST16, which has been associated with both human patients and animals, particularly pigs. Further detailed population genetic analysis of *E. faecalis* is needed to evaluate whether this and other clones shared by humans and animals might favour transfer of antimicrobial resistance from farms to hospitals.

Even though resistance genes of clinical relevance have been widely reported in enterococci isolated from animals, comparison of data on prevalence of antimicrobial resistance in animal, meat and human clinical isolates indicates that zoonotic risks associated with horizontal gene transfer from animal to human enterococci greatly differ depending on geographical region and are restricted to specific types of resistance and animal sources. The major risk seems to be associated to horizontal transfer of gentamicin resistance genes through consumption of poultry meat, especially in the USA, where resistance to this first line agent is relatively frequent in isolates from broiler and turkey meat. Moreover, farm-to-fork transmission of gentamicin resistance genes is plausible, since aminoglycosides are used both in hospitals and in livestock production, but hardly ever used for systemic antimicrobial therapy in the primary health care sector because of parenteral administration and high toxicity. A possible poultry reservoir of quinupristin/dalfopristin-resistant *E. faecium* cannot be excluded on the basis of prevalence data. However, the lack of epidemiological data on strains of human and animal origin hampers quantification of this zoonotic risk. VRE strains causing human infections are not obviously linked to animals as indicated by the fact that they are prevalent in countries where avoparcin has never been used in livestock and VRE are rare or absent in livestock. Indigenous anaerobes in the patient's digestive tract seem to be a more important source of VanB operons than farm animals. As for VanA operons, farm animals have been a reservoir of these vancomycin resistance determinants, as indicated by the recovery of indistinguishable Tn*1546* variants in clinical and animal VREf isolates. However, this zoonotic risk has been significantly reduced by the ban of avoparcin and by the consequent decrease of VRE in livestock.

Little is known about the zoonotic risks associated with direct exposure to animals, including farm and companion animals. The risk of foodborne transmission is significantly higher for poultry meat than for other food products of animal origin, mainly due to lower hygienic standards and higher risk of carcass contamination in poultry slaughtering. Although horizontal transfer of resistance genes from animal

to human enterococci has been demonstrated to occur in human digestive tract under *in vivo* conditions, the magnitude and clinical significance of this phenomenon remain unclear. Further insights into the ecology and epidemiology of MGEs carrying resistance genes of clinical relevance are needed to clarify the public health impact of horizontal gene transfer of antimicrobial resistance from animal to human enterococci.

References

Aarestrup FM (1995) Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb Drug Resist 1:255–257

Aarestrup FM, Kruse H, Tast E et al (2000a) Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb Drug Resist 6:63–70

Aarestrup FM, Agerso Y, Gerner-Smidt P et al (2000b) Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn Microbiol Infect Dis 37:127–137

Aarestrup FM, Butaye P, Witte W (2002) Nonhuman reservoirs of enterococci. In: Gilmore MS (ed) The enterococci: pathogenesis, molecular biology and antibiotic resistance. ASM, pp 55–99

Agerso Y, Lester CH, Porsbo LJ et al (2008) Vancomycin-resistant *Enterococcus faecalis* isolates from a Danish patient and two healthy human volunteers are possibly related to isolates from imported turkey meat. J Antimicrob Chemother 62:844–845

Ahmad A, Ghosh A, Schal C et al (2011) Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. BMC Microbiol 11:23

Al-Ahmad A, Maier J, Follo M et al (2010) Food-borne enterococci integrate into oral biofilm: an in vivo study. J Endod 36:1812–1819

Ammerlaan HS, Harbarth S, Buiting AG et al (2013) Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis 56:798–805

Anonymous (1997) Commission directive 97/6/EC. Off J Eur Commun Legis L 35:11–13

Anonymous (1998a) Council Regulation 2821/98. Off J Eur Commun Legis L 351:4–8

Anonymous (1998b) Commission Regulation 2788/98. Off J Eur Commun Legis L 347:31–32

Anonymous (2003) Commission Regulation (EC) No 1831/2003. Off J Eur Union L 268:29–43

