

Expert Opinion

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General

Treatment and prevention of enterococcal infections – alternative and experimental approaches

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Enterococci are one of the leading organisms isolated from infections of hospitalised patients and the third most common cause of nosocomial bloodstream infections. They contribute significantly to patient mortality and morbidity as well as healthcare costs. The emergence of resistance against virtually all clinically available antibiotics and the ability to transfer these resistance determinants to other pathogens demonstrates the urgency for an improved understanding of enterococcal virulence mechanisms and the development of alternative treatment and prevention options. This article reviews new antimicrobials, vaccine targets, bacteriophage therapy, as well as treatments targeting virulence factors and biofilm, for their potential to treat and/or prevent enterococcal infections. Although clinical isolates often cause serious infections, so-called 'non-pathogenic' strains are used as therapeutics in the form of probiotics. Understanding the differences between true pathogens and beneficial commensals may help to evaluate future treatment and prophylactic options.

Keywords: bacteriophage therapy, biofilm, enterococci, probiotics, therapeutic antibodies, vaccine, virulence factors

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1. Introduction

Enterococci are Gram-positive bacteria that inhabit the gastrointestinal (GI) and female genital tract of humans and many animals. They are found in soil, plants and water, as well as in food products, including fermented milk products, cheeses and sausages [1,2]. Enterococci are thought to be relatively avirulent bacteria that live as commensals and make up a significant proportion of the normal gut flora of healthy humans and animals (10^8 colony-forming units [cfu]/g faeces) [3]. Within the last few decades, however, infectious diseases caused by enterococci, especially urinary tract, surgical wound and intra-abdominal infections, as well as endocarditis, bacteraemia and meningitis, have increased in number and severity. Today, enterococci are the second most common pathogen isolated from nosocomial infections in the US [4] and contribute significantly to patient mortality, prolonged hospital stay [5] and healthcare costs. *Enterococcus faecalis* accounts for ~ 80% of the clinical strains isolated, *Enterococcus faecium* for the majority of the rest [2].

Because of the intrinsic resistance of enterococci and their ability to acquire resistance against almost all available antibiotics, enterococcal infections are an increasing clinical challenge. In addition, enterococci can transfer resistance determinants to other more virulent bacteria, for example, staphylococci [6], which further increases the pathogenic importance of enterococci.

Several well-defined patient populations, such as cancer patients undergoing chemotherapy, patients with haematological malignancies [7], AIDS patients, postsurgical patients, transplant recipients [8,9], patients receiving dialysis [10,11] and patients in intensive care units [12,13], show an increased risk of serious enterococcal infections. From 1989 to 1999, the percentage of vancomycin-resistant enterococci (VRE) associated with nosocomial infections in intensive care unit patients in the US rose from 0.4 to 25.2% [14]. It has been shown that infections caused by antibiotic-resistant enterococci have a several-fold higher mortality than antibiotic-susceptible strains [15]. Therapeutic options for serious enterococcal infections are limited. We must, therefore, expand our repertoire of available reagents and explore new therapeutic directions.

This review summarises different preventive and therapeutic options against enterococcal infections that may be available in the future. The dual role of enterococci as serious pathogens on the one hand and as therapeutic probiotics on the other is also discussed.

2. Antimicrobial therapy of enterococcal infections

Enterococci are very hardy bacteria that can survive under harsh environmental conditions. They tolerate hypertonic, hypotonic, acidic and alkaline environments. They grow in a wide temperature range (10 – 45°C) as well as under aerobic and anaerobic conditions [16,17].

Enterococci are intrinsically resistant to a number of antibiotics, including cephalosporins, penicillinase-resistant penicillins, co-trimoxazole, clindamycin and low-level aminoglycosides [18].

Enterococci are also particularly capable of acquiring antibiotic resistance. Because of the increase in multiresistant isolates, traditional therapies are increasingly failing and new antibiotics are needed. Recently, VRE and multi-drug resistant (MDR) enterococci have shown resistance to almost all antibiotics available at present, including vancomycin [19–22] and teicoplanin [23,24]. Enterococci often acquire antibiotic resistance through exchange of resistance-encoding genes carried on plasmids, conjugative transposons [3] or pathogenicity islands [25,26].

