Review Article
Enterococci and Antibiotic Resistance

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Abstract

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Enterococci are widely distributed in nature. Intestinal tract of humans and animals is the natural habitat of these organisms. They gain entry into raw material and foodstuffs through the water supply, food animals, or unhygienic conditions of the production and handling.

Enterococci have been known to be resistant to most antibiotics used in clinical practice. Multidrug-resistant and vancomycin-resistant enterococci are commonly isolated from humans, animal sources, aquatic habitats, agricultural run-off which indicates their ability to enter the human food chain. Vancomycin-resistant enterococci are emerging as a global threat to public health.

Enterococcus sp., *antimicrobial resistance, transmission, animals, human, food chain*

Microbial resistance to antibiotics is a world-wide problem in human and veterinary medicine. It is generally accepted that the main risk factor for the increase in the antibiotic resistance is an extensive use of antibiotics. This has lead to the emergence and dissemination of resistant bacteria and resistance genes in animals and humans. In both populations antibiotics are used for therapy and prophylaxis of infectious diseases (van den Bogaard and Stobberingh 2000a). The main sector of resistance-increasing medicine usage, in regard to human health, lies within the health care sector. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogens, but also in bacteria transmitted from animals to humans.

Most studies show that not only the level of resistance of pathogenic bacteria, but also of commensal bacteria increases. Commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens. Resistant commensal bacteria of food animals might contaminate, as zoonotic bacteria, meat (products) and so reach the intestinal tract of humans. Monitoring the prevalence of resistance in indicator bacteria such as faecal *Escherichia coli* and enterococci in different populations, animals, patients and healthy humans, makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa. Only in countries that use or used avoparcin as antimicrobial growth promoter (AMPG), is vancomycin resistance common in intestinal enterococci, not only in exposed animals, but also in the human population outside hospitals. Resistance genes against antibiotics, that are or have only been used in animals i.e. nourseothricin, apramycin etc. were found soon after their introduction, not only in animal bacteria but also in the commensal flora of humans, in zoonotic pathogens such as salmonellae, but also in strictly human pathogens such as shigellae.

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Moreover, since the EU ban of avoparcin, a significant decrease has been observed in several European countries in the prevalence of vancomycin resistant enterococci in meat, in faecal samples of food animals and healthy humans, which underlines the role of antimicrobial usage in food animals in the selection of bacterial resistance and the transport of these resistance via the food chain to humans (van den Bogaard and Stobberingh 2000a).

Usage of antibiotics in agriculture

Antimicrobial agents are usually used as the therapeutic agents against bacterial infections, for treatment of subclinical or clinical mastitis during lactation or as a cure/preventive at dry-off. They may be also used as grow-promoting and prophylactic agents in animals. Bacitracin, chlortetracycline, tylosin, avoparcin, neomycin, oxytetracycline and others are used for this purpose. The doses are lower than those required for therapeutic use. Inappropriate use of these grow-promoting antibiotics (AGP) are the major contributor to the emergence of antibiotic-resistant bacteria (Khachatourians 1998).

In the EU and many other countries, drugs, that have been registered for therapeutic use in humans and/or animals are not allowed to be used as growing-promoters. However, many of the compounds used as grow-promoting are analogues of and show cross resistance with therapeutic antibiotics (van den Bogaard 1999).

Other practices may also affect the use of antibiotics and the development of resistance. For example spraying of crops, particularly fruit trees, to eliminate surface bacteria is also implicated.

Antimicrobial resistance

According to WHO the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agens (Lochmann 1994). Antimicrobial resistance in bacteria may emerge by several pathways. A bacterium of a normally susceptible species might become resistant by mutation or acquisition of the new genes. Some bacterial species are normally and inherently resistant to certain antibiotics, whereas other are sensitive. Sensitivity has 3 requirements: a target for reaction, a mechanism for transport into the cell before the antibiotic action takes place and absence of enzymes that could inactivate or modify the antibiotic. A change in any of these prerequisites could render an antibiotic-sensitive bacterium resistant to the drug (Levy 1992)

Enterococcus spp.

The enterococci are widely distributed, being found in air, water, sewage, soil, vegetation. The primary source of enterococci is the intestine of humans and warm-blooded animals. Enterococci survive in environmental conditions that destroy other microorganisms of sanitary significance.

Due to their resistance to freezing, low pH, and moderate heat treatment, the enterococci have been suggested as an indicator in some types od food products (Banwart 1989). Contrary to other faecal bacteria that are released into the environment (e. g. *Escherichia coli* that traditionally has been used as indicator of faecal contamination), the enterococci can survive for a long time also outside their natural intestinal hosts.

Even though the enterococci are not regarded as highly pathogenic organisms, they are among the most common organisms encountered in nosocomial infections. These bacteria are responsible for infections as endocarditis, urinary tract infections and hospital acquired bacteriemia. Most of the isolates belong to the species *Enterococcus faecalis* and *Enterococcus faecium.*

However, enterococci are also present as dominant microflora in many traditional fermented foods in which they play an important role with regard to texture and taste. They are also specifically applied as starter cultures in specific fermentations (Tsakalidou 1993).

Antibiotic resistance of enterococci

Enterococci have been known to be resistant to most antibiotics used in clinical practice. They are naturally resistant to cephalosporins, aminoglycosides and clindamycin and may also be resistant to tetracyclines and erythromycin. They are intermediate sensitive to penicillin and ampicillin and glycopeptides. The strains that produce b-lactamase are rare (Urbášková 1999).

Enterococci are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (Kühn et al. 2000). *Enterococcus faecalis* has been reported to transfer plasmids harbouring antibiotic-resistance traits to other enterococci and to *Listeria monocytogenes* in water treatment plants (Marcinek et al. 1998). *Enterococcus faecium* conjugative transposons can be transferred from animal bacteria to human ones. Such conjugative trasposons can also transfer vancomycin resistance to *Staphylococcus aureus*, streptococci and lactobacilli. Multidrug-resistant and vancomycin-resistant enterococci are commonly isolated from humans, sewage, aquatic habitats, agricultural run-off and animal sources, which indicates their ability enter to human food chain (Rice et al.1995)

Vancomycin resistant enterococci (VRE) are emerging as a global threat to public health. VRE threatens to compromise effective treatment of infections caused by these multi-resistant bacteria particularly in seriously ill patients who may need treatment with vancomycin where other antibiotics have failed.

VRE were first isolated from sewage treatment plants in England and a small town in Germany and later from manure samples from pig and poultry farm (McDonald et al. 1997). Bates et al. (1993) recovered VRE from livestock faeces and from uncooked chicken samples purchased from retail outlets. Klare et al. (1995) suggested a possible relationship between the recovery of these microorganisms and the use of avoparcine, a glycopeptide antimicrobial drug used as livestok feed additive in many European countries. Avoparcin is an antibiotic that acts in the same way as vancomycin, except that it is used solely for veterinary practice. VRE are cross resistant to avoparcin and to teicoplanin. There may be a link among the use of avoparcin, the selection for VRE and the colonization of humans by these bacteria through the food chain (Khachatourians 1998).

Vancomycin and teicoplanin are used for the treatment of infections caused by Gram-positive bacteria in case of resistance or allergy to b-lactams. These antibiotics act by blocking cell wall formation and resistance is due to synthesis of modified late peptidoglycan precursors. Glycopeptide resistance can be intrinsic or acquired and strains may be resistant to vancomycin and teicoplanin, or to vancomycin only (Gholizadeh and Courvalin 2000). Resistance to glycopeptides is phenotypically and genotypically heterogenous. Four types of acquired resistance have been described in enterococci, (Arthur et al. 1996). They can be distinguished on the basis of transferability, resistance level, and the range of glycopeptides to which the strains are resistant. The VanA-type is characterised by inducible high-level resistance to both vancomycin and teicoplanin and is due to aquisition of Tn1546-like transposons (Arthur et al. 1993). VanB-type enterococci are resistant to variable levels of vancomycin and teicoplanin (Périchon et al. 1997). VanE-type is characterised by low-level resistance to vancomycin and susceptibility to teicoplanin (Fines et al. 1999). The VanC-typ is a low level intrinsic resistance found in *Enterococcus galinarum, Enterococcus casseliflavus* and *Enterococcus flavescens* and is to vancomycin (Leclercq et al. 1992; Navarro et al. 1994). Glycopeptide resistance in enterococci that are already resistant to other antibiotics resultes in difficult therapeutic problems.