Arias CA, Murray BE (2008) Emergence and management of drug-resistant enterococcal infections. Expert Rev Anti Infect Ther 6:637–655

Arias CA, Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. Nat Rev Microbiol 10:266–278

Arias CA, Contreras GA, Murray BE (2010) Management of multi-drug resistant enterococcal infections. Clin Microbiol Infect 16:555–562

Arias CA, Panesso D, McGrath DM et al (2011) Genetic basis for in vivo daptomycin resistance in *enterococci*. N Engl J Med 365:892–900

Bager F, Madsen M, Christensen J et al (1997). Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev Vet Med 31:95–112

Barros J, Andrade M, Radhouani H et al (2012) Detection of *vanA*-containing *Enterococcus* species in faecal microbiota of gilthead seabream (*Sparus aurata*). Microbes Environ 27:509–511

Bates J, Jordens Z, Selkon JB (1993) Evidence for an animal origin of vancomycin-resistant enterococci. Lancet 342:490–491

Berchieri A (1999) Intestinal colonization of a human subject by vancomycin-resistant *Enterococcus faecium*. Clin Microbiol Infect 5:97–100

Biavasco F, Foglia G, Paoletti C et al (2007) VanA-type enterococci from humans, animals, and food: species distribution, population structure, Tn*1546* typing and location, and virulence determinants. Appl Environ Microbiol 73:3307–3319

Bourdon N, Fines-Guyon M, Thiolet JM et al (2011) Changing trends in vancomycin-resistant enterococci in French hospitals, 2001–2008. J Antimicrob Chemother 66:713–721

Braga TM, Pomba C, Lopes MF (2013) High-level vancomycin resistant *Enterococcus faecium* related to humans and pigs found in dust from pig breeding facilities. Vet Microbiol 161:344–349

Butaye P, Devriese LA, Haesebrouck F (2001) Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. Antimicrob Agents Chemother 45:1374–1378

Cercenado E et al (2010) Emerging linezolid resistance: dissemination of the *cfr* gene among *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium* and *Enterococcus faecalis* and inability of the Etest method for detection. Abstr. C2-1490. Abstract presented at the 50th interscience conference on antimicrobial agents and chemotherapy, Boston

Cetinkaya Y, Falk P, Mayhall CG (2000) Vancomycin-resistant enterococci. Clin Microbiol Rev 13:686–707

Chow JW (2000) Aminoglycoside resistance in enterococci. Clin Infect Dis 31:586–589

Christiansen KJ, Turnidge JD, Bell JM et al (2007) Prevalence of antimicrobial resistance in *Enterococcus* isolates in Australia, 2005: report from the Australian Group on Antimicrobial Resistance. Commun Dis Intell Q Rep 31:392–397

Coque TM, Tomayko JF, Ricke SC et al (1996) Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob Agents Chemother 40:2605–2609

Courvalin P (2006) Vancomycin resistance in gram-positive cocci. Clin Infect Dis 42(Suppl 1):S25–S34

Cremniter J, Mainardi JL, Josseaume N et al (2006) Novel mechanism of resistance to glycopeptide antibiotics in *Enterococcus faecium*. J Biol Chem 281:32254–32262

Damborg P, Top J, Hendrickx AP et al (2009) Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. Appl Environ Microbiol 75:2360–2365

DANMAP (2011) Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. www.danmap.org. Accessed 1 Sept 2013

Das I, Fraise A, Wise R (1997) Are glycopeptide-resistant enterococci in animals a threat to human beings? Lancet 349:997–998

de Been M, van Schaik W, Cheng L et al (2013) Recent recombination events in the core genome are associated with adaptive evolution in *Enterococcus faecium*. Genome Biol Evol 5:1524–1535

de Garnica ML, Valdezate S, Gonzalo C et al (2013) Presence of the *vanC1* gene in a vancomycin-resistant *Enterococcus faecalis* strain isolated from ewe bulk tank milk. J Med Microbiol 62:494–495

De Graef EM, Decostere A, De Leener E et al (2007) Prevalence and mechanism of resistance against macrolides, lincosamides, and streptogramins among *Enterococcus faecium* isolates from food-producing animals and hospital patients in Belgium. Microb Drug Resist 13:135–141

De Kraker ME, Jarlier V, Monen JC et al (2013) The changing epidemiology of bacteraemias in Europe: trends from the European antimicrobial resistance surveillance system. Clin Microbiol Infect 19:860–868

De Leener E, Martel A, De Graef EM et al (2005) Molecular analysis of human, porcine, and poultry *Enterococcus faecium* isolates and their *erm*(B) genes. Appl Environ Microbiol 71:2766–2770

de Regt MJ, van Schaik W, van Luit-Asbroek M et al (2012) Hospital and community ampicillin-resistant *Enterococcus faecium* are evolutionarily closely linked but have diversified through niche adaptation. PLoS ONE 7:e30319

Del Campo R, Ruiz-Garbajosa P, Sanchez-Moreno MP et al (2003) Antimicrobial resistance in recent fecal *enterococci* from healthy volunteers and food handlers in Spain: genes and phenotypes. Microb Drug Resist 9:47–60

Deshpande LM, Fritsche TR, Moet GJ et al (2007) Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. Diagn Microbiol Infect Dis 58:163–170

Devriese LA, Ieven M, Goossens H et al (1996) Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob Agents Chemother 40:2285–2287

Diarra MS, Rempel H, Champagne J et al (2010) Distribution of antimicrobial resistance and virulence genes in *Enterococcus* spp. and characterization of isolates from broiler chickens. Appl Environ Microbiol 76:8033–8043

Diaz L, Kiratisin P, Mendes RE et al (2012) Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. Antimicrob Agents Chemother 56:3917–3922

Donabedian SM, Thal LA, Hershberger E et al (2003) Molecular characterization of gentamicin-resistant *Enterococci* in the United States: evidence of spread from animals to humans through food. J Clin Microbiol 41:1109–1113

Donabedian SM, Perri MB, Vager D et al (2006) Quinupristin-dalfopristin resistance in *Enterococcus faecium* isolates from humans, farm animals, and grocery store meat in the United States. J Clin Microbiol 44:3361–3365

Donabedian SM, Perri MB, Abdujamilova N et al (2010) Characterization of vancomycin-resistant *Enterococcus faecium* isolated from swine in three Michigan counties. J Clin Microbiol 48:4156–4160

Dowzicky MJ, Chmelarova E (2011) Global in vitro activity of tigecycline and linezolid against Gram-positive organisms collected between 2004 and 2009. Int J Antimicrob Agents 37:562–566

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2013) The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2011. EFSA J 11(5):3196. www.efsa.europa.eu. Accessed 1 Sept 2013

Fard RM, Heuzenroeder MW, Barton MD (2011) Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. Vet Microbiol 148:276–282

Fontana R, Caneari P, Lleò MM et al (1990) Mechanisms of resistance of enterococci to beta-lactam antibiotics. Eur J Clin Microbiol Infect Dis 9:103–105

Freitas AR, Novais C, Ruiz-Garbajosa P et al (2009) Clonal expansion within clonal complex 2 and spread of vancomycin-resistant plasmids among different genetic lineages of *Enterococcus faecalis* from Portugal. J Antimicrob Chemother 63:1104–1111

Freitas AR, Coque TM, Novais C et al (2011a) Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn *1546* on indistinguishable plasmids. J Clin Microbiol 49:925–931

Freitas AR, Novais C, Correia R et al (2011b) Non-susceptibility to tigecycline in enterococci from hospitalised patients, food products and community sources. Int J Antimicrob Agents 38:174–176

Frye JG, Jackson CR (2013) Genetic mechanisms of antimicrobial resistance identified in *Salmonella* enterica, *Escherichia coli*, and *Enteroccocus* spp. isolated from U.S. food animals. Front Microbiol 4:135

Galimand M, Schmitt E, Panvert M et al (2011) Intrinsic resistance to aminoglycosides in *Enterococcus faecium* is conferred by the 16S rRNA m5C1404-specific methyltransferase EfmM. RNA 17:251–262

Galloway-Pena J, Roh JH, Latorre M et al (2012) Genomic and SNP analyses demonstrate a distant separation of the hospital and community-associated clades of *Enterococcus faecium*. PLoS ONE 7:e30187

Getachew Y, Hassan L, Zakaria Z et al (2013) Genetic variability of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans, chickens, and pigs in Malaysia. Appl Environ Microbiol 79:4528–4533

Ghosh A, Dowd SE, Zurek L (2011) Dogs leaving the ICU carry a very large multi-drug resistant enterococcal population with capacity for biofilm formation and horizontal gene transfer. PLoS ONE 6:e22451

Ghosh A, Kukanich K, Brown CE et al (2012) Resident cats in small animal veterinary hospitals carry multi-drug resistant enterococci and are likely involved in cross-contamination of the hospital environment. Front Microbiol 3:62

Gilmore MS, Lebreton F, van Schaik W (2013) Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. Curr Opin Microbiol 16:10–16

Giraffa G (2002) Enterococci from foods. FEMS Microbiol Rev 26:163–171

Goossens H (1998) Spread of vancomycin-resistant *enterococci*: differences between the United States and Europe. Infect Control Hosp Epidemiol 19:546–551

Graham JP, Price LB, Evans SL et al (2009) Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci Total Environ 407:2701–2710

Guardabassi L, Schwarz S, Lloyd DH (2004) Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 54:321–332

Guardabassi L, Perichon B, van Heijenoort J et al (2005) Glycopeptide resistance *vanA* operons in *Paenibacillus* strains isolated from soil. Antimicrob Agents Chemother 49:4227–4233

Hammerum AM (2012) Enterococci of animal origin and their significance for public health. Clin Microbiol Infect 18:619–625

Hammerum AM, Flannagan SE, Clewell Db et al (2001) Indication of transposition of a mobile DNA element containing the *vat*(D) and *erm*(B) genes in *Enterococcus faecium*. Antimicrob Agents Chemother 45:3223–3225

Hammerum AM, Lester CH, Neimann J et al (2004) A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is possibly related to pig isolates. J Antimicrob Chemother 53:547–549

Hammerum AM, Agerso Y, Garcia-Migura L et al (2009) Evaluation of the quinupristin/dalfopristin breakpoints for *Enterococcus faecium*. Int J Antimicrob Agents 34:288–290

Harada T, Tsuji N, Otsuki K et al (2005) Detection of the *esp* gene in high-level gentamicin resistant *Enterococcus faecalis* strains from pet animals in Japan. Vet Microbiol 106:139–143

Hegstad K, Mikalsen T, Coque TM et al (2010) Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. Clin Microbiol Infect 16:541–554

Herrero IA, Fernandez-Garayzabal JF, Moreno MA et al (2004) Dogs should be included in surveillance programs for vancomycin-resistant enterococci. J Clin Microbiol 42:1384–1385

Hershberger E, Donabedian S, Konstantinou K et al (2004) Quinupristin-dalfopristin resistance in gram-positive bacteria: mechanism of resistance and epidemiology. Clin Infect Dis 38:92–-98

Hidron AI, Edwards JR, Patel J et al (2008) NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 29:996–1011

Hollenbeck BL, Rice LB (2012) Intrinsic and acquired resistance mechanisms in enterococcus. Virulence 3:421–433

Howden BP, Holt KE, Lam MM et al (2013) Genomic insights to control the emergence of vancomycin-resistant enterococci. MBio 4. doi:10.1128/mBio.00412-13

Humphries RM, Kelesidis T, Tewhey R et al (2012) Genotypic and phenotypic evaluation of the evolution of high-level daptomycin nonsusceptibility in vancomycin-resistant *Enterococcus faecium*. Antimicrob Agents Chemother 56:6051–6053

Jackson CR, Fedorka-Cray PJ, Davis JA et al (2009) Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. J Appl Microbiol 107:1269–1278

Jackson CR, Fedorka-Cray PJ, Davis JA et al (2010) Mechanisms of antimicrobial resistance and genetic relatedness among enterococci isolated from dogs and cats in the United States. J Appl Microbiol 108:2171–2179