One major concern is the ability of enterococci to transfer resistance determinants to other more virulent bacteria, such as staphylococci [27]. In 2003, the first fully vancomycin-resistant *Staphylococcus aureus* strain was isolated from a patient who previously had been colonised with VRE. This suggests an *in vivo* exchange of resistance traits [6]. The vancomycin resistance determinant carried by this strain was the *vanA* gene locus, one that frequently confers resistance in *E. faecalis* and *E. faecium*.

Examples of new antibiotics that are clinically available include linezolid, an oxazolidinone, and the streptogramin Synercid®, a combination of the streptogramins quinopristin–dalfopristin. These drugs have been used successfully in the treatment of multiresistant Gram-positive infections, but

resistance in some strains has already been reported [28–34]. NVP PDF-713, a novel peptide deformylase inhibitor, has recently been shown to retain activity against Gram-positive pathogens that are resistant to oxazolidinones and streptogramins. This compound appears to be a promising clinical candidate [35]. Daptomycin, oritavancin and tigecycline are also new antimicrobials that seem to have good activity against VRE isolates and other drug-resistant Gram-positive pathogens [30,36,37]. It is likely, however, that enterococci will rapidly develop resistance against these new antimicrobials as well.

One of the major causes for the development of antibiotic resistance is the overuse and misuse of antibiotics [38]. Restriction of the use of antibiotics and written guidelines can help decrease the selective pressure of resistant strains, but are sometimes difficult to implement [18]. Restriction of antimicrobials as growth promoters in animal husbandry (e.g., avoparcin, which may have played a role in the spread of vancomycin resistance) is equally important [39]. The use of rapid diagnostic techniques, for example, polymerase chain reaction in detection of VRE [40,41], may promote a more focused approach to therapy.

Broad spectrum antibiotic treatment can lead to several complications besides the well-known drug side effects. They not only affect the disease-causing bacteria, but also eliminate components of the normal physiological mucosal microflora. Selective pressure can initiate the development of resistance through otherwise rare mutations or genetic exchange events. The normal resistance of the intestinal tract to colonisation with exogenous organisms (such as MDR enterococci [42] or multiresistant Gram-negative pathogens) is part of the physiological barrier function of the gut. Overgrowth with certain organisms can lead to problems such as pseudomembranous enterocolitis (caused by *Clostridium difficile*) or fungal vaginosis (caused by *Candida* species). It has been shown that the number of VRE in the stool increases several-fold when antibiotics, particularly those with anti-anaerobic activity, are administered to patients or mice [43,44]. This increase in numbers of VRE in the gut is associated with an increased risk of systemic enterococcal infections [45].

3. Vaccination

Vaccination against bacterial pathogens has commonly been used to prevent and treat infectious diseases. Bacterial capsules are well-defined virulence factors that protect the microorganisms against phagocytosis. The capsules consist of polysaccharides and usually elicit a T cell-independent immune response that is characterised by a predominance of IgM antibodies and by the lack of memory B cells. These antigens have frequently been used in the development of effective vaccines.

The majority of clinical strains of enterococci possess a capsule that has been shown to be the target of opsonic antibodies [46]. The structure of one capsular serotype has been elucidated using nuclear magnetic resonance spectroscopy and gas chromatography/mass spectrometry. It has been shown to

consist of a kojibiose that is linked by a 1,2 glycosidic bond to a glucose-glycerol-diphosphate backbone [47]. This antigen is a cell wall-associated teichoic acid, and purified material has been used to immunise animals [48]. Rabbit sera raised against this polysaccharide effectively killed the homologous strain at a dilution of 1:5000 in an opsonophagocytic assay and also killed 28% of a collection of vancomycin-resistant *E. faecium* strains tested [46].

The prophylactic and therapeutic efficacies of antibodies against this antigen were tested in a mouse model of systemic enterococcal infections [48]. Animals were injected with enterococci in the tail vein and sacrificed after 5 days. Colony counts from spleen, kidney and liver were then compared. Active immunisation with purified polysaccharide prior to challenge with bacteria produced a statistically significant reduction with animals immunised with an irrelevant antigen [48]. The application of rabbit sera raised against purified teichoic acid 24 h before, 4 h after and 24 h after challenge led to 3 log – 4 log reduction in colony counts as compared with animals that had received normal rabbit sera [48]. This protective effect was also seen when the animals were challenged with three heterologous strains (one *E. faecalis*, two VRE). A statistically significant reduction in colony counts in all organs was achieved even when the protective serum was administered up to 4 days after bacterial challenge.

So far, the structure of just one enterococcal polysaccharide has been elucidated [47]. The authors recently developed a simple enzyme-linked immunosorbent assay procedure to serotype enterococcal isolates and have identified four different serogroups using rabbit antisera raised against these antigens. Of 29 clinical and laboratory strains tested, 55% were assigned to one of these serogroups [49]. The development of a broadly reactive enterococcal vaccine based on a limited number of structurally diverse capsular polysaccharides therefore seems feasible.

Two groups have described genetic loci putatively responsible for carbohydrate antigen production. Xu *et al.* identified a polysaccharide biosynthesis gene cluster in *E. faecalis* and developed insertional mutants with diminished virulence in a mouse peritonitis model [50]. Hancock and Gilmore found a different locus containing a cell wall polysaccharide biosynthetic operon in *E. faecalis*. They also created an insertion mutant, which was found to be more susceptible to human neutrophil-mediated killing [51]. Neither group was able to isolate enough polysaccharide material from their prototype strains for structural analysis. It is not clear which of these loci is responsible for the production of the capsular polysaccharide the authors have described. However, preliminary results indicate that the locus described by Hancock and Gilmore may be associated with the seroreactivity the authors observed, as restriction-length polymorphism in this locus can be used to distinguish two major groups among the four different serotypes characterised so far [49]. The role and the exact cellular location of the polysaccharide antigen described by Xu is not clear. Hancock and Gilmore suggest that this antigen is

rather small (molecular weight of 50kDa) and may be hidden inside the cell wall. The preliminary compositional analysis indicates that this material consists of four sugar molecules, one of which appears to be a deoxysugar (such as rhamnose or fucose). This material is not immunogenic when injected into mice or rabbits, but could probably be used as a vaccine target when conjugated to a protein antigen.

Protective antibodies directed against surface proteins have been studied in a number of bacteria [52-54]. These antigens, either alone or conjugated to polysaccharide antigens, may provide alternative mechanisms in the treatment and prevention of enterococcal infections.

Burnie and colleagues raised antibodies against an immunodominant ATP-binding cassette (ABC) transporter complex. Sera from patients with enterococcal infections were used to identify this antigen by immunoblotting. The patients showed an immunodominant cluster of antigens at 34, 54 and 97 kDa. Survival was significantly correlated with reaction to the 34- and 97-kDa bands. Antibodies directed against these bands provided protection to mice in a systemic infection model [55]. ABC transporters share highly conserved sequences, and antibodies against similar proteins have been observed in convalescent sera from patients with systemic enterococcal infections [55].

A number of recent studies have identified potential vaccine targets in different Gram-positive bacteria. So far, there are no comparable studies in enterococci, but as some of these proteins are found in enterococci as well, they may also be useful in preventing and/or treating enterococcal infections. Immunisation with FimA, a surface-associated protein of *Streptococcus parasanguis*, has been shown to confer protection against the development of endocarditis in rats [54]. Enterococci also possess FimA-like proteins that are reactive with polyclonal anti-FimA serum. This creates hope for an endocarditis vaccine candidate. Eighty one per cent of the isolates from bacteraemia patients expressed proteins co-migrating with FimA that reacted with anti-FimA serum. However, no animal protection studies with enterococci have been performed so far [54]. Penicillin-binding proteins (PBPs) are another class of possible vaccine targets. A DNA-vaccine raised against PBP2a, a PBP from methicillin-resistant *S. aureus* (MRSA), has recently been shown to be effective against sublethal doses of MRSA in an animal model [56]. Altered PBP5 is present in high-level ampicillin-resistant enterococci [57] and may also be a useful vaccine candidate, although no animal studies have yet been carried out.

