REVIEW ARTICLE

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Vancomycin resistant enterococci in farm animals – occurrence and importance

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The view on enterococci has over the years shifted from harmless commensals to opportunistic but important pathogens mainly causing nosocomial infections. One important part of this development is the emergence of vancomycin resistance enterococci (VRE). The term VRE includes several combinations of bacterial species and resistance genes of which the most clinically important is *Enterococcus faecium* with vanA type vancomycin resistance. This variant is also the most common VRE among farm animals. The reason for VRE being present among farm animals is selection by extensive use of the vancomycin analog avoparcin for growth promotion. Once the use of avoparcin was discontinued, the prevalence of VRE among farm animals decreased. However, VRE are still present among farm animals and by spread via food products they could potentially have a negative impact on public health. This review is based on the PhD thesis *Vancomycin Resistant Enterococci in Swedish Broilers – Emergence, Epidemiology and Elimination* and makes a short summary of VRE in humans and food producing animals. The specific situation regarding VRE in Swedish broiler production is also mentioned.

Keywords: *VRE*; *epidemiology*; *vancomycin*; *vanA*; *Enterococcus faecium*; *gene transfer*

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Vancomycin resistant enterococci

The term vancomycin resistant enterococci (VRE) includes several combinations of bacterial species and resistance genes. Some of which are highly important as pathogens and reservoirs of antimicrobial resistance genes, others where the resistance is intrinsic and a part of that species characteristics. To bring order in the complexity, each part of the term VRE is dealt with and elucidated separately.

Vancomycin

Vancomycin is a glycopeptide antimicrobial produced by the soil bacteria *Streptomyces orientalis* (1). It was developed and introduced in the 1950s (2). Glycopeptides interfere with the cell wall production resulting in a destabilized cell wall and lysis of the bacteria (2). When the bacterial cell wall is synthesized, polysaccharide-pentapeptide complexes are linked together via a trans-peptidation reaction in which the end amino acid of the pentapeptide is removed (2). Glycopeptides interfere with this process by binding tightly to the D-Alanyl-D-Alanin (D-Ala-D-Ala) end of the pentapeptide and hiding it from the transpeptidase that is to catalyse the cross-linking in the peptidoglycan synthesis (2).

Vancomycin is active against most Gram positive bacteria whereas the majority of Gram negatives are resistant (3, 4). It is considered a drug of 'last resort' and has been classified as critically important for human medicine for treatment of patients with severe infections with multi-drug resistant *Enterococcus* spp. and meticillin resistant *Staphylococcus aureus* (MRSA) as the main indications (5). Vancomycin is also used for intestinal infections, especially pseudomembranous colitis caused by *Clostridium difficile* where the poor absorption of vancomycin when administered orally is advantageous (6).

Enterococci

Enterococci are intestinal bacteria colonizing humans and other mammals as well as birds, reptiles and insects (7–9). They are Gram positive, facultative anaerobes, catalase negative and non sporeforming cocci occurring either as single bacteria, in pairs or in short chains (10, 11). They can sustain various adverse conditions and can survive for several months in the environment (12, 13).

Until 1984 enterococci were considered a part of the genus *Streptococcus*, even though they were first described and tentatively named enterococci (*entérocoque*) in 1899 (11, 14). Today, 40 different species of enterococci have been described (15). The species most frequent in the intestines of humans are *Enterococcus faecalis,* and to a lesser extent *E. faecium* whereas the most common species in various farm animals are *E. faecium* together with *E. cecorum*, *E. faecalis*, and to some extent *E. hirae* (3, 16, 17).

Even though the first description in 1899 referred to enterococci as potential pathogens they were for a long time regarded as harmless intestinal bacteria without clinical importance (10, 14). Nowadays however, enterococci are recognised as important opportunistic pathogens, especially causing nosocomial infections such as urinary tract infections, wound infections and endocarditis (10). The clinically most important species in human medicine are *E. faecalis* and *E. faecium* (18). Of these, *E. faecalis* is the most pathogenic species but *E. faecium* is of increasing importance as it is generally more frequently resistant to antimicrobials (3).

Enterococcal resistance to vancomycin

Until today, nine different variants of vancomycin resistance in enterococci have been described (vanA, B, C, D, E, G, L, M and N; Table 1) (19–22). Among those, the three most common variants are the vanA, B and C types with *E. faecium* carrying the *vanA* genotype as the most common combination (10, 18). An additional variant (vanF) has also been described but thus far only in *Paenibacillus popilliae* (23). Since the vanF variant has a high similarity in amino acid sequences to the vanA variant, *P. popilliae* has been suggested as a possible origin for vancomycin resistance in enterococci (23). Other plausible sources are various glycopeptide producing organisms, even if genetic differences make an older common source more likely (24).

Common to all variants of vancomycin resistance in enterococci is the ability to cause a change in the structure of the pentapeptide incorporated in the three dimensional web of peptidoglycans composing the bacterial cell wall: from the original D-Ala-D-Ala to either D-Ala-D-Lactate (D-Ala-D-Lac) or D-Ala-D-Serine (D-Ala-D-Ser) (20). This shift results in a reduced affinity for vancomycin by 1000 and seven times respectively (10).

In all different variants of vancomycin resistance are several genes involved in the alteration of the cell wall structure which results in the resistance. The number and organisation of these genes are somewhat similar among the different variants. For the vanA variant, the genes are organized as in Fig. 1. *vanS* is a sensor gene which in the presence of a glycopeptide phosphorylate, and thus activate the regulator gene *vanR* (20). After activation of the gene complex, *vanH* mediates production of lactate from pyruvate which *vanA* uses to synthesize the alternative D-Ala-D-Lac end of the pentapetide (3). It is essential for resistance that production of the normal D-Ala-D-Ala end of the pentapetide does not continue. This is resolved by the *vanX* and *vanY* genes where *vanX* hydrolyzes and thereby interrupts the production of the pentapeptides, and *vanY* cleaves the pentapeptides that might still be produced (3, 25). In the absence of a glycopeptide, *vanS* initiates dephosphorylation of *vanR* resulting in deactivation of the gene (20).The function of the *vanZ* gene is not understood (20).

Clinical impact of VRE

The first cases of infection with vancomycin resistant entrerococci were seen in 1986 (26, 27). Since then, VRE

Table 1. Characteristics of different types of vancomycin resistance described among *Enterococcus* spp

			Range of MIC (mg/L)				
	Sort	Modified target	Vancomycin	Teicoplanin	Expression	Location	Transferable
vanA	Acquired	D-Ala-D-Lac	64–1000	16–512	Inducible	Chromosome or plasmid	Yes
vanB	Acquired	D-Ala-D-Lac	4–1000	0.5–1	Inducible	Chromosome or plasmid	Yes
vanC	Intrinsic	D-Ala-D-Ser	2–32	0.5–1	Constitutive or inducible	Chromosome	No
vanD	Acquired	D-Ala-D-Lac	64–128	4–64	Constitutive or inducible	Chromosome	No
vanE	Acquired	D-Ala-D-Ser	(6) 8–32	0.5	Inducible	Chromosome	No
vanG	Acquired	D-Ala-D-Ser	16	0.5	Inducible	Chromosome	Yes
vanL	Acquired	D-Ala-D-Ser	8	<8	Inducible	Chromosome	No
vanM	Unknown	D-Ala-D-Lac	>128	64 to >256	Inducible	Unknown	Yes
vanN	Acquired	D-Ala-D-Ser	16	0.5	Constitutive	Plasmid	Yes

MIC, minimum inhibitory concentration (mg/L).
D-Ala-D-Lac = D-Alanyl-D-Lactate, D-Ala-D-Ser = D-Alanyl-D-Serine.
Adapted from Lebreton et al., 2011 (22); CLSI, 2010 (91); Xu et al., 2010 (19); Boyd et al., 2008 (21) and Courvalin, 2006 (20).

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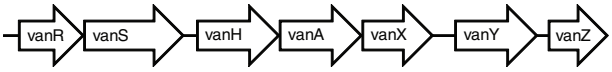

Fig. 1. Organization of the genes involved in the vanA variant of vancomycin resistance in enterococci. Adapted from Courvalin, 2006 (20).

have spread around the world and are now one of the most important causes of nosocomial infections, even if asymptomatic intestinal colonization is much more common than morbidity (24). In most parts of the world, VRE of the vanA variant are the most prevalent among human cases (18). But in some countries (e.g. Australia and Sweden) the majority of human cases are of the vanB type (28–31).

When infections with VRE do occur, the consequences are often worse compared to infections with vancomycin susceptible enterococci. Associations have been seen between infections with VRE and increases in therapy failure, length of hospital stay as well as mortality (24, 32). However, studies where no significant difference is seen also exist (33).

VRE in farm animals

Feeding animals low doses of antimicrobials may in certain conditions increase their productivity, for example by improving feed conversion and decreasing morbidity and mortality caused by clinical and subclinical infections (34). The growth promoting effect of antimicrobials was first discovered when broiler chickens were fed the fermentation leftovers after production of antimicrobials (35, 36). Notable is that all use of growth promoters in Sweden was forbidden in 1986 by the Feedingstuffs Act (SFS 1985:295).

The glycopeptide avoparcin was first introduced for growth promotion in 1975 (37). At that time it was used extensively in most parts of Europe and the rest of the world with the notable exception of Canada and USA where avoparcin never has been approved for animals (38). Avoparcin was mainly used for broilers and pigs but to some extent also for turkeys, veal calves and other animals (37, 39, 40). The extent of avoparcin use is demonstrated by data from Denmark where 24 kg of vancomycin was used in human medicine in 1994, and in the same year more than 24,000 kg of avoparcin was used for growth promotion (39). A similar example is Australia, where from 1992 to 1996 less than 600 kg of vancomycin but over 62,000 kg of avoparcin was imported (41).

As avoparcin confers cross-resistance to vancomycin the (mis)use of avoparcin selected for VRE (42). Hence, VRE, i.e. *E. faecium* carrying the *vanA* genotype was common in the intestinal flora of farm animals in Europe during the 1990s (43, 44). By contrast, since avoparcin has never been approved in Canada and USA, VRE had until 2008 never been isolated from farm animals in USA (45).

When the connection between avoparcin and VRE in farm animals was confirmed, the use of avoparcin was discontinued as a precautionary measure to avoid further spread of VRE to the community and into hospital settings (46). Apart from Sweden, the use of avoparcin in Europe ceased first in Denmark, Finland and Norway (47). Later it ceased in Germany and finally in the whole of the European Union as a consequence of the Commission Directive 97/6/EC (46). Avoparcin has later been banned or phased out also in other parts of the world (37, 48, 49). Once the use of avoparcin had been discontinued, the prevalence of VRE in farm animals rapidly declined (50–52).

The decreased occurrence of VRE in animals after the use of avoparcin was discontinued reinforces the theory that if the selective pressure is removed, the antimicrobial resistance will disappear (53, 54). However, when using selective media (i.e. media with vancomycin) VRE could and can still be readily detected in samples from farm animals (55–61). Furthermore, a recent study modelling persistence of VRE indicates that it will be present among farm animals for a long period of time which is also in agreement with today's view on the timeframe of reversal of antimicrobial resistance (54, 62).

Different theories about why VRE persist among farm animals have been presented. In Denmark, the use of the macrolide tylosin in pigs was suggested to co-select for vancomycin resistance among enterococci since the genes encoding the two resistances were located on the same plasmid (63). A similar but weaker correlation with co-selection by copper resistance has also been suggested (64). Another explanation that has been suggested is that plasmid addiction systems located on the same plasmid as the *vanA* gene would force the bacteria to retain the resistance (65).

The Swedish paradox

In Sweden, avoparcin was only used for a short period of time from the end of the 1970s until 1984 and the yearly usage by the end of that period was between 7,000 and 9,000 kg (66). Furthermore, all use of growth promoters in Sweden was forbidden in 1986 by the Feedingstuffs Act (SFS 1985:295).

In accordance with the low selective pressure for vancomycin resistance by avoparcin use that enterococci in Swedish farm animals had been exposed to, VRE were not isolated in samples from Swedish broilers or pigs in the middle of the 1990s (67, 68). However, in a study conducted 1998 to 2000 the first VRE from Swedish farm animals were isolated (69). In the following years, the occurrence of VRE increased and in samples analysed within the Swedish Veterinary Antimicrobial Resistance Monitoring programme (SVARM), the proportion of

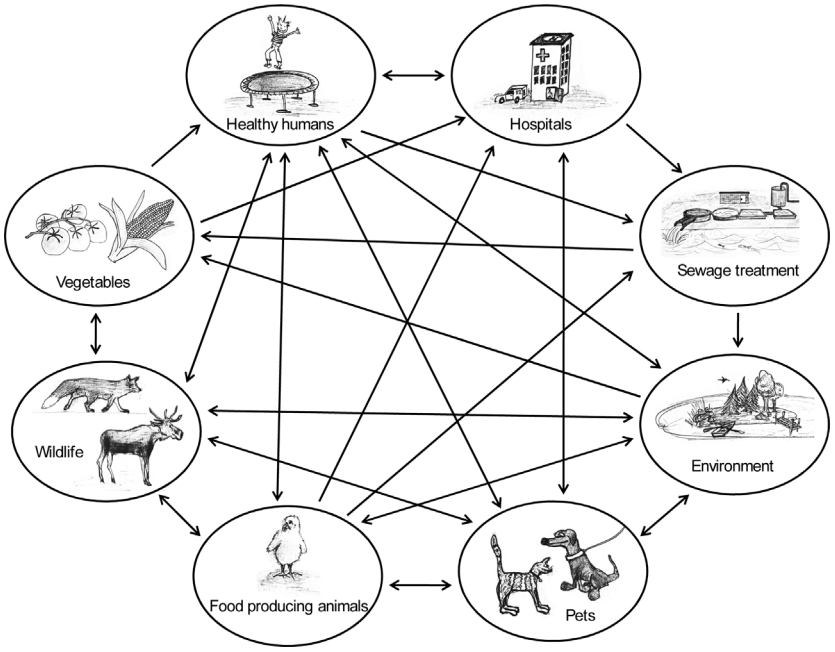

Fig. 2. Various routs by which zoonotic bacteria can spread between animals and humans. The same routes apply also for resistance genes. Illustrations by K. Dahl.

broilers colonized with VRE increased from less than 1% in 2000 to over 40% in 2005 (70). In addition, this increase was caused by the spread of one clone of *E. faecium* with the *vanA* gene (70). It is however important to keep in mind that the increase is observed only when samples are cultured on selective media, i.e. on agar containing vancomycin, indicating that the proportion of enterococci that are vancomycin resistant is low (70).

The reason(s) for the increased occurrence of VRE in Swedish broiler production is unknown. For example, plasmid addiction systems do not seem to be common among VRE from Swedish broilers and hence are probably not an important factor in the epidemiology (71). However, it has been shown that both the level of VRE contamination and the proportion of broilers colonized with VRE differ among farms (72). This was taken as an indication that if the factor(s) causing these differences were identified, it might be possible to reduce the occurrence of VRE among Swedish broilers. Unpublished results also indicate that altered disinfection routines might be a way forward to achieve this.

VRE as a foodborne zoonosis

The World Health Organisation defines a zoonosis as 'any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa' (73). The transmission from animals to humans can be either direct or indirect (Fig. 2). One route for indirect transfer is contaminated animal or vegetable food products (74, 75). The scenario regarding spread of zoonotic agents via the food chain, i.e. foodborne zoonoses, is that organisms which are pathogenic to man and originating from animals contaminate human food products. Meat products could for example be contaminated by faecal material at the slaughterhouses whereas vegetables may be contaminated in the field by manure or sewage water used for fertilization and irrigation.

Normally, it is the agent *per se* that is zoonotic (e.g. Norovirus, *Salmonella* and *Campylobacter* spp.). However, regarding antimicrobial resistant bacteria it is not only pathogens that could have a zoonotic potential, but also the genes encoding antimicrobial resistance in commensals such as *Escherichia coli* and *Enterococcus* spp. which may transfer to more pathogenic organisms (75, 76). Here, the scenario is that once in the human intestine, the bacteria might colonize and persist or their presence may only be transient (77, 78). Even if the animal derived bacteria colonize the human intestine only for a short time, this can be sufficient for resistance genes to be transferred to other strains better adapted to colonize humans (79). These human adapted strains can then persist for long periods and also spread to other people. The zoonotic potential of certain antimicrobial resistance genes means any resistant bacteria present in farm animals may be considered a reservoir for resistance that can spread affecting both veterinary and human medicine (80).

Regarding VRE, both spread of the resistant bacteria and for some of the variants also spread of the resistance genes via horizontal transfer could occur and has been subject to extensive reviews (37, 81, 82). Both ways are

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possible for the vanA variant and is among other things of importance for the zoonotic potential.

Similar strains of VRE have been isolated from farm animals and humans (83). Furthermore, when the use of avoparcin was discontinued in Europe, not only did the occurrence of VRE among farm animals decrease but there was also a subsequent decrease in the occurrence of VRE in food of animal origin and in the prevalence of human colonization with VRE (51, 84). However, hospital isolates of *E. faecium* generally cluster in subgroups which are separate from those found in animals (85, 86). Taken together, even though strains adapted to animals can cause infections in humans, these events are of limited importance for public health.

Transfer of the *vanA* gene is often mediated by a transposon, a mobile genetic element that can be incorporated either in the bacterial chromosome or on plasmids (20, 87). More specifically the genes are located in and transferred by the transposon Tn*1546* or closely related genetic elements (20). One example of gene transfer between animal and human adapted enterococci is when Jensen showed that VRE from pigs and broilers have specific variants of the Tn*1546* transposon and that VRE from healthy humans can have any of the two variants (88). This was taken as an indication that the dissemination rout was probably from pigs and poultry to humans. Furthermore, the possibility for *in vivo* transfer of vancomycin resistance from VRE of animal origin to enterococci of human origin in the intestines of humans has been described (79). Transfer in mice of the *vanA* gene to hospital adapted enterococci has also been demonstrated (89). Taken together, this indicates that the most important way by which the presence of VRE among farm animals impact on public health is via transfer of resistance genes to strains already adapted to humans and/or hospitals.

To what extent the presence of VRE among farm animals has actually affected the situation in public health will probably never be determined. However, it is clear that both occasional infections with animal associated strains and gene transfer from such strains can happen. Furthermore, one can only speculate on what happened when the first hospital adapted VRE emerged. Such thoughts have been tested in a mathematical model by Smith et al. (90). It was concluded that agricultural use of antimicrobials can lead to the spread of antimicrobial resistance from animals to humans, either by spread of resistant strains or by gene transfer between animal adapted and human adapted strains. Furthermore, Smith et al. state that these events will have the largest impact on public health if they occur when that particular resistance is still rare among human adapted strains. So, if the first hospital adapted VRE emerged by horizontal gene transfer of the resistance genes into a vancomycin susceptible hospital adapted enterococci – where did then the resistance genes come from? And if they came from an animal associated VRE, is not then all hospital adapted VRE animal associated strictly speaking?

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