Jensen LB (1998) Differences in the occurrence of two base pair variants of Tn*1546* from vancomycin-resistant *enterococci* from humans, pigs, and poultry. Antimicrob Agents Chemother 42:2463–2464

Jensen LB, Hammerum AM, Aerestrup FM et al (1998) Occurrence of *satA* and *vgb* genes in streptogramin-resistant *Enterococcus faecium* isolates of animal and human origins in the Netherlands. Antimicrob Agents Chemother 42:3330–3331

Jensen LB, Willems RJ, van den Bogaard AE (2003) Genetic characterization of glycopeptide-resistant enterococci of human and animal origin from mixed pig and poultry farms. APMIS 111:669–672

Jensen LB, Garcia-Migura L, Valenzuela AJ et al (2010) A classification system for plasmids from enterococci and other Gram-positive bacteria. J Microbiol Methods 80:25–43

Jung WK, Hong SK, Lim JY et al (2006) Phenotypic and genetic characterization of vancomycin-resistant enterococci from hospitalized humans and from poultry in Korea. FEMS Microbiol Lett 260:193–200

Jung YH, Shin ES, Kim O et al (2010) Characterization of two newly identified genes, *vgaD* and *vatG*, conferring resistance to streptogramin A in *Enterococcus faecium*. Antimicrob Agents Chemother 54:4744–4749

Kelesidis T, Chow AL (2013) Proximity to animal or crop operations may be associated with de novo daptomycin-non-susceptible *Enterococcus* infection. Epidemiol Infect 15:1–4

Kieke AL, Borchardt MA, Kieke BA et al (2006) Use of streptogramin growth promoters in poultry and isolation of streptogramin-resistant *Enterococcus faecium* from humans. J Infect Dis 194:1200–1208

Klare I, Heier H, Claus H et al (1993) Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. FEMS Microbiol Lett 106:23–29

Klare I, Heier H, Claus H et al (1995) *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol Lett 125:165–171

Klare I, Badstubner D, Konstabel C et al (1999) Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist 5:45–52

Klare I, Konstabel C, Badstubner D et al (2003) Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. Int J Food Microbiol 88:269–290

Klare I, Witte W, Wendt C et al (2012) Vancomycin-resistant enterococci (VRE). Recent results and trends in development of antibiotic resistance. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 55:1387–1400

Kuch A, Willems RJ, Werner G et al (2012) Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe. J Antimicrob Chemother 67:551–558

Kuhn I, Iversen A, Burman LG et al (2003) Comparison of enterococcal populations in animals, humans, and the environment-a European study. Int J Food Microbiol 88:133–145

Larsen J, Schonheyder HC, Lester Ch et al (2010) Porcine-origin gentamicin-resistant *Enterococcus faecalis* in humans, Denmark. Emerg Infect Dis 16:682–684

Larsen J, Schonheyder HC, Singh KV et al (2011) Porcine and human community reservoirs of *Enterococcus faecalis*, Denmark. Emerg Infect Dis 17:2395–2397

Lebreton F, Depardieu F, Bourdon N et al (2011) D-Ala-d-Ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 55:4606–4612

Lebreton F, van Schaik W, Manson MA et al (2013) Emergence of epidemic multidrug-resistant *Enterococcus* faecium from animal and commensal strains. MBio 4. doi:10.1128/mBio.00534-13

Leclercq R, Derlot E, Duval J et al (1988) Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N Engl J Med 319:157–161

Lester CH, Hammerum AM (2010) Transfer of *vanA* from an *Enterococcus faecium* isolate of chicken origin to a CC17 *E. faecium* isolate in the intestine of cephalosporin-treated mice. J Antimicrob Chemother 65:1534–1536

Lester CH, Frimodt-Moller N, Sorensen TL et al (2006) In vivo transfer of the *vanA* resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother 50:596–599

