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Enterococcal infections: host response, therapeutic, and prophylactic possibilities

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Abstract

The emergence of resistance against multiple antibiotics and the increasing frequency with which *Enterococcus faecalis* and *Enterococcus faecium* are isolated from hospitalized patients underscore the necessity for a better understanding of the virulence mechanisms of this pathogen and the development of alternatives to current antibiotic treatments. The genetic plasticity of enterococci and their ability to rapidly acquire and/or develop resistance against many clinically important antibiotics and to transfer these resistance determinants to other more pathogenic microorganisms makes the search for alternative treatment and preventive options even more important. A capsular polysaccharide antigen has recently been characterized that is the target of opsonic antibodies. A limited number of clinically relevant serotypes exist, and the development of an enterococcal vaccine based on capsular polysaccharides may improve our ability to prevent and treat these infections. Additional enterococcal surface antigens, including ABC transporter proteins and other virulence factors, such as aggregation substance (AS), may also be useful targets for therapeutic antibodies.
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1. Introduction

Enterococci are physiologic commensals of the gastrointestinal and female genital tracts of humans and several mammals and birds [1]. They are extremely versatile and well suited for survival under harsh conditions [2]. Under most circumstances, enterococci do not cause any harm to the host, despite living in abundance in the intestinal lumen (10^5–10^8 colony-forming units per gram of feces) [3,4]. Some enterococcal strains are used as probiotic agents and are believed to have beneficial effects on a number of gastrointestinal and systemic diseases [5–7]. However, on some occasions, the commensal relationship with the host is disrupted with the consequence that enterococci cause serious diseases [8]. Enterococci are intrinsically not as virulent as other Gram-positive organisms such as *Staphylococcus aureus*, pneumococci, or group A streptococci, which makes the study of their pathogenicity more difficult. A number of putative virulence factors for enterococci have been described, although their relevance to disease development is often not as obvious as for other pathogens. Enterococci are endogenously resistant and are known to have acquired further resistance mechanisms to multiple antibiotics [9], allowing them to prevail in hospital and nursing home settings. The immense difficulties in treating serious enterococcal infections underscore the importance of understanding virulence factors that may be targeted by alternative therapeutics. The rapid increase in enterococcal strains resistant to vancomycin (VRE) and other antibiotics [3,9] and their ability to pass this trait on to other pathogens, i.e. *S. aureus*, indicates an urgent and expanding clinical problem.

2. Enterococcal infections

Enterococci are the third most common pathogen isolated from bloodstream infections [9], the single most frequently reported type of pathogen in surgical-site infections in intensive care units [10], and the second most common nosocomial pathogen in the US [11]. Enterococci are responsible for three to four cases of nosocomial bloodstream infections per 10,000 hospital discharges [12]. These bacteria contribute significantly to patient mortality as well as to additional hospital stay [13]. The ability of enterococci to acquire, accumulate, and transfer genetic elements such

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Table 1
Predominant enterococcal infections in specific patient populations

Immunocompetent patients	Immunocompromised patients	Procedure-related infections
Urinary tract infections	Bacteremia/sepsis	Urinary tract infections
Endocarditis		Intraabdominal infections
		Meningitis

as plasmids and transposons via conjugation is one of the major reasons for their increased importance as nosocomial pathogens [2]. Transfer of resistance determinants from enterococci to other more virulent Gram-positive bacteria, like staphylococci, has been observed in vitro [2]. The first isolation of a fully vancomycin-resistant *S. aureus* strain in a patient previously colonized with VRE suggests the possibility of an in vivo exchange of resistance traits [14].

Enterococci can cause a variety of clinical syndromes including endocarditis, bacteremia, meningitis, intraabdominal, wound, and urinary tract infections. There are well-defined patient populations (e.g. liver-transplant patients [15], neonates [16], and patients with hematological malignancies [17]) who would clearly benefit from improved treatment options for enterococcal infections (Table 1).

3. Pathogenicity of enterococci

The mechanisms by which peaceful commensals are transformed into life-threatening pathogens are not well understood. One hypothesis is that enterococci normally colonize the intestinal tract and are held in check by host mechanisms, but at some point develop traits to occupy new niches or exploit a possibly weakened host immune system [18]. This imbalance could lead to translocation of organisms from the intestinal lumen into the bloodstream, eventually resulting in systemic spread. Successful evasion of the host defense can eventually lead to increased pathogenicity in the host and subsequent disease [19]. Additional sources of infections include intravenous, urinary, or biliary catheters, foreign bodies, the urinary tract, surgical wounds, or the oral cavity [8,18]. Studies have shown that enterococci can also be transmitted through the hands of healthcare workers, clinical instruments [20], or from patient to patient [21].

4. Colonization

Enterococci normally colonize the gastrointestinal tract of healthy humans. A number of adhesion factors of enterococci have been identified that confer binding to mucosal and other epithelial surfaces and facilitate colonization or the formation of vegetations. Adhesion to host tissues is considered a prerequisite for the establishment of infection by many bacteria. For example, in endocarditis, firm attachment to endocardial epithelium is a precondition of successful colonization, considering the high flow rates inside the heart [22,23].

Table 2
Prevalence of virulence genes of enterococcal isolates from different sources

Virulence factors	Clinical isolates	Stool isolates from healthy volunteers
Aggregation substance (*AsaI*)	50–90% [34–37,60,63]	30–60% [34,36,37]
Esp	5–100% [35,36,42,59,60]	3–40% [35,36,42]
Cytolysin/hemolysin	11–70% [34–37,59,60,63]	0–25% [34–37]
Gelatinase	55–100% [34,36,59,60,63]	27–66% [34–36]

Aggregation substance (AS) is one enterococcal virulence factor that seems to mediate the specific binding of enterococci to intestinal epithelium [24], renal epithelial cells [25], human neutrophils [26], and macrophages [27]. AS is a surface-bound glycoprotein encoded on sex-pheromone plasmids that mediates aggregation between bacteria and facilitates plasmid transfer [28]. AS augments internalization of enterococci [24,29,30] and intracellular survival [27,31] and has been associated with an increased mass in valvular vegetations in rabbit endocarditis models [32,33]. In some studies, AS seems to be more common in clinical versus stool isolates [34,35], while other studies found no difference [36,37] (Table 2).

Another cell surface protein, Ace (adhesin of collagen from *Enterococcus faecalis*), which exhibits strong similarities with the *S. aureus* collagen-binding protein Cna, has recently been identified [38]. This *E. faecalis*-specific surface component belongs to the MSCRAMM family, mediates binding to certain collagens [38], and may play a role in the pathogenesis of endocarditis [39].

Similarly, *E. faecalis* adhesin (EfaA), a serum-inducible surface protein that shows extensive similarities with several adhesins of streptococci [40], is a putative endocarditis antigen and demonstrated a potential biological role in a mouse peritonitis model [41].

Another putative colonization factor is the enterococcal surface protein Esp [42], a cell-wall associated protein, that shows structural similarities with the *Streptococcus agalacticae* (GBS) Rib [43], C alpha protein of GBS [44], R28 of *Streptococcus pyogenes* (GAS) [45], and the *S. aureus* biofilm-associated protein BAP [46]. Esp was found to be enriched in clinical versus stool or food isolates in several studies [36,42,47–49], though this could not be confirmed by others [35] (Table 2). Esp has been shown to contribute to the colonization and persistence of some *E. faecalis* strains during ascending urinary tract infection [50]. It also seems to play a role in mediating primary attachment of enterococci to surfaces and in biofilm formation [51].

5. Secreted virulence factors

Enterococci also secrete molecules that are putative virulence factors. For example, cytolysin/hemolysin is a bacterial toxin that is encoded by an operon consisting of eight genes [52–56] localized on a pheromone-responsive plasmid [8] or on the chromosome [57,58]. Cytolysin shows hemolytic (against human, horse, and rabbit erythrocytes) and bacteriocidal activity against other Gram-positive bacteria [34]. It is thought to play an important role in human infections, in which it is produced in 11–70% of strains [34–37,59–63], compared to 0–25% in stool isolates [34–37] (Table 2). Cytolysin also contributes to enterococcal virulence in all animal models [3,32,64–66] and a *C. elegans* model studied [67]. It has recently been shown to be regulated by a quorum-sensing mechanism involving a two-component regulatory system [55].

Gelatinase (GelE) is an extracellular zinc metallo-endopeptidase secreted by *E. faecalis* that shares homologies with gelatinase of *Bacillus* species and *P. aeruginosa* elastase [34]. It is co-transcribed with the serine protease SprE and regulated by the quorum-sensing *fsr* locus, which shows homology to the *S. aureus agr* locus and is expressed in late exponential phase at high cell densities [68–71]. GelE can hydrolyze gelatin, casein, hemoglobin, and other bioactive peptides, which provides clues for its potential role as a virulence factor in enterococci [72,73]. Gelatinase can also cleave sex pheromones, which are known to be potent chemo-attractants [74], and might therefore modulate the host response [75]. It might also play an important role in the severity of systemic disease, as shown in several independent animal studies [32,76–80]. GelE was also shown to be enriched in clinical isolates in some studies (55–100% in clinical isolates versus 27–66% in stool isolates from healthy volunteers [34,36,59,60]), but contradicting observations have also been reported [35] (Table 2). Further investigations are needed to explore possible therapeutic uses for the above-mentioned enterococcal virulence mechanisms.

Burnie et al. [81] examined sera of patients with enterococcal infections to identify enterococcal antigens that might be associated with protective antibodies. They identified an immunodominant ABC transporter complex that was recognized by antibodies from patients. Antibodies raised against parts of this complex conferred protection to mice in a systemic infection model. ATP-binding cassette (ABC) transporter proteins are cell membrane-associated ex- and import systems that transport a variety of molecules, including nutrients and drugs [82–84]. They have also been associated with polysaccharide biosynthesis in *E. faecalis* [85]. ABC transporters have been implicated as virulence factors in staphylococcal infections in several studies [86–88] and as immunodominant antigens in infections due to *E. faecalis* [89] and *S. aureus* [90]. MsrC from *Enterococcus faecium*, another ABC transporter, which is homologous to MsrA of *S. aureus*, is associated with macrolide resistance [91,92]. ABC transporters share highly conserved sequences and therefore seem to be promising targets for the development of protective antibodies.

6. Translocation

Enterococci possess the ability to translocate from the intestinal lumen to mesenteric lymph nodes, the liver, and the spleen [93–96]. However, the mechanisms responsible have not been fully elucidated. Enterococci are thought to be phagocytosed by tissue macrophages or intestinal epithelial cells and transported across the intestinal wall into the lymphatic system [75]. Olmsted et al. [29] showed that internalization of enterococci by cultured intestinal cells is significantly increased in the presence of AS, although this is most likely only one of several factors that control internalization efficiency. No study to date has been able to suggest any therapeutic approaches to prevent infection at this level of interaction between host and enterococci.

7. Host response against enterococcal infections

Surprisingly little is known about host defense mechanisms against enterococcal infections, and only a few studies have attempted to investigate this area systematically. In order to survive in the host, enterococci must successfully avoid specific and non-specific host defense mechanisms. Most Gram-positive pathogens possess factors such as anti-phagocytic polysaccharide capsules, surface proteins such as the M-protein of GAS, or toxins to ensure survival in the host. After translocation or introduction into the bloodstream, enterococci are susceptible to neutrophil-mediated killing carried out mainly by complement and opsonizing antibodies [97–100]. Certain strains of enterococci have also been shown to be capable of surviving within phagocytic cells [27,31,101,102], which might serve as vehicles for enterococci to translocate across the intestinal wall and disseminate into distant organs. The failure of phagocytic cells to kill intracellular enterococci might lead to systemic spread [93]. Whether phagocytosis of enterococci represents a successful host defense mechanism or a means of immune response evasion for enterococci remains to be demonstrated.

Arduino et al. [99,100] studied the resistance of *E. faecium* to neutrophil-mediated phagocytosis using a fluorescence microscopic ingestion assay. While all *E. faecalis* strains studied were internalized, only 50% of the *E. faecium* strains were phagocytosed. Exposure to pronase, trypsin, or phospholipase C did not affect the bacterium's resistance to phagocytosis, while treatment with periodate eliminated the resistance to phagocytosis.

The authors concluded that a carbohydrate structure was responsible for the resistance to phagocytic killing, although they did not isolate or chemically characterize a specific factor. By electron microscopy, they identified

small electron-dense clumps in *E. faecium* as well as in *E. faecalis* that may be consistent with capsular material [99].