Enterococci exhibit also inherent low-level aminoglycoside resistance. The MICs are between 2 and 16 μg/ml (Murray 1990). Some of enterococcal strains have acquired high-level aminoglycoside resistance (HLAR) The MICs are $\geq$ 2 000 μg/ml (Sahm et al. 1991). Rice et al. (1995) investigated 248 environmental isolates of enterococci for HLAR. The highest percentage of the resistance was seen for kanamycin, closely followed by tobramycin and to a lesser degree by streptomycin and gentamycin. Multiple antibiotic resistance patterns were observed in 95% of HLAR isolates. The most frequently occurring multiple resistance pattern was HLAR to both kanamycin and tobramycin, followed by multiple resistance to streptomycin, kanamycin and tobramycin. All isolates that exhibited gentamycin resistance were also resistant to kanamycin and tobramycin, but not to streptomycin. These environmental enterococci may contribute to the dissemination of HLAR strains to the human population.

Enterococci and animals

Enterococci naturally occur in large numbers in the intestines of mammals, birds, reptiles and insects. Devriese et al. (1992) found in faeces of nonruminating calves several species of enterococci: *E. avium, E. cecorum, E. durans, E. faecalis, E. faecium*, and. *E hierae. E. faecalis* were most frequent. Enterococci were infrequent in dairy cows. The same authors (Devriese et al.1991) identified intestinal enterococcal flora of poultry.

Many authors confirmed the occurrence of antibiotic resistant strains of *Enterococcus* sp. isolated from animals. One important reason is the amount of anti-microbial agent used in animals. According to FEDESA, the European Federation of Animal Health, the sales of anti-microbials in the EU in 1997 were 3 949 tonnes for animal health and 1599 tonnes as growth promoters (Ungemach 2000). Another critical issue is the use of in-feed or in water medication with prophylactic purposes.

Widespread resistance to chloramphenicol, macrolides, kanamycin, streptomycin and tetracycline was found among isolates of *E. faecalis* and *E. faecium* isolated from humans, broilers and pigs (Aarestrup et al. 2000a).

Resistance of *Enterococcus* sp. from the mammary gland was tested to penicillin, cloxacilin, cephapirin, ceftiofur, novobiocin, enrofloxacin, erythromycin and pirlimycin. The MIC for 90% isolates were 4, 64, 32, 64, 4, 1, 4, and 4 (Wats et al. 1995). Epidemiological study of Aarestrup et al. (2000b) showed a statistically significant association between the use of avilamycin for growth promotion and occurrence of avilamycin-resistant *E. faecium* on broiler farms. On the other hand Davis and Roberts (1999), did not find any evidence that the feeding of this growth promoter caused selection of enterococci resistant to tylosin and avilamycin.

Two main classes of antimicrobial agents have been the target of the most studies: fluoroquinolons and glycopeptides. Both types of compounds are first-line drugs for the treatment of many life-threatening infections in humans. Fluoroquinolones were introduced for veterinary use in the early 1990s (Mateu and Martin 2001).

Glycopeptides like avoparcin have been used extensively as growth promoters in the Europe and several authors have suggested them as being responsible for the development of vancomycin-resistant enterococci in animals (Borgen et al. 2000).

Van den Bogaard et al. (2000b) showed that in Netherlands, within 2 years of stopping the use of avoparcin, the prevalence and numbers of VRE have decreased significantly, not only in the faecal flora of food animals but also in the endogenous flora of healthy humans.