Lim SK, Kim TS, Lee HS et al (2006) Persistence of *vanA*-type *Enterococcus faecium* in Korean livestock after ban on avoparcin. Microb Drug Resist 12:136–139
Liu Y, Wang Y, Wu C et al (2012) First report of the multidrug resistance gene *cfr* in *Enterococcus faecalis* of animal origin. Antimicrob Agents Chemother 56:1650–1654
Liu Y, Wang Y, Schwarz S et al (2013) Transferable multiresistance plasmids carrying *cfr* in *Enterococcus* spp. from swine and farm environment. Antimicrob Agents Chemother 57:42–48
Lopez M, Saenz Y, Rojo-Bezares B et al (2009) Detection of *vanA* and *vanB2*-containing *enterococci* from food samples in Spain, including *Enterococcus faecium* strains of CC17 and the new singleton ST425. Int J Food Microbiol 133:172–178
Lopez M, Cercenado E, Tenorio C et al (2012) Diversity of clones and genotypes among vancomycin-resistant clinical *Enterococcus* isolates recovered in a Spanish hospital. Microb Drug Resist 18:484–491
Mainardi JL, Legrand R, Arthur M et al (2000) Novel mechanism of beta-lactam resistance due to bypass of DD-transpeptidation in *Enterococcus faecium*. J Biol Chem 275:16490–16496
Manson JM, Keis S, Smith JM et al (2003) Characterization of a vancomycin-resistant *Enterococcus faecalis* (VREF) isolate from a dog with mastitis: further evidence of a clonal lineage of VREF in New Zealand. J Clin Microbiol 41:3331–3333
Martone WJ (1998) Spread of vancomycin-resistant enterococci: why did it happen in the United States? Infect Control Hosp Epidemiol 19:539–545
McBride SM, Fischetti VA, Leblanc DJ et al (2007) Genetic diversity among *Enterococcus faecalis*. PLoS ONE 2:e582
Moyaert H, De Graef EM, Haesebrouck F et al (2006) Acquired antimicrobial resistance in the intestinal microbiota of diverse cat populations. Res Vet Sci 81:1–7
Murray BE (1990) The life and times of the *Enterococcus*. Clin Microbiol Rev 3:46–65
Murray BE (1992) Beta-lactamase-producing enterococci. Antimicrob Agents Chemother 36:2355–2359
NARMS (2011) National Antimicrobial Resistance Monitoring System. Retail Meat Report. www.fda.gov. Accessed 1 Sept 2013
Nilsson O (2012) Vancomycin resistant enterococci in farm animals—occurrence and importance. Infect Ecol Epidemiol 2. doi:10.3402/iee.v2i0.16959
Novais C, Sousa JC, Coque TM et al (2004) In vitro activity of daptomycin against *enterococci* from nosocomial and community environments in Portugal. J Antimicrob Chemother 54:964–966
Novais C, Coque TM, Costa MJ et al (2005) High occurrence and persistence of antibiotic-resistant enterococci in poultry food samples in Portugal. J Antimicrob Chemother 56:1139–1143
Novais C, Freitas AR, Silveira E et al (2013) Spread of multidrug-resistant *Enterococcus* to animals and humans: an underestimated role for the pig farm environment. J Antimicrob Chemother. doi:10.1093/jac/dkt289
Ono S, Muratani T, Matsumoto T (2005) Mechanisms of resistance to imipenem and ampicillin in *Enterococcus faecalis*. Antimicrob Agents Chemother 49:2954–2958
Oravcova V, Zurek L, Townsend A et al (2013) American crows as carriers of vancomycin-resistant enterococci with *vanA* gene. Environ Microbiol. doi:10.1111/1462-2920.12213
Oteo J, Cuevas O, Navarro C et al (2007) Trends in antimicrobial resistance in 3469 enterococci isolated from blood (EARSS experience 2001–2006, Spain): increasing ampicillin resistance in *Enterococcus faecium*. J Antimicrob Chemother 59:1044–1045
Palmer KL, Godfrey P, Griggs A et al (2012) Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. MBio 3:e00318–11
Pantosti A, Del Grosso M, Tagliabue S et al (1999) Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet 354:741–742
Poeta P, Costa D, Rodrigues J et al (2005) Study of faecal colonization by *vanA*-containing *Enterococcus* strains in healthy humans, pets, poultry and wild animals in Portugal. J Antimicrob Chemother 55:278–280

