Enterococcus faecium infections: where bacterial virulence meets innate immunity
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INTESTINAL ENTEROCoccus FAECIUM
COLONIZATION IMPROVES HOST DEFENSE
DURING POLYMICROBIAL PERITONITIS

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Abstract

Background: Vancomycin resistant *Enterococcus faecium* (VRE) is increasingly found colonizing and infecting hospitalized patients. Enterococci are frequently isolated from polymicrobial infections originating from the intestines. The impact of VRE on these infections and vice versa is not clear. Methods: Mice were intestinally colonized with VRE during oral vancomycin treatment; control mice received oral vancomycin only. Fourteen days after VRE colonization, cecal ligation and puncture (CLP) was performed in all mice to induce polymicrobial peritonitis in the presence or absence of VRE colonization. Results: VRE colonization per se was not associated with systemic dissemination of VRE. CLP resulted in systemic VRE infection in all VRE colonized mice, with high VRE loads in peritoneal fluid, blood, liver and lungs. Forty-eight hours after CLP, VRE infected mice had significantly lower bacterial loads in all organs tested when compared to mice not infected with VRE. Additionally, lower inflammatory parameters were measured in VRE infected mice. CLP-induced transient liver and kidney damage, with a faster recovery in VRE colonized mice. Conclusions: VRE infection, originating from a natural source (the intestinal tract), does not worsen the outcome of CLP-induced polymicrobial peritonitis and sepsis, but rather facilitates bacterial clearance and attenuates host inflammatory responses.
Introduction

Since the first isolation of vancomycin resistant Enterococcus faecium (VRE) in the mid-1980s, enterococci have emerged from being a physiological commensal of the gastrointestinal tract to an important drug-resistant pathogen, with increasing rates of colonization and infection worldwide [1-3]. In the US enterococci currently represent the third leading cause of health care-associated infections of which about 33% are VRE [4]. The origin of infections, including bacteremia, most often is the gastrointestinal tract, colonized by hospital acquired strains of VRE [5, 6]. Enterococci isolated from secondary peritonitis most commonly are part of a mixed flora; the pathogenicity of these bacteria during these infections is still not clear [7-10].

In experimental animal models, synergy between enterococci and other pathogens has been reported. Higher mortality rates and an increased incidence of intra-abdominal abscesses were observed when Enterococcus faecalis was part of the inoculum in a rat model of polymicrobial peritonitis [11-13]. The majority of enterococcal infections are caused by E. faecalis, and consequently most clinical and experimental data concern this Enterococcus species. However, in parallel with the increase in nosocomial enterococcal infections, a partial replacement of E. faecalis by multiresistant E. faecium has occurred in European and US hospitals [14, 15]. To investigate the pathogenicity of VRE during intra-abdominal infection with mixed flora, we used the model of cecal ligation and puncture (CLP) in mice first colonized by VRE. As such we induced a polymicrobial peritonitis and subsequent systemic infection caused by a mixed endogenous microbial flora that did or did not contain VRE. This resembles the clinical situation of patients colonized by hospital strains of E. faecium and subsequent intestinal damage, e.g. postsurgical intestinal leakage [9,16].

Materials and Methods

Mice

Specific pathogen-free 10-wk-old female C57BL/6 mice were purchased from Harlan Sprague-Dawley (Horst, The Netherlands). The Animal Care and Use Committee of the University of Amsterdam approved all experiments.
**Bacterial strain**

VRE strain, E155, was used in all experiments. This clinical isolate from the Cook County Hospital, Chicago, IL, belongs to a genetic subpopulation of hospital-associated *E. faecium*, that is responsible for the worldwide emergence of nosocomial multiresistant *E. faecium*, characterized by high-level quinolone and ampicillin resistance, a pathogenicity island, containing the variant *esp* gene, and the presence of five cell surface protein genes [6, 17]. For all experiments the bacteria were grown overnight on agar sheep blood (BA) plates and then grown for approximately 3.5 hours in Todd-Hewitt (TH) broth (Difco, Detroit, MI) to midlogarithmic phase at 37˚C, while shaking.