Resistant bacteria from animals can infect or reach the human population by direct contact, and also via food products of animal origin. The resistant bacteria can colonize humans and/or transfer their resistance genes to other bacteria belonging to the endogenous flora of humans. The genes encoding the resistance can be transferred to pathogenic bacteria, and disseminated into the environment from animals to foods of animal origin.

Some authors paid attention to the transmission of the resistant enterococci from animals to humans. Van den Bogaard et al. (1997) found that VRE could be detected in 50% of faecal samples from turkeys and 39% of faecal samples from turkey farmers. Furthermore VRE were isolated from 20% of faecal samples from turkey slaughterers and 14% from urban residents from the same area. Similar results obtained Aarestrup et al. (2000a) who compared antimicrobial resistance phenotypes and resistance genes in enterococci from humans, broilers and pigs.

Enterococci can be spread by faecal-oral transmission, contact with infected body fluids or contact with contaminated surfaces. Noskin et al. (2000) found that enterococci are capable of prolonged survival (at least one week) on fabric seat cushions and can be transferred to hands.

Enterococci and humans

Virulence of enterococci is not well understood, but adhesins, haemolysin, hyaluronidase, aggregation substance and gelatinase are putative virulence factors (Franz et al. 1999).

In recent years enterococci have emerged as pathogens in association with serious nosocomial infections. A major problem in enterococcal infections is vancomycin resistance (Rice 2001). Vancomycin is a powerful antibiotic that is often the drug of the last resort. It is generally limited to be used against gram positive bacteria, that are resistant to β-lactam-antibiotics and other ones (Mayhall 1996). The risk factor for the appearence of VRE in hospitalized patients is heavy use of vancomycin, third-generation cephalosporines and similarly active β-lactams (Edmond et al. 1995). There may be a link among the use of glycopeptide avoparcin as growth promotor for farm animals, the selection for vancomycin resistant enterococci and the colonisation (infection) of humans by these bacteria through the food chain. The route of the transmission of VRE from livestock to human has not been proven, but if VRE colonization occurs in a community without hospital exposure, this possibility should be considered (Aarestrup et al. 2000a) . The relationship between VRE colonization of the food animals and VRE colonization of humans was suggested by Bates et al. (1994), who recovered VRE with identical ribotypes from retail chicken carcasses and humans.

Reinert et al. (1999) found resistance to vancomycin in 1,5% from 730 enterococcal isolates in Germany.

In Sweden VRE were found only sporadically (Hallgren et al. 2000). The occurrence of VRE in Czech Republic was first described in 1997 (Kolář et al. 1997). Their frequency reached 3.7%.

Sievert et al. (2002) described the first clinical isolate of *Staphylococcus aureus* that was fully resistant to vancomycin. The presence of vanA in this VRSA suggests that the resistance determinant might have been acquired through exchange of genetic material from the vancomycin-resistant *enterococcus* also isolated from the same clinical material.

VRE poses a serious threat to certain high-risk patients. It is necessary to control the spread of these dangerous organisms and judicious use of vancomycin should be promoted.

Enterococci and foodstuffs

Enterococci may gain acces into raw material and food products from primary habitats such intestines of animals and humans, and from sources associated with unsanitary conditions of the production and handling with foods. The resistant enterococci can be potentially transferred from food animals to human via food chain (Mateu and Martia 2001).

Quednau et al. (1998) isolated 279 strains of *Enterococcus* sp. from the retailed chicken and pork. Seventy-three per cent of *Enterococcus* isolates from Swedish chicken were resistant to one or more of the tested antibiotics. The corresponding values for Swedish pork, Danish chicken and pork were 9%, 55% and 14%, respectively.

Enterococci isolated from sausages and raw milk exhibited resistance to tetracycline, chloramphenicol, gentamicin, and erythromycin. In this study, several *E. faecium* and *E. faecalis* strains were showen to transfer tetracycline resistance genes into chromosome of *Lactococcus lactis* subs. *lactis* var. *diacetylactis* (Perreten 1996). Ten strains of genus *Enterococcus* were isolated from the Egyptian Dominati cheese. All strains were resistant against oxacillin and caphazolin and sensitive against ampicillin, amoxicillin, and cefaclor (Hematiet al. 1997). All enterococci isolated from raw milk cheese showed resistance to 1 of 12 antibiotics. The resistance genes for tetracycline, chloramphenicol and erythromycin were found to be identical to genes found in resistant enterococci from human infections (Perreten and Teuber 1995).