8. Enterococcal polysaccharides

Little is known about capsular polysaccharides in enterococci or their roles in colonization or persistence. Since 1935, there have been reports on serological typing systems for enterococci (formerly group D streptococci). Initially 31 subtypes of "enterococci" were described [103]. However, the main goal of these studies was the epidemiological investigation of outbreaks rather than the taxonomic classification of isolates. Only crude extracts of bacteria were used to prepare immunizing suspensions. The streptococcal group D antigen is expressed by most enterococci. Unlike the cell-wall carbohydrates characterizing the serogroup A to C antigens, the group D antigen is a glycero-phosphate polymer [104]. Lancefield recognized additional cell-wall or surface carbohydrates and referred to these as type-specific antigens [105]. These antigens were considered to be the structural and chemical counterparts of the group-specific substances in streptococci groups A, B, C, E, F, and G. Type-specific enterococcal antigens contain glucosamine, rhamnose, and glucose [106]. Bleiweis et al. [107] attempted an analysis of the chemical composition of the type antigen from *E. faecalis* type 1. By extraction with lysozyme, they identified material that consisted of 22.5% rhamnose, 11.9% hexosamine, 14.4% glucose, 4.2% muramic acid, 11.7% alanine, 5.5% glutamic acid, and 5.8% lysine. They suggested that the type 1 antigen contained a rhamnose polymer covalently linked to a second moiety, a ribitol phosphate [108].

In 1964, Sharpe [109] proposed a typing system for *Streptococcus faecalis* based on cell-wall type antigens that included 11 serogroups. Her antigen preparations were unaffected by trypsin but were inactivated by periodate [109]. However, no systematic seroepidemiologic study reported to date has used the above-mentioned system. In 1992, Maekawa et al. [110] proposed a new serotyping system for *E. faecalis* that included nine of Sharpe's type strains. It distinguished a total of 21 serotypes, with four types being responsible for 72% of the typeable strains [110,111]. However, this system used formalin-killed bacteria to immunize rabbits instead of chemically defined antigen preparations (i.e. polysaccharide antigens) to produce typing sera. This serotyping system is therefore not based on defined antigenic structures such as capsules or other cell-wall antigens. In recent years, a number of studies have focused on polysaccharide antigens in enterococci [85,89,112]. By expressing chromosomal DNA fragments in *Escherichia coli*, Xu et al. [112] were able to identify clones that produced an antigen detectable by convalescent human sera. However, they were not able to isolate this material from the parent strain, and thus its structure remains unknown. The fact that two of the polysaccharide genes are a putative glycosyl transferase and a putative rhamnose biosynthesis gene indicate that this locus may be responsible for the synthesis of the enterococcal type antigen described by Lancefield and others. Insertional mutants of these two genes were shown to have diminished virulence in a mouse peritonitis model [112]. Hancock et al. [113] identified a serotype-specific cell-wall polysaccharide biosynthetic operon. This operon consists of 11 ORFs, and mutants with insertions into certain of these genes lacked a high-molecular weight antigen. One of the created mutants, HG101, with insertion in the *cps*I gene, was more readily cleared from a subcutaneous infection model and was found to be more susceptible to human neutrophil-mediated killing in an opsonophagocytosis assay compared to the wild-type FA2-2. Genetic evidence and preliminary carbohydrate analysis indicated a teichoic acid-like surface molecule consisting of glycerol phosphate, glucose, and galactose. Although some phenotypic effects have been observed in the mutants described above [112,113], it cannot be concluded from these studies that the antigens are indeed present on the surface of enterococci. It has not been shown for either of the polysaccharides that antibodies directed against these structures are protective.

9. Vaccine potential of enterococcal antigens

Data from our laboratory showed that about 57% of pathogenic enterococci (90 out of 157 strains) possess a capsule and that the capsule may be used to immunize animals as well as protect them against systemic infection [114,115]. A high-molecular weight polysaccharide fraction isolated from strain *E. faecalis 12030* inhibited opsonic killing activity of immune rabbit sera raised against both *E. faecalis* and *E. faecium* strains. The crude antigen could be divided into two distinct polysaccharide fractions by ion-exchange chromatography, and analysis of these purified materials by NMR spectroscopy indicated that the first peak consisted of four distinct monosaccharides (see Fig. 1). This first fraction most likely contained amino sugars and deoxyhexoses and is probably identical with the type-specific antigen. The second polysaccharide consisted of a glycerol-teichoic acid-like molecule with a backbone structure of –6 a-D-glucose-1-2-glycerol-3-PO_4 substituted on carbon two of the glucose molecule with a 2-1-linked molecule of D-glucose (Fig. 2) [114]. Immunoblot and ELISA experiments indicated that the immuno-reactivity of the immune rabbit sera was directed against the second polysaccharide. Rabbits immunized with the purified glycerol/glucose polymer material developed specific high-titer antibodies that mediated bacterial killing in an opsonophagocytic assay. This killing activity could be abolished by absorption of the immune rabbit sera with the purified polymer. However, pretreatment of this polysaccharide with Na-periodate prior to absorption rendered the polysaccharide unable to affect killing activity. Immune-electron microscopy studies clearly indicate that those polysaccharide-specific antibodies have

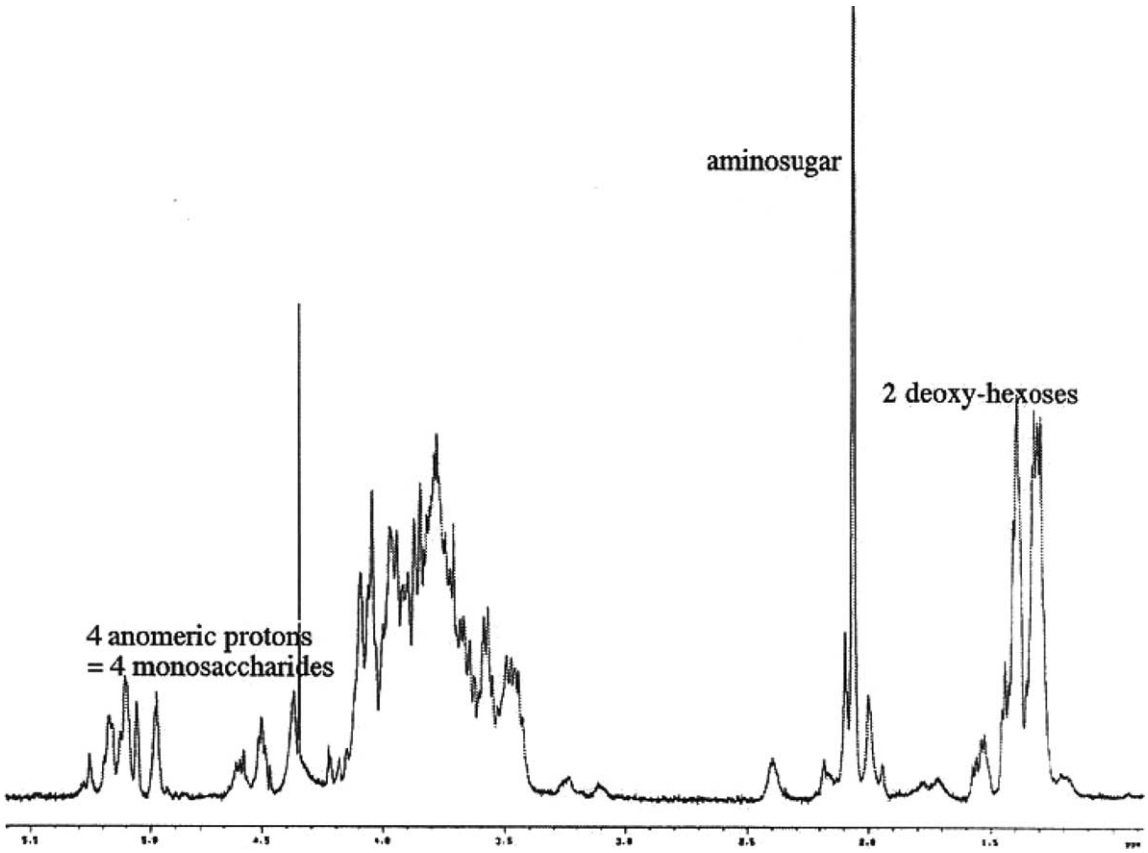

Fig. 1. NMR spectroscopy of the putative-type antigen from *E. faecalis* 12030.

a capsule-like structure (see Fig. 3) [116]. Evaluation of protective efficacy was carried out in mice that were intravenously (i.v.) challenged with live enterococci [115]. In non-immune mice, i.v. inoculations resulted in high bacterial levels in kidney, spleen, and liver 5 days after challenge. Mice immunized with four 10 μg doses of capsular polysaccharide (CP) antigen were protected against challenge with the homologous *E. faecalis* strain. Opsonic IgGs were induced in high titers by immunizing rabbits with the purified CP, and passive transfer of this antiserum to mice produced significantly lower bacterial counts in organs than did normal rabbit serum or sterile saline. Antibodies to the polysaccharide isolated from *E. faecalis* strain 12030 were protective against another *E. faecalis* strain and against two serologically related, vancomycin-resistant clinical *E. faecium* isolates. Antibodies to this CP antigen were also effective as a therapeutic reagent in mice when passive therapy was initiated up to 4 days after challenge with live bacteria [115].

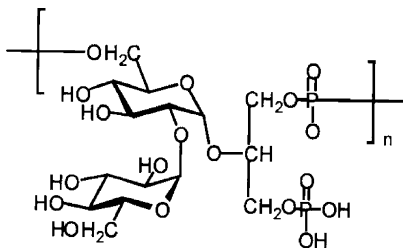

Fig. 2. Chemical structure of the capsular teichoic acid from *E. faecalis* 12030.

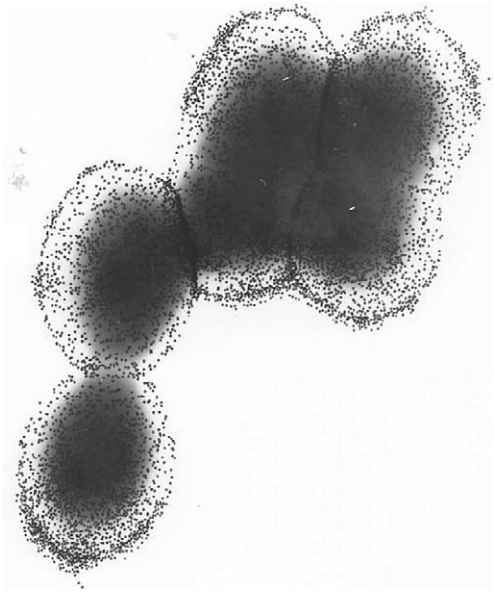

Fig. 3. Immune electron microscopy of *E. faecalis* 12030 with immunogold-labeled rabbit sera raised against the purified capsular polysaccharide.

10. Other potential vaccine candidates

So far only the ABC transporters and the CP described above have been studied as targets of therapeutic antibodies in an appropriate animal model [81]. However, all of the above-mentioned putative virulence factors could theoretically be used as vaccine targets. A recombinant aggregation substance has been used to immunize rabbits, and the application of these hyperimmune sera protected mice against weight loss and kidney infections in a bacteremia model (Krueger, manuscript in preparation). Protective antibodies directed against surface proteins have been studied in a number of bacteria, and the possibility of conjugating a capsular polysaccharide to one of these proteins would provide targets against two different pathophysiologic mechanisms included in the same vaccine [117,118]. Further studies to evaluate these possibilities are necessary.

11. Possible usage of an enterococcal vaccine

The development of an enterococcal vaccine to prevent and/or treat systemic infections depends on a number of factors, but must take into account the patient populations most likely to be at risk for infections due to enterococci. A number of recent studies established specific risk factors in well-defined patient populations [119–127], and the prevention of infections in high-risk patients could lead to reduced mortality and reduced hospital stay, making the cost–benefit favorable for this possibly very expensive treatment. Passive immunotherapy using hyperimmunoglobulins would be the therapy of choice, since most patients at risk are likely to need protection for only a limited period (i.e. several weeks), and in most instances there would not be sufficient time to actively immunize these patients in advance. Passive immunotherapy has been used in the prevention and treatment of a number of bacterial and viral diseases [128]. The generation of antibodies with new technologies such as phage display and the genetic manipulation of mammals that express human antibody molecules are promising techniques to explore in the future. Highly specific monoclonal antibodies [129] directed against enterococcal antigens could be a useful addition and/or alternative for the prevention and/or treatment of enterococcal infections in susceptible patients.

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