**Colonization model**

A mouse model of VRE gastrointestinal colonization was used as described [18]. All mice received oral vancomycin (250 μg/mL) in drinking water during the entire experiment. After 5 days of vancomycin treatment one group of mice received gastric inoculation of $10^7$ CFU VRE suspended in 300 μl TH broth and a control group received 300 μl of sterile TH broth. For quantification of intestinal VRE fresh stool was plated on Slanetz-Bertley (SB) agar plates (Oxoid, Badhoevedorp, The Netherlands), supplemented with vancomycin (6 μg/mL) (SBv). After 14 days of VRE colonization 8 mice per group were sacrificed to check for cecal bacterial flora and VRE numbers, directly prior to induction of CLP. Therefore cecal contents was plated on SBv agar and on BA, MacConkey (McC) (Difco, Detroit, MI) and colistin nalidixic acid (CNA) agar (BD, Breda, The Netherlands) plates for quantification of total aerobic, gram-negative and gram-positive bacteria, respectively.

**Cecal ligation and puncture**

Fourteen days after VRE colonization or inoculation of TH broth CLP was performed as previously described [19]. Two hours after the procedure and every 8 hours thereafter, mice received s.c. imipenem/cilastatin (Tienam; 0.5 mg/0.5 mL, Merck Sharp & Dohme BV, Haarlem, The Netherlands) [20]. Imipenem/cilastatin has no activity against the VRE strain used. Nine mice per group per time point were included and sacrificed 24 and 48 hours after the CLP procedure.

**Preparation of blood samples and homogenates**

Mice were anesthetized by inhalation of isoflurane (Abbot, Laboratories Ltd., Kent, UK)/ $O_2$ (2%/ 2 liter), a peritoneal lavage was performed with 5 ml sterile phosphate-buffered saline using a 18-gauge needle; peritoneal lavage fluid (PLF) was collected...
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in polypropylene tubes (BD, Breda, The Netherlands). Blood was drawn by cardiac puncture and transferred to heparin-gel vacutainer tubes. Liver and lungs, and in control experiments the intestines, were harvested. To correct for the differences in organ weight, four times the weight (in milligrams) in microliters of sterile saline was added. The organs were homogenized at 4°C with a tissue homogenizer (Biospect Products, Bartlesville, UK). For intestinal cytokine measurements homogenates were lysed in 1 volume of lysis buffer (300 mM NaCl, 15 mM Tris, 2 mM mgClH2O, 2 mM Triton X-100, pepstatin A, leupeptin, and aprotinine (20 ng/mL), pH 7.4) on ice for 30 minutes and spun down. Supernatants and plasma were frozen at -20°C until assayed.

**Determination of bacterial outgrowth**

Bacterial numbers were determined in PLF, blood, liver and lung homogenates. Serial 10-fold dilutions were made of each sample of the homogenates, PLF and blood in sterile saline, then 50 μl of each dilution was plated. All organs were plated on BA, McC, CNA, SB and SBv agar plates. The plates were incubated at 37°C under 5% CO₂, and CFU were counted after 20 (BA and McC) or 44 (CNA and SBv) hours.

**Typing bacteria by multiple-locus variable-number tandem repeat analysis**

Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) was used to identify VRE from stool as *E. faecium* strain E155. The MLVA typing was performed as described previously [21].

**Cell counts and differentials**

Total cell numbers were counted from each PLF using a hemocytometer (Beckman Coulter, Fullerton, CA). Differential cell counts were performed on cytospin preparations, stained with a modified Giemsa stain (Diff-Quick; Dade Behring). PLF supernatants were stored at -20°C until determination of cytokines.

**Assays**

Macrophage inflammatory protein (MIP)-2, cytokine-induced neutrophil chemoattractant (KC) and LPS-induced C-X-C chemokine (LIX) were measured in PLF by ELISA’s (R&D Systems, Minneapolis, MN). Tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10 and monocyte chemoattractant protein (MCP)-1 were measured in PLF and plasma by using a cytometric bead array (CBA) multiplex assay (BD Biosciences, San Jose, CA). Serum amyloid A (SAA) was measured by ELISA (Biosource International). All tests were performed according to the manufacturers’ instructions. Complement 3 (C3) was
detected by sandwich ELISA [22]. Aspartate aminotransferase (ASAT) and creatinin were determined with commercially available kits (Sigma-Aldrich, St. Louis, MO), using a Hittachi analyzer (Boehringer Mannheim, Mannheim, Germany).

Organ pathology
Intestinal, liver and lung sections were fixed in 4% buffered formaldehyde, then embedded in paraffin and analyzed by a pathologist as described [19].

Statistical analysis
All data are expressed as mean ