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Enterococci from foods

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Abstract

Enterococci have recently emerged as nosocomial pathogens. Their ubiquitous nature determines their frequent finding in foods as contaminants. In addition, the notable resistance of enterococci to adverse environmental conditions explains their ability to colonise different ecological niches and their spreading within the food chain through contaminated animals and foods. Enterococci can also contaminate finished products, such as fermented foods and, for this reason, their presence in many foods (such as cheeses and fermented sausages) can only be limited but not completely eliminated using traditional processing technologies. Enterococci are low grade pathogens but their intrinsic resistance to many antibiotics and their acquisition of resistance to the few antibiotics available for treatment in clinical therapy, such as the glycopeptides, have led to difficulties and a search for new drugs and therapeutic options. Enterococci can cause food intoxication through production of biogenic amines and can be a reservoir for worrisome opportunistic infections and for virulence traits. Clearly, there is no consensus on the acceptance of their presence in foodstuffs and their role as primary pathogens is still a question mark. In this review, the following topics will be covered: (i) emergence of the enterococci as human pathogens due to the presence of virulence factors such as the production of adhesins and aggregation substances, or the production of biogenic amines in fermented foods; (ii) their presence in foods; (iii) their involvement in food-borne illnesses; (iv) the presence, selection and spreading of antibiotic-resistant enterococci as opportunistic pathogens in foods, with particular emphasis on vancomycin-resistant enterococci.

Keywords: Enterococcus; Enterococcal virulence; Food-borne pathogen; Opportunistic pathogenicity; Antibiotic resistance; Fermented food

Contents

1. Introduction

Bacteria of the genus *Enterococcus* or enterococci (formerly the 'faecal' or Lancefield group D streptococci) are ubiquitous micro-organisms, but have a predominant habitat in the gastrointestinal tract of humans and animals. Because of their high heat tolerance and survival under

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adverse environmental conditions, enterococci can colonise diverse niches and may then serve as indicators of the sanitary quality of food. Indeed, enterococci commonly occur in large numbers in vegetables, plant material and foods, especially those of animal origin such as fermented sausages and cheeses. In processed meats, enterococci are generally not desirable because they cause spoilage. On the contrary, enterococci have important implications in the dairy industry. They play an acknowledged role in the development of organoleptic characteristics during the ripening of many cheeses and they have been also used as components of cheese starter cultures [1]. Some enterococci of food origin also share a number of useful biotechnological traits (e.g. bacteriocin production, probiotic characteristics), which led to earlier applications in fermented foods.

Unfortunately, enterococci have recently assumed major importance in clinical microbiology as well. Enterococci have traditionally been regarded as low-grade pathogens. However, there is no consensus on the significance of their presence in foodstuffs. Their newly accentuated ambiguity concerning the relationships of enterococci with human beings is related to their enteric *habitat*, their entering the food chain, their antibiotic resistance and their possible involvement in food-borne illnesses due to the presence of virulence factors, such as the production of adhesins and aggregation substances. Over the last two decades, enterococci have emerged as important hospital-acquired pathogens in immune-suppressed patients and intensive-care units. The rise in hospital-acquired enterococcal infections has been in part due to the increased use of broad-spectrum antibiotics and the rising number of severely ill patients. Enterococci are not only intrinsically resistant to several antibiotics, but are also characterised by a potent and unique ability to exchange genetic material. The increasing prevalence of strains resistant to ampicillin, aminoglycosides and glycopeptides and the acquisition of resistance to the few antibiotics available for treatment (such as vancomycin) pose serious difficulties in clinical therapy.

In addition, selective pressure exerted by the use of antibiotics as growth promoters in food animals appears to have created large reservoirs of transferable antibiotic resistance in various ecosystems. With the emergence of glycopeptide resistance in *Enterococcus faecium* outside hospitals, a large reservoir of transferable resistance (vanA gene cluster) was identified in animal husbandry due to the use of avoparcin as a feed additive. The spread of resistance, which enters the human enterococcal flora via the food chain, and the transfer of this trait to pathogenic species (i.e. the recent emergence of *Staphylococcus aureus* with decreased sensitivity to vancomycin) indicate the need for greater control of the use of glycopeptide antibiotics in animal feed.

Therefore, the barrier separating enterococci as inoffensive contaminants from pathogens appears most fragile.

2. Taxonomy

The identification of the enterococci has always been problematic. Numerous enterococcal isolates, especially from an environmental source, often remain unidentified when their identification is based on phenotypic traits alone. It is difficult to unequivocally categorise isolates into one of the *Enterococcus* species by physiological tests because heterogeneity in phenotypic features is very high, regardless of the origin of the isolate [2–5].

The problem with the taxonomy of enterococci is generally that they are a heterogeneous group of Gram-positive cocci sharing many characteristics with the genera *Streptococcus* and *Lactococcus*. This explains why food-associated enterococci have often been considered to belong to the 'lactic' microflora. On the basis of 16S rRNA cataloguing, the genus *Streptococcus* was separated during the 1980s into the three genera *Enterococcus*, *Lactococcus* and *Streptococcus*. Consequently, bacteria previously named '*Streptococcus faecalis*', '*Streptococcus faecium*', *Streptococcus avium* and *Streptococcus gallinarum* were transferred in 1984 to the revised genus *Enterococcus* as *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus avium* and *Enterococcus gallinarum*, respectively [6]. Since this transfer, the total number of species presently included in the *Enterococcus* genus on the basis of chemotaxonomic and phylogenetic studies is 19. This situation continues to fluctuate from time to time as individual species are moved into other genera or new taxa are discovered. More recently, other species of enterococci have been proposed on the basis of chemotaxonomic studies and phylogenetic evidence provided by 16S rDNA sequencing [7,8]. It is highly probable that phylogenetic system of the genus *Enterococcus* has not yet been completely elucidated and that some re-classifications may be necessary in the near future.

3. Enterococci, emerging pathogens

Over the last two decades, enterococci, formerly viewed as organisms of minimal clinical impact, have emerged as important hospital-acquired pathogens in immunosuppressed patients and intensive care units. Enterococci do not possess the common virulence factors found in many other bacteria, but they have a number of other characteristics, e.g. the resistance to antimicrobial agents, that may contribute to their virulence and make them effective opportunistic pathogens. Foodborne enterococci have not yet been clearly involved as direct causes of clinical infections [9]. In this context, reports of hospital-acquired infections attributed to enterococci are difficult to interpret because these bacteria are generally identified in mixed cultures with other primary pathogens, such as staphylococci and others [10]. Enterococci have been implicated in cases of food poisoning, e.g. by production of biogenic

amines, based on their isolation in high numbers from suspect foods, but this statement still has not found direct support.

3.1. Clinical epidemiology

The incidence of enterococcal infections has increased in recent years accounting for approximately 10% of hospital-acquired infections in the USA [10,11]. Enterococci are now among the most common nosocomial pathogens; they have been implicated as an important cause of endocarditis, bacteraemia, urinary tract, central nervous system, intra-abdominal and pelvic infections [12]. Epidemiological data also indicate that *E. faecalis* is the most common species among the enterococci isolated from human illnesses while *E. faecium*, which is associated with the majority of the remaining enterococcal infections, may pose a larger antibiotic-resistant threat [13–17].

Enterococci from the gut account for 5–15 and 4% of the causes of infective endocarditis and bacteraemia, respectively, whereas urinary tract infections are the most common enterococcal, hospital-acquired infections [9,10]. In addition, there is strong evidence that enterococci causing bacteraemias commonly originate from the urinary tract. Malone et al. [18] observed that in 24% of enterococcal bacteraemias the isolate originated from a urinary tract infection. In addition to these well-documented infections, the incidence of intra-abdominal infections caused by vancomycin-resistant enterococci is increasing [19]. Enterococci may also contribute to cause abdominal and pelvic abscess formation and sepsis [20].

3.2. Factors determining the increasing virulence of the enterococci

The clinical significance of enterococci in human infections is poorly understood because of the scarcity of well-documented reports confirming their occurrence in mixed cultures. The increasing resistance of enterococci to antibiotics and the presence of active mechanisms of gene transfer are exacerbating the increasing findings of these bacteria as nosocomial opportunists. However, the antibiotic resistance alone cannot explain the virulence of these bacteria in the absence of pathogenicity factors.

3.2.1. Presence of virulence factors and genetic exchange mechanisms

Virulence traits in enterococci include adherence to host tissue, invasion and abscess formation, resistance to and modulation of host defense mechanisms, secretion of cytolysins and other toxic products and production of plasmid-encoded pheromones [12,14,21]. A number of genes encoding for virulence factors (especially in *E. faecalis*) have been sequenced and characterised and their effects have been shown in human and animal studies. Recent molecular screenings of *Enterococcus* virulence determinants indicated that medical *E. faecalis* strains had more virulence determinants than did food strains, which, in turn, had more than starter strains. Multiple determinants, e.g. those involved in adherence, cytolysin and pheromone production mechanisms, were harboured mostly by *E. faecalis* and, to a lesser extent, by *E. faecium* [21,22].

Many of these enterococcal virulence traits, such as haemolysin–cytolysin production, the adhesion ability and the antibiotic resistance (see later), have been shown to be transmissible by gene transfer mechanisms [23–26]. Often, the same plasmid may encode a sex pheromone response and either antibiotic resistance or haemolysin production genes [12,26,27]. The exchange of genetic material in *E. faecalis* was shown to be highly facilitated by the sex pheromone response. In a recent study, multiple pheromone-encoding genes were identified in both clinical and food enterococcal strains, indicating the potential of these latter to acquire other sex pheromone plasmids. Transconjugation in which starter strains acquired virulence determinants from medical strains was also demonstrated [21]. Sex pheromones are also thought to act as virulence factors by eliciting an inflammatory host response [28–30].

3.2.2. Antibiotic resistance

Virulence of enterococci is strongly enhanced by their frequent resistance to commonly used antibiotics. Antibiotic resistance, which can be both intrinsic and acquired, makes enterococci effective opportunists in nosocomial infections.

Enterococci show intrinsic resistance to cephalosporins, lincosamides, many β-lactams and low levels of aminoglycosides [10,20,31,32]. Intrinsic resistance to many antibiotics suggests that treatment of infection could be difficult. In addition to these constitutive resistances, enterococci have acquired genetic determinants that confer resistance to all classes of antimicrobials, including chloramphenicol, tetracyclines and glycopeptides. The major risk related to these latter resistance traits is that they are for the most part transferable. The genes coding for all of these antibiotic-resistant traits may be transferred by pheromone-mediated, conjugative (often multiresistant) plasmids or transposons to both enterococci and more virulent pathogens, such as *S. aureus* [10,33]. Within acquired antibiotic resistances, vancomycin-resistant enterococci (VRE) are possibly the most serious concern that has recently emerged in human clinical infections. Two distinct forms of transferable vancomycin-resistant phenotypes have been described in enterococci: the VanA phenotype (associated with a high level of inducible resistance to vancomycin and cross resistance to teicoplanin) and the VanB phenotype (usually displaying variable levels of inducible resistance only to vancomycin).

4. Possible involvement of the enterococci in food-borne illnesses or food poisoning

4.1. Presence of the enterococci in foods

Enterococci can be readily isolated from foods, including a number of traditional fermented foods. A clear picture of the microbial ecology of these bacteria easily explains their presence in foods. Enterococci constitute a large proportion of the autochthonous bacteria associated with the mammalian gastrointestinal tract. Once rejected from the environment by means of human faeces or animal ejecta, they are able to colonise diverse niches because of their exceptional aptitude to resist or grow in hostile environments. Therefore, enterococci are not only associated with warm-blooded animals, but they also occur in soil, surface waters and on plant and vegetables. By intestinal or environmental contamination they can then colonise raw foods (e.g. milk and meat) and multiply in these materials during fermentation. They can also contaminate finished products during food processing. Therefore, many fermented foods made from meat and milk (especially fermented meats and cheeses) contain enterococci.

A wide variety of fermented meat products is produced in many parts of the world. In Europe the predominant types are Italian salami and German raw sausage with numerous national and regional variants. The technology for the production of most of these products is essentially similar. After a period of fermentation to biologically stabilise the product, processed meats are typically salted or smoked, and for the most part eaten raw [34]. In these conditions enterococci, which usually contaminate raw meats in the range of 10^2–10^4 CFU g^{-1} [35] and are very resistant to extremes in temperature, pH and salinity, may multiply to high numbers and act as spoiling agents in processed meats. For example, the fermented meat products salami and Landjager were found to contain enterococci at numbers ranging from 10^2 to 10^5 CFU g^{-1} [35]. It was therefore suggested that a proper heat treatment during processing, such as in the case of cooked, unfermented meats, would be necessary to eliminate enterococci as spoilage microflora in fermented meats [36].

In many cases, however, enterococci are a spoilage problem also in cooked, processed meats because they are able to survive heat processing, especially if initially present in high numbers [12]. To this regard, both *E. faecalis* and *E. faecium* have been implicated in the spoilage of pasteurised canned hams [37,38]. Gordon and Ahmad [39] stated that *E. faecium* can survive cooking to 68°C for 30 min during normal 'frankfurter' production Furthermore, great potential exists for recontamination with enterococci, both in raw and properly cooked products, from intestinal or environmental sources. Therefore, the presence of enterococci in fermented or non-fermented meat products appears unavoidable by present day applied technologies.

The presence of enterococci in dairy products has long been considered an indication of insufficient sanitary conditions during the production and processing of milk. To the contrary, many authors suggest that certain strains of enterococci in some cheeses may be highly desirable on the basis of their positive contribution to flavour development during the cheese ripening. This beneficial role led to the inclusion of enterococcal strains in certain starter cultures. Enterococci occur in a variety of cheeses, especially artisanal cheeses produced in southern Europe from raw or pasteurised milk, and in natural milk starters. The isolation of enterococci from natural milk starter cultures, which are still widely used for many Italian soft cheeses made with raw or pasteurised milk, can be explained by their thermal resistance. In fact, natural milk cultures are made by pasteurising a good quality raw milk and by incubating it at 42–44°C for 12–15 h, thus promoting the natural selection of thermophilic and heat-resistant lactic acid bacteria, usually belonging to *Streptococcus thermophilus* and *Enterococcus* spp. [1]. The presence of enterococci in pasteurised cheeses is generally due to recontamination after the heat treatment and to their heat resistance. Levels of enterococci in different cheeses at the end of ripening may range from 10^5 to 10^7 CFU g^{-1} [35]. *E. faecium* and *E. faecalis* are the most prevalent species recovered [1,12]. The recovery and persistence of the enterococci in some cheeses during ripening can be attributed to their wide range of growth temperatures and their tolerance to pH and salt. Clearly, the presence of enterococci is ineluctable also in many dairy products.

4.2. Antibiotic resistance in foods

The extremely high level of antibiotic resistance observed in enterococci and their widespread finding in raw foods are two key elements contributing to the frequent recovery of antibiotic-resistant enterococci (ARE) in both unfermented and fermented foods. ARE have been found in meat products, dairy products, ready-to-eat foods and even within enterococcal strains proposed as probiotics [36,40–44].

In previous studies on European cheeses, enterococci mainly belonging to *E. faecalis* and *E. faecium* and resistant, in different proportions, to penicillin, tetracycline, chloramphenicol, erythromycin, gentamicin, lincomycin, rifampicin, fusidic acid and vancomycin were detected; a prevalence of multiple drug resistance was also observed [36]. Although ARE are found in both pasteurised and, to a much higher extent, raw milk cheeses, their presence in these latter products may represent a more serious risk of expanding antibiotic resistance through the food chain. Strains with high-level resistance to kanamycin and gentamicin were recently isolated from French raw milk cheeses and hospitalised patients [45].

The same picture emerges from data on meat products. Seventy-three percent of the *Enterococcus* isolates from

Swedish retailed chicken were resistant to one or more different antibiotics such as tetracycline, erythromycin and vancomycin; the corresponding values for Swedish pork, Danish chicken and Danish pork were 9%, 55% and 14%, respectively [41]. Enterococci resistant to one or more antibiotics including bacitracin, chloramphenicol, erythromycin, gentamicin, penicillin, rifampicin, streptomycin and tetracycline were isolated from minced meat, raw meat sausages, ham and tenderloin beef [36,42].

The overall data on antibiotic resistance within food-associated enterococci open the question of their entering the food chain. There is strong epidemiological evidence of a link between the use of antibiotics in human medicine and animal husbandry and the emergence, spreading and persistence of resistant strains in animal products [46,47]. The prevalence of ARE in farm animals and their meat is always high, and multiple drug resistance ranges from 60 to 100% [36]. Antibiotic-resistant enterococci can be provided to the human gut by contaminated foods (especially of animal origin) and environment, including people and animals. A recent epidemiological study carried out in France, which showed common pulsed field gel electrophoresis (PFGE) patterns in antibiotic-resistant *E. faecalis* from humans and cheeses, suggests that cheeses may serve as a reservoir of ARE with characteristics that allow them to persist and spread in the community [45]. Food-associated enterococci could therefore be a reservoir for antibiotic resistance. Once ingested, ARE can survive gastric passage and multiply, thus leading to sustained intestinal carriage [48].

The emergence of ARE in nosocomial infections poses the problems of (i) the role played by these bacteria, as possible natural food reservoirs, in the dissemination of antibiotic-resistant traits in the environment, and (ii) the risk for human health of using antimicrobial drugs in agriculture. These two aspects, with almost exclusive emphasis on the selection and microbial ecology of VRE in foods, will be treated later.

4.2.1. The resistance to glycopeptides: a case study

Among ARE, the emergence, selection and spreading of enterococci resistant to the glycopeptide antibiotics vancomycin and teicoplanin in a hospital environment is a clinical emergence. The use of this class of antimicrobials is of utmost importance in clinical therapy against multiple antibiotic-resistant strains or in the case of allergy to other antibiotics, e.g. ampicillin and penicillin [10]. Glycopeptide antibiotics often represent the 'last therapeutic option' against nosocomial pathogens and, for this reason, new drugs are rapidly being evaluated as candidates for replacing vancomycin; some of the most promising include semisynthetic glycopeptides, quinupristin-dalfopristin, oxazolidinones, everninomycins and daptomycin [49–51].

Although nosocomial acquisition and subsequent colonisation of VRE has been emphasised among hospitalised persons, colonisation appears to occur frequently in persons not associated with the health care setting [52–55]. Several reports carried out in European countries and the USA in recent years indicate that colonisation with VRE frequently occurs in the community, and that many animal, food and environmental reservoirs can act as community sources for VRE outside the health care setting. In this mechanism, the transport of these resistances via the food chain to humans appears most probable.

4.2.2. Presence, selection and spreading of VRE in foods

High level VRE strains, especially belonging to *E. faecium*, were found at high frequencies in beef, poultry, pork and other meat products [42,56–61] and also in industrial and artisan cheeses [36,44]. The vanA type glycopeptide resistance, which confers coupled resistance to both vancomycin and teicoplanin, seems the most frequent vancomycin-resistant phenotype among food-associated VRE.

The chronic use of antibiotics as growth promoters in livestock is a recognised factor acting as a selective agent in promoting resistant enterococci. With the emergence of glycopeptide resistance in enterococci (especially *E. faecium*) outside hospitals, a large reservoir of transferable resistance (vanA gene cluster) was identified in animal husbandry due to the use of avoparcin as a feed additive. Similar mechanisms have been suggested for streptogamin, avilamycin and tylosin resistance. Streptogamin resistance has been found in *E. faecium* of animal and clinical origin. Because virginiamycin has been used as a growth promoter in animal feed but streptogamins have rarely been used in human therapy, this again appears to be another example of resistance of animal origin [47].

The fact that vancomycin resistance is common not only in animals fed with avoparcin as a growth promoter, but also in the human population outside hospitals, makes clear that either a clonal spread of resistant strains or a transfer of resistance genes between animal and human bacteria may occur. Epidemiological studies of farms that use avoparcin have shown a significant association with the presence of VRE in animal stools. VRE generally reach foods through environmental contamination from various sources. VRE have been found in waste water samples from sewage treatment plants, livestock faeces, uncooked chicken samples, manure samples from pig and poultry farms [12,36,62]. These findings suggest that a possible link between the use of avoparcin and other antibiotics used in livestock, the selection of VRE (or ARE), and humans becoming colonised via the food chain exists.

To prove such a link is beset with many difficulties: it is necessary to explain the presence of VRE within non-hospitalised people in the United States where avoparcin has never been approved for use. To this regard, it was suggested that other modes of community transmission, such as household contact (e.g. food preparation and pets) may