4. Bacteriophage therapy

Bacteriophages (or phages) are bacterial viruses that can infect and destroy bacteria. They were described as early as the beginning of the 19th century and have a long history as therapeutic agents in Eastern Europe and the former Soviet Union (reviewed in [58]). A combination of several factors, including the publications of phage research mainly in non-English

journals, the failure of many studies to meet the existing rigorous standards for clinical trials, and the overwhelming success of antibiotics introduced in the 1940s, combined to produce the near abandonment of bacteriophage therapies in the Western world. The desperate need for alternatives to antibiotics, in addition to marked success in utilising phage therapy in multiple studies in Eastern Europe and the former Soviet Union over several decades, has sparked renewed interest in bacteriophage therapy.

It is possible to generate phages with lytic activity against specific bacterial strains. They have been used successfully to treat infections caused by a variety of pathogens in humans [58] and animal models [59-62]. However, studies focusing on enterococcal infections have been limited. One human study of phage therapy performed by Sakandelidze and colleagues reported clinical improvement in the treatment of infectious allergoses caused by a number of pathogens, including enterococci. The success in the phage-treated group was superior to that in the group treated with antibiotics (clinical improvement of 86 versus 48%) [63]. In addition, researchers from the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia have reported an 80% success rate in the treatment of enterococcal infections (reported in [64], no details mentioned). Recently, a promising study reported successful treatment of VRE infection in a murine model [65]: Biswas *et al.* demonstrated 100% rescue of mice infected with a lethal dose of vancomycin-resistant *E. faecium* by using a single bacteriophage injection directed against VRE 45 min after bacterial challenge. When the bacteriophage injection was delayed 25 h after challenge with VRE, 50% of mice were still rescued. Blood bacterial titres in the mice treated with phage therapy were 200-fold lower in comparison to PBS-treated control mice 20 h after initiation of the experiment. Given these encouraging preliminary results, additional experiments examining the bacteriophage treatment of enterococcal infections are highly warranted. However, some experts have pointed out that resistance development is very frequent and bacteriophage therapy may soon become obsolete.

Theoretically, bacteriophage therapy possesses several advantages over antibiotic therapy (for comparison of advantages and disadvantages of treatment options see Table 1). For example, bacteriophages target pathogens more specifically, avoiding disruption of the physiological microflora and selection for resistant bacterial strains. They can replicate at the site of infection and parallel the kinetic behaviour of their target, requiring less frequent administration and avoiding the danger of insufficient tissue penetration. Phages can be developed in the event that the pathogen becomes resistant, theoretically making them able to defeat every bacterium. However, the production, as well as the safety controls, of a new phage (as with any therapeutic regimen) is a long process.

In addition, possible disadvantages of bacteriophage therapy should not be underestimated. These include: the necessity of identifying the source of infection before phage therapy

can be started; possible side effects due to insufficiently purified phage solutions; an inappropriately narrow host range resulting in treatment failure; the risk of anaphylactic reactions; and the development of phage-neutralising antibody. Nevertheless, bacteriophage therapy offers exciting potential in the treatment of severe infections for which antibiotics are no longer effective.

5. Protection against disease conferred by probiotics

The organisms comprising the flora of the human GI tract are extremely diverse and complex [66], including as many as 400 different species. They play a crucial role in the health of the host by protecting against disease and by helping to maintain a functional mucosal barrier. The physiological GI flora also modulate both innate and acquired immunity at local and systemic levels [67,68]. When this precarious balance between the host and the microflora is disrupted, diseases can emerge. Specific microorganisms, so-called probiotics, comprising mainly lactic acid bacteria, have been repeatedly shown to exert beneficial influences on host health and physiology [69,70]. The scientific concept of probiotic therapy is almost a century old, but controlled studies of the mechanisms behind these effects are still sparse. Probiotic bacterial preparations are widely used in livestock nutrition, and may be useful against a variety of human pathogens as well.

Various enterococcal strains have been used as human and animal probiotics [1,70,71]. The commercially available probiotic Vitacanis®, containing *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and an *E. faecium*, was developed to treat intestinal disorders in cats and dogs, and was shown to confer protection against *Salmonella enterica* subspecies *enterica* serovar *Typhimurium* in a mice model. It is of interest that, of the three microorganisms in this preparation, only *E. faecium* provided sufficient protection and increased survival in the challenged mice, while *L. acidophilus* increased the survival time only and *S. cerevisiae* showed no effect [72]. The mechanisms by which probiotics enhance GI defence of the host and mediate protection are not fully understood and have sparked considerable interest in recent years (reviewed in [73-75]). Several studies in animals have demonstrated the beneficial effect of various probiotic preparations containing enterococci on colonisation with potentially dangerous bacteria (*Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, *Clostridium* spp.) [76-79].

Benyacoub and colleagues examined the effect of a probiotic *E. faecium* strain (SF68) on young dogs [71]. They found that SF68 had an overall beneficial effect on specific immune functions of the test group, that is, increasing their faecal IgA and circulating IgG and IgA levels specific for the canine distemper virus vaccine, as well as the proportion of mature B cells. This adjuvant effect of an enterococcal probiotic on the immune system implies new opportunities for the utilisation of enterococci in probiotic nutrition [71] and clearly

Table 1. Advantages and disadvantages of possible treatment options against enterococcal infections.

Treatment option	Advantages	Disadvantages
Antibiotics	very effective, immediate effects, large spectrum available and approved (although choices declining due to rapid resistance development in enterococci), well-known and tested for decades	side effects, resistance development, nonspecific and affecting physiological microflora
Infection control procedures	effective prophylaxis, prevention of spread	only prophylactic, difficulties with compliance
Decolonisation	decreases risk of infection prophylactically, prevention of spread of multiresistant strains	often not very effective, affects physiological microflora, only preventive effect, development of resistance
Vaccination		
Capsular polysaccharide	promising results in animal model, successfully used against many other pathogens, passive immunisation can probably be used prophylactically as well as to fight infection	active immunisation in patients most likely not feasible, side effects in humans unknown, influence on physiological flora unknown, structures of most strains still unknown
ABC transporter complex	successful in animal model, highly conserved sequences	same as above
FimA-like proteins	anti-FimA serum shown to protect against streptococcal endocarditis in rats, FimA-like proteins of enterococci react with anti-FimA sera	no studies in enterococcal infections available
Penicillin-binding proteins	preliminary success of PBP2a in animal model against MRSA infection, PBPs also present in enterococci, targets multiresistant organisms while sparing physiological microflora	no studies in enterococcal infections available
Bacteriophage therapy	very specific, avoiding disruption of physiological microflora and resistance development, works specifically at site of infection, producible against virtually each bacterium	limited experience, identification of causative pathogen before administration of therapy necessary, very narrow target, risk of treatment failure, risk of anaphylactic reactions, development of neutralising phage antibodies, side effects due to phage preparation procedures
Antivirulence factor therapy	would specifically target disease-causing mechanism	too little known about specific mechanisms in enterococci
Antibodies against AS	can theoretically reduce endocarditis risk	no effect in animal model
Anticytolysin therapy	approachable regulatory pathway	purely speculative at this point
Antibiofilm mechanisms		
Biofilm production (e.g., RIP in staphylococci)	possibly assist in treatment of severe infections in hospitalised patients	too little known about similar mechanisms in enterococci
Surface design of medical devices	prevention of colonisation	associated toxicities effects on enterococci unknown
Antibiotic-lock techniques	can potentially rescue medical device, high antibiotic doses without systemic effect on patient available	has no systemic effect in infection

ABC: ATP-binding cassette; AS: Aggregation substance; MRSA: Methicillin-resistant *Staphylococcus aureus*; PBP: Penicillin-binding protein; RIP: RNAlII inhibiting peptide.

deserves further investigation. Oral applications of enterococcal preparations have been shown in humans to reduce the number of relapses in chronic sinusitis [80], and an increase in circulating IgG titres has been seen after 3 weeks of oral application of 10^7 cfu of *E. faecalis* [81].

Our growing awareness of the role of enterococci in diseases has raised many questions regarding their safety for use in foods and as probiotics [1,2,82]. It has been recommended that every strain used as a food additive be carefully evaluated for virulence factors and antibiotic resistance [70,83,84]. Some have suggested that enterococcal strains used as starter cultures and probiotics should be completely devoid of all known or putative virulence traits and antibiotic resistance genes [70]. Another method determining the virulence and the potential utility of probiotic strains is an opsonophagocytic assay [85]. The resistance to opsonophagocytic killing of bacteria indicates the likeliness of survival in the bloodstream and the ability to cause serious disease in the host, and promises to be a useful additional tool in evaluating probiotic strains.

The administration of avirulent and harmless probiotic strains of enterococci might be beneficial in out-competing more virulent strains, or possibly VRE, with which a patient at risk might be colonised, thus decreasing the risk of a serious VRE infection. Enterococci in food have also been shown to inhibit potentially pathogenic bacteria, such as *Listeria monocytogenes*, *Vibrio cholerae*, *Bacillus* spp., *S. aureus* and *Clostridia*, through the production of bacteriocins [86]. However, it should be emphasised that even 'harmless' probiotic strains may cause life-threatening disease, especially in immunocompromised patients, and administration of such strains must be approached with caution [2]. Furthermore, the possibility of genetic exchange between food or probiotic isolates and medical isolates has been documented [87].

6. Pathophysiology of enterococci

The sequence of an enterococcal infection is thought to be initiated either by the colonisation of the GI tract with a virulent strain or by transformation of a previous avirulent strain into a more aggressive isolate, which can then persist for long periods and serve as a reservoir for transmission [39,88,89]. This event is followed by translocation of enterococci through the mucosal barrier, which may be disrupted by iatrogenic measures (such as immunosuppression, chemotherapy or antibiotic therapy). The bacteria are subsequently disseminated throughout the body, ultimately resulting in a systemic infection [2].

Enterococci can be disseminated on healthcare workers' hands [90], medical instruments [91] and via environmental contamination [92,93]. Infection control procedures, such as isolation precautions (as recommended by the Hospital Infection Control Practices Advisory Committee, HICPAC [94]), hand washing and education about the transmission of VRE, should be used to control possible reservoirs of VRE in the hospital environment [18].

GI colonisation with VRE has been associated with subsequent VRE infections. One proposed method to decrease this risk is the decolonisation of patients using oral and/or systemic antibiotics. The rationale is that this procedure would decrease both patient susceptibility and the potential to spread VRE to the environment, other carriers or other patients. Several antimicrobials, including bacitracin, gentamicin, tetracycline, doxycycline, novobiocin and rifampin, have been used in such a decolonisation scheme, with varying success [18]. Ramoplanin is a new antimicrobial that can be administered orally, is not absorbed systemically, and can reduce VRE colonisation to undetectable levels [95]. Therefore, it appears to be a promising candidate and is undergoing Phase III clinical trials at present [18].

Only a small percentage of colonised patients will develop a serious systemic enterococcal infection. However, in certain specific clinical situations, VRE-colonised patients are at increased risk for developing an infection. For liver transplant patients, the risk has been shown to be 11.3%, and for paediatric oncology patients, 8.2% [96,97]. This underscores the importance of active surveillance in high-risk patient groups to prevent transmission and outbreaks [39].

Little is known about the transition of enterococci from 'harmless' commensals to virulent pathogens. Several authors have suggested a number of possible enterococcal virulence factors (see review [98]). In most studies, clinical enterococcal isolates have been shown to possess a higher virulence potential than food, commensal or environmental strains (reviewed in [84,99]).

A number of these putative virulence factors are thought to be involved in adhesion and colonisation, including aggregation substance (AS), extracellular surface protein (Esp), adhesin of collagen (Ace) and *E. faecalis* adhesin (EfaA). These factors are considered prerequisites for the establishment of colonisation of the GI tract and other epithelia and for causing infections such as endocarditis or urinary tract infections. Most studies show that they are more commonly found in clinical isolates than in food or commensal strains [99].

AS is one factor that has been extensively studied [98]. It mediates adhesion of enterococci to intestinal epithelium [100] and other human cells [101-103], probably through interaction with mucosal fibronectin [104]. AS augments internalisation [100,104-106] and translocation [107], and also seems to play an important role in enterococcal endocarditis [108,109]. Antibodies targeted against the N-terminal domain of AS may offer a promising new therapeutic tool for vaccine development to prevent enterococcal endocarditis, although they have not conferred protection in a rabbit endocarditis model [110].

The ability to form a biofilm, a community of microbes embedded in an organic polymer matrix adhering to a surface [111], is another factor in conferring adhesion or colonisation. The colonisation of the GI tract may be dependent on biofilm formation as well, which allows bacteria to persist in the gut despite constant peristalsis. This hypothesis is

supported by the increased ability of a biofilm-producing *E. faecalis* strain to colonise the GI tract of mice, as compared with its biofilm-reduced mutant (manuscript in preparation).

Biofilm production is recognised as a virulence factor in a number of pathogens. Several authors have described the ability of enterococci to produce biofilm [112-117]. Many bacteria, including enterococci, can colonise and form biofilms on foreign bodies and medical devices such as indwelling catheters, respiratory tubes, artificial heart valves or synthetic joints. Bacteria in a mature biofilm are significantly more resistant to antibiotics or antiseptics than their planktonic counterparts. Patients with foreign material implanted or inserted into their bodies frequently develop infections and bacteraemia resulting from seeding of bacteria from the colonised material. These foreign body infections often require removal of the device, systemic antibiotic therapy, or both.

Nosocomial enterococcal bloodstream infections are often associated with medical devices, and intravascular catheter-related *E. faecalis* strains produce significantly more biofilm than isolates from other origins [118]. A biofilm-negative transposon-mutant of a strong biofilm-producing *E. faecalis* wild-type strain demonstrated significantly reduced virulence in a mouse bacteraemia model [119]. This indicates an important role for biofilm formation in enterococcal infections as well.

Several attempts have been made to understand the mechanisms of biofilm formation [73] and to target this mechanism to disrupt biofilm formation *in vivo*. Quorum-sensing mechanisms appear to play an important role in biofilm formation of *S. aureus* and *S. epidermidis*, and a number of recent discoveries have been made that could lead to clinical applications. RNAIII inhibiting peptide (RIP) is a protein that can interfere with the signalling pathways in staphylococcal biofilm formation and toxin production. RIP has been shown to inhibit biofilm formation and toxin production in several animal models [120-122]. In combination with antibiotics, it leads to clearance of staphylococcal infections more effectively than antibiotic treatment alone.

The mechanisms involved in biofilm formation of enterococci are less well-understood than those of staphylococci or *Pseudomonas aeruginosa*, but similar regulators might be identified and successfully used to treat enterococcal infections associated with biofilm formation.

Preventing the formation of biofilms on medical devices is an important goal in controlling nosocomial infections. The design of graft materials resistant to colonisation [123], or the coating of materials with antibiotics, quaternary ammonium compounds, silver ions or iodine, have been tested (summarised in [124]). Attaching the compound poly(4-vinyl-N-alkylpyridinium-bromide) to glass has recently been shown to kill Gram-positive and Gram-negative airborne organisms, and might be useful in the design of medical equipment [124].

Another mechanism for preventing or clearing biofilm formation on catheters is the 'antibiotic-lock technique'. In this technique, highly concentrated antibiotic solutions are instilled into the lumen of the catheter [125-127]. Several studies

are underway at present to identify which antibiotics and doses have the greatest effect on biofilm eradication [128,129] and to determine whether this method can lead to salvage of some infected catheters [126].

Even in healthy immunocompetent animals and humans, enterococci have been shown to occasionally translocate through the GI barrier without causing disease [130]. It is unclear what mechanism enterococci use to translocate and whether this represents an increased infection risk or just a physiological phenomenon. Recently, it was shown that dendritic cells (DCs) in the intestine sample organisms of the intestinal microflora, transport these organisms across the epithelial monolayer and deliver them to lymphoid tissues, where an efficient immune response can take place [131]. This mechanism seems to be of physiological importance, as it might activate the innate immune system to develop adaptive immunity to a challenging pathogen, and as it has been shown to selectively induce IgA production by DCs [132]. On the other hand, certain pathogens, for example, virulent enterococci, might exploit this pathway to translocate across the mucosal barrier and cause infection [131,133]. Whether this mechanism will provide an approach to alter translocation of virulent enteric organisms is speculative and should be investigated further.

It is not known which factors play a role in the dissemination of enterococci and distinguish those that cause infections from those that do not. Several molecules secreted by enterococci have been implicated in enterococcal virulence and promoting dissemination. Cytolysin/haemolysin (Cyl), the protease gelatinase (GelE) and extracellular superoxide production have all been associated with infections (summarised in [99]). Cytolysin possesses haemolytic, tissue damaging and bacteriocidal capacities [134] and is more common in clinical isolates [99]. Its expression is regulated by a quorum-sensing mechanism involving a two-component regulatory system [135]. An extracellular protease activates both subunits of cytolysin and potentially could be inhibited, making this pathway an interesting new therapeutic target [17]. Newly described virulence factors include an *araC*-like regulator, a *gls24* homologue and conjugated bile acid hydrolase (*cbh*) [98]. These are part of an enterococcal pathogenicity island [26] and have been reported to play a role in stress response, carbon metabolism and pathogenesis in a number of bacteria [136,137]. They are significantly more common in clinical isolates than in commensal isolates found in the stool of healthy volunteers [98].

Our understanding of the mechanisms and exact roles of enterococcal virulence factors is still limited, and further investigation of virulence determinants is needed to explore possible targets for the development of alternative or additional therapies.

7. Conclusion

Effective control of infections caused by enterococci, particularly multi-drug-resistant enterococci, is urgently called for.

However, this will require a much deeper understanding of the interactions between the commensal enterococcus and its host, the reasons leading to disruption of this balance, and the mechanisms by which enterococci cause serious disease. Strict guidelines on the use of existing and new antibiotics in human and veterinary medicine, as well as in animal husbandry, are critical in preventing development of further resistance. Infection control and hospital hygiene measures, including isolation of patients with MDR enterococcal infections, effective procedures for washing and disinfecting hands [138], and surface decontamination, play an important role in the control of transmission of MDR strains [17,18,139].

The search for new therapeutics, including vaccines, bacteriophages, antivirulence factors and antibiofilm agents, are crucial, as these approaches may be less subject to resistance development and treatment failure. Enterococci function both as direct therapeutic agents and as probiotics. They also play an important part in the food industry as aromatisers and are used in the production of cheeses and sausages. Although it is likely that pathogenic strains differ greatly from food and probiotic strains, a thorough understanding of these differences may help to establish future prophylactic and treatment options.

8. Expert opinion

Enterococci are among the most significant causes of nosocomial infections. These pathogens will increase in importance because of their unique ability to acquire and retain resistance determinants. No known antimicrobial is yet in sight that can guarantee efficient treatment without rapid development of resistance.

Vaccines will eventually be the predominant alternative approach for treatment and prevention of enterococcal infections in high-risk patient populations. Only a limited number of serotypes seem to exist, making the development of a broadly active polyvalent vaccine a realistic goal. Although it seems clear that enterococci contain several different polysaccharide antigens, the precise role and the cellular location of these carbohydrates remain to be defined.

A number of protein antigens may be promising vaccine targets. AS seems to play an especially important role in the

attachment/adhesion of enterococci to host tissue, but ABC transporters appear to be equally attractive. A combined approach (i.e., the conjugation of these proteins with capsular polysaccharides) may be the ideal solution because it not only improves the immunogenicity of the carbohydrate antigen, but also specifically targets important steps in the pathogenesis of enterococcal infections.

Although phage therapy represents an exciting and completely different approach to the treatment of bacterial infections, many unanswered questions and potential difficulties remain. However, increasing interest in this area and promotion of this approach by government funding are likely to answer many of these questions and will help establish the value of this approach.

The ability of enterococci to form biofilms has recently been demonstrated by a number of researchers. Although the exact role of biofilm in the pathogenesis of enterococcal infections has not yet been elucidated, it seems likely that this property plays an important role in the establishment of enterococcal endocarditis and, potentially, also in the ability of enterococci to colonise the GI tract. Although there are no established therapies to counteract quorum-sensing mechanisms, for example, interest in such compounds is growing because they could be used to prevent biofilm formation and establishment of certain infections. These drugs could be given prophylactically and would help control potentially invasive enterococci by diminishing their potential to colonise.

A better understanding of the GI microflora and its physiological role in establishing and maintaining a competent immune response is of great importance. Recent work has attempted to elucidate the specific role of components of the physiological GI flora and of host cells such as DCs, Paneth cells and M-cells. The mechanism by which these bacteria are sampled and presented to the immune system may be part of the pathway by which enterococci gain access to the bloodstream, thereby causing systemic infections such as bacteraemia and endocarditis. A better understanding of these mechanisms may help prevent systemic spread of enterococci and possibly other GI bacteria as well. Probiotic preparations may be used to colonise the GI tract selectively with strains that have beneficial effects such as inhibition of potential pathogens.

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