Poulsen LL, Bisgaard M, Son NT et al (2012) *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. Emerg Infect Dis 18:1096–1100

Quinones D, Kobayashi N, Nagashima S (2009) Molecular epidemiologic analysis of *Enterococcus faecalis* isolates in Cuba by multilocus sequence typing. Microb Drug Resist 15:287–293

Rice LB, Carias LL, Rudin S et al (2005) *Enterococcus faecium* low-affinity *pbp5* is a transferable determinant. Antimicrob Agents Chemother. 49:5007–5012

Rodrigues J, Poeta P, Martins A et al (2002) The importance of pets as reservoirs of resistant *Enterococcus* strains, with special reference to vancomycin. J Vet Med B Infect Dis Vet Public Health 49:278–280

Ruiz-Garbajosa P, Bonten MJ, Robinson DA et al (2006) Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. J Clin Microbiol 44:2220–2228

Sacco E, Hugonnet JE, Josseaume N et al (2010) Activation of the L, D-transpeptidation peptidoglycan cross-linking pathway by a metallo-D, D-carboxypeptidase in *Enterococcus faecium*. Mol Microbiol 75:874–885

Sarti M, Campanile F, Sabia C et al (2012) Polyclonal diffusion of beta-lactamase-producing *Enterococcus faecium*. J Clin Microbiol 50:169–172

Shen J, Wang Y, Schwarz S (2013) Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. J Antimicrob Chemother 68:1697–1706

Simjee S, White DG, McDermott PF et al (2002) Characterization of Tn*1546* in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. J Clin Microbiol 40:4659–4665

Simjee S, Zhang Y, McDermott PF et al (2006) Heterogeneity of *vat*(E)-carrying plasmids in *Enterococcus faecium* recovered from human and animal sources. Int J Antimicrob Agents 28:200–205

Simonsen GS, Haaheim H, Dahl KH et al (1998) Transmission of VanA-type vancomycin-resistant *enterococci* and *vanA* resistance elements between chicken and humans at avoparcin-exposed farms. Microb Drug Resist 4:313–318

Singh KV, Murray BE (2005) Differences in the *Enterococcus faecalis lsa* locus that influence susceptibility to quinupristin-dalfopristin and clindamycin. Antimicrob Agents Chemother 49:32–39

Sletvold H, Johnsen PJ, Simonsen GS et al (2007) Comparative DNA analysis of two *vanA* plasmids from *Enterococcus faecium* strains isolated from poultry and a poultry farmer in Norway. Antimicrob Agents Chemother 51:736–739

Soltani M, Beighton D, Philpott-Howard J et al (2000) Mechanisms of resistance to quinupristin-dalfopristin among isolates of *Enterococcus faecium* from animals, raw meat, and hospital patients in Western Europe. Antimicrob Agents Chemother 44:433–436

Somarajan SR, Murray BE (2013) Could a phosphotransferase system provide the means to control outbreaks of *Enterococcus faecium* infection? J Infect Dis 207:1633–1636

Sorensen TL, Blom M, Monnet DL et al (2001) Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. N Engl J Med 345:1161–1166

Sparo M, Urbizu L, Solana MV et al (2012) High-level resistance to gentamicin: genetic transfer between *Enterococcus faecalis* isolated from food of animal origin and human microbiota. Lett Appl Microbiol 54:119–125

Spiliopoulou I, Damani A, Chini V et al (2011) Linezolid-resistant enterococci in Greece: epidemiological characteristics. Chemotherapy 57:181–185

Stobberingh E, van den Bogaard AE, London N et al (1999) Enterococci with glycopeptide resistance in turkeys, turkey farmers, turkey slaughterers, and (sub)urban residents in the south of The Netherlands: evidence for transmission of vancomycin resistance from animals to humans? Antimicrob Agents Chemother 43:2215–2221

Sundsfjord A, Simonsen GS, Courvalin P (2001) Human infections caused by glycopeptide-resistant *Enterococcus* spp.: are they a zoonosis? Clin Microbiol Infect 7(Suppl 4):16–33