act as concomitant factors in spreading of resistance outside hospitals [62]. It is also difficult to prove that animal, environmental and human strains are identical by means of molecular typing. To date, molecular typing of strains only suggests a link [40,63]. Human vancomycin-resistant *E. faecium* (VREF) isolates were indistinguishable from isolates deriving from non-human sources, which may suggest that VRE from animals may pass to humans via the food chain [64]. Conversely, no PFGE-deduced genetic overlap was found when VREF from humans were compared with VREF from poultry [57]. Molecular typing by PFGE carried out on VRE isolated from hospitalised patients and non-hospitalised controls in France revealed a different pattern for each VRE that originated from an individual subject [65]. Similarly, different PFGE patterns between strain 686B, a VRE causing nosocomial outbreaks in three hospitals located in the Northern Italy, and various VRE isolated from food were observed (Fontana, Goglio and Scagnelli, personal communication). Therefore, although a connection between the occurrence of VRE in meat and nosocomial infections has not yet been clearly proved, epidemiological data would suggest that both clonal dissemination through the food chain and horizontal gene transfer between a variety of different strains are involved in the VRE spreading outside the hospital. Concerning gene transfer route of transmission, recent molecular studies on sequence polymorphism of Tn*1546*, encoding vancomycin resistance, within VRE from humans, pigs and poultry suggest that the primary transmission is from animals to humans and not the other way around [66]. On the other hand, although many reports of cases of VRE infection suggested inter-strain transmission of resistance genes, cross colonisation of single resistant clones seem the most frequent mechanisms of VRE spreading within farms or hospitals [11,61,64,67–70].

4.3. Production of biogenic amines in fermented foods

High levels of biogenic amines in many fermented foods, such as fermented sausages, cheeses, wines, beers, olives and fish products, involved in food intoxication may be a clinical concern. Food intoxication caused by ingestion of biogenic amines determines a number of symptoms of increasing complexity which include headache, vomiting, increase of blood pressure and even allergic reactions of strong intensity.

Microbial agents involved in biogenic amine production in foods may belong to either starter or contaminating microflora. Cheeses may represent a good substrate for production and accumulation of biogenic amines, especially tyramine, from enterococci able to decarboxylate free amino acids into the matrix. The ability to produce biogenic amines in cheese and fermented sausages has been reported for bacteria of the genus *Enterococcus* [1,71–74].

5. Concluding remarks

There is evidence that enterococci could find potential application in the processing of some fermented dairy products [1,75]. On the other hand, the emergence of many enterococci resistant to glycopeptides and other antibiotics and the finding of virulence traits within both clinical and food-borne isolates pose the presence of enterococci in foods as questionable. In the past, it has been suggested that the application of enterococci in foods could proceed once pathogenic strains and non-pathogenic strains are defined on the basis of careful selection and case-by-case studies. The presence of safer strains within food-borne enterococci was also emphasised [1,76]. However, the finding of effective gene transfer mechanisms within enterococci weakens these statements. Transconjugation in which starter enterococci acquire virulence determinants from medical enterococci was recently demonstrated [21]. In the case of VRE, the greatest threat is the potential to transfer their resistance genes to more pathogenic Gram-positive bacteria, which could produce truly frightening pathogens. To this regard, glycopeptide resistance has not yet transferred in vivo to other significant pathogens, but experimental transfer to *S. aureus* has been achieved in vitro [77].

The finding of non human VRE suggests the presence of community and animal reservoirs for these bacteria, where they have been selected through the use of avoparcin as a feed additive. Once VRE contaminate foodstuffs, food-borne resistant strains may cause human colonisation through the food chain. The emergence of VRE in hospital patients may reflect further selection of these organisms in the hospital environment by antibiotic usage, from which nosocomial spread might occur. As a first barrier, it is then desirable to decrease food contamination by antibiotic-resistant strains through a more limited use of antibiotics by veterinarians. To this regard, it is worth noting that the avoparcin withdrawal has been successful in reducing the occurrence of VRE in foods and food animals, although their decrease appears slow as a consequence of their survival in adverse environmental conditions [44,58,59,70,78,79]. However, this measure should be complemented by a more prudent use of antibiotics in human medicine.

Other possible solutions should include more effective control measures for the presence and typing of enterococci in food and environmental sources in order to prevent, or at least limit, the spread of the pathogenic strains. Further studies of community transmission are also needed. If transmission with enterococci from unrecognised community sources can be identified and controlled, an increase in the incidence of colonisation and infection among hospitalised patients could be prevented. These long-term policies should be based on international prospective monitoring systems for the surveillance in humans, animals, carcasses and foods. In this context, the

still unclear taxonomy of enterococci will demand molecular tools to reach a more effective identification and characterisation of natural isolates in epidemiological and clinical studies. A grouping of the strains on the basis of virulence traits, as well as case-by-case studies on overall phenotypic and genotypic characteristics, could enable us to trace pathogenicity schemes within the enterococcal population and better comprehend the microbial ecology of this heterogeneous group of bacteria.

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