According to the study of Sorensen et al. (2001) the ingestion of resistant *E. faecium* of animal origin leads to detectable concent rations of the resistant strains in stool of the health volunteers for up to 14 days after ingestion and therefore food animals appear to be an important reservoir of the transferable antibiotic resistance.

Many studies deal with the occurrence of vancomycin-resistant enterococci in foodstuffs and with the possibility of animal-human transfer of vancomycin resistance. Pavia et al. (2000) investigated antimicrobial resistance of enterococci in samples of meat sold in retail outlets of Catanzaro. Enterococci were isolated from 45% of the samples, mostly from the chicken meat. Overall, 29% of the samples were contaminated with VRE. 10-12% of *E. faecalis* isolated from the Danish chicken and pork but none of the Swedish isolates were resistant to vancomycin (Ahrne et al. 1996). 242 (79%) of the 305 retail chicken products from the Netherlands were contaminated with VRE. VRE isolated from chicken samples were also compared by pulsed field gel electrophoresis wit human VRE isolates. The highest homology between VRE from chicken products and human strains was 60% (van den Braak et al. 1998). In another study, Simonsen et al. (1998) examined faecal VRE strains from poultry farmers and their broilers at five avoparcine exposed Norwegian farms. Animal and human *E. faecium* strains at one farm were genetically closely related with indistinguishable Van A elements. The results indicate that the transmission of VanA glycopeptide resistance in enterococci between humans and animals can occur. Jensen et al. (1998) examined 38 high-level vancomycin resistant *E. faecium* isolates of human and animal origin from Europe and the United States. Their results were similar and indicated either horizontal gene transfer between *E. faecium* organisms of human and animal origin or the existence of a common reservoir for glycopeptide resistance.

On the other hand the interesting opinion has been demonstrated by Lemcke and Bütte (2000). Out of 1 643 enterococcal isolates from 115 poultry and 50 pork samples, 420 isolates could be identified as vancomycin resistant. Comparing VanA-positive food isolates with those from different human sources by means of the pulsed field gel electrophoresis it could clearly be demonstrated that they do not show homologousfingerprints according to the source of origin. It is therefore unlikely that there is a close genetic relationship between isolates from foodstuffs and humans.

In conclusion, antimicrobial agents have been used widely in animals in order to treat and prevent infections. They have been also used as growth promoters in animals feed in subtherapeutic concentrations. This practice can facilitate the emergence of resistant bacteria. They can be transmitted to humans through the food chain.

Enterococci have been known to be resistant to the most antibiotics. The resistance pattern of enterococci isolated from food products reflects to the same extent animal application of antimicrobial agents.

In the present situation the amount of antibiotics and the development of the resistance in important pathogens should be closely monitored. Furthermore, resistance monitoring in certain non- pathogenic intestinal bacteria, including enterococci, which may serve as the reservoir for the resistance genes is probably more important than hitherto anticipated. More attention shoud be given to further epidemiological studies of resistant bacteria clones, improvement of hygienic practices of the production and handling with foodstuff and more rational use of the antibiotics in veterinary and human medicine.

Enterokoky a rezistence k antibiotikům

Enterokoky jsou mikroorganismy široce rozšířené v zevním prostředí. Přirozeně osidlují trávicí trakt lidí a zvířat. Suroviny a potraviny mohou být kontaminovány vodními zdroji, potravními zvířaty a při nehygienických podmínkách výroby.

Enterokoky jsou známy svou rezistencí k většině antibiotik, používaných v klinické praxi. Multirezistentní a vankomycin rezistentní enterokoky jsou běžně izolovány od lidí, zvířat, z vodního a zemědělského prostředí což indikuje jejich schopnost pronikat do potravního řetězce. Vankomycin resistentní enterokoky představují vážné nebezpečí pro lidské zdraví.

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