The Surveillance Network, USA (2013) www.cddep.org/map. Accessed 1 Sept 2013

Torres C, Reguera JA, Sanmartin MJ et al (1994) *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. J Antimicrob Chemother 33:553–561

Torres C, Tenorio C, Portillo A et al (2003) Intestinal colonization by *vanA*- or *vanB2*- containing enterococcal isolates of healthy animals in Spain. Microb Drug Resist 9(Suppl 1):S47–S52

Tran TT, Panesso D, Gao H et al (2013) Whole-genome analysis of a daptomycin-susceptible *Enterococcus faecium* strain and its daptomycin-resistant variant arising during therapy. Antimicrob Agents Chemother 57:261–268

Tremblay CL, Letellier A, Quessy S et al (2011) Multiple-antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* from cecal contents in broiler chicken and turkey flocks slaughtered in Canada and plasmid colocalization of *tet*O and *erm*B genes. J Food Prot 74:1639–1648

Tremblay CL, Letellier A, Quessy S et al (2012) Antibiotic-resistant *Enterococcus faecalis* in abattoir pigs and plasmid colocalization and cotransfer of *tet*(M) and *erm*(B) genes. J Food Prot 75:1595–1602

Tzavaras I, Siarkou VI, Zdragas A et al (2012) Diversity of *vanA*-type vancomycin-resistant *Enterococcus faecium* isolated from broilers, poultry slaughterers and hospitalized humans in Greece. J Antimicrob Chemother 67:1811–1818

Uttley AH, Collins CH, Naidoo J et al (1988) Vancomycin-resistant enterococci. Lancet 1:57–58

van Belkum A, van den Braak N, Thomassen R et al (1996) Vancomycin-resistant enterococci in cats and dogs. Lancet 348:1038–1039

van den Bogaard AE, Mertens P, London NH et al (1997a) High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in The Netherlands: is the addition of antibiotics to animal feeds to blame? J Antimicrob Chemother 40:454–456

van den Bogaard AE, Jensen LB, Stobberingh EE (1997b) Vancomycin-resistant enterococci in turkeys and farmers. N Engl J Med 337:1558–1559

van den Bogaard AE, Bruinsma N, Stobberingh EE (2000) The effect of banning avoparcin on VRE carriage in The Netherlands. J Antimicrob Chemother 46:146–148

van den Bogaard AE, Willems RJ, London N et al (2002) Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 49:497–505

van Schaik W, Top J, Riley DR et al (2010) Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island. BMC Genomics 11:239

Waites KB, Duffy LB, Dowzicky MJ (2006) Antimicrobial susceptibility among pathogens collected from hospitalized patients in the United States and in vitro activity of tigecycline, a new glycylcycline antimicrobial. Antimicrob Agents Chemother 50:3479–3484

Wegener HC, Madsen M, Nielsen N et al (1997) Isolation of vancomycin resistant *Enterococcus faecium* from food. Int J Food Microbiol 35:57–66

Welton LA, Thal LA, Perri MB et al (1998) Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. Antimicrob Agents Chemother 42:705–708

Werner G, Klare I, Witte W (1998) Association between quinupristin/dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. Eur J Clin Microbiol Infect Dis 17:401–402

Werner G, Coque TM, Franz CM et al (2013) Antibiotic resistant enterococci-tales of a drug resistance gene trafficker. Int J Med Microbiol 303:360–379

Willems RJ, Hanage WP, Bessen DE et al (2011) Population biology of gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS Microbiol Rev 35:872–900

Willems RJ, Top J, van Schaik W (2012) Restricted gene flow among hospital subpopulations of *Enterococcus faecium*. MBio 3:e00151–12

Woodford N, Adebiyi AM, Palepou MF et al (1998) Diversity of VanA glycopeptide resistance elements in enterococci from humans and nonhuman sources. Antimicrob Agents Chemother 42:502–508

Zhang X, Paganelli FL, Bierschenk D et al (2012) Genome-wide identification of ampicillin resistance determinants in *Enterococcus faecium*. PLoS Genet 8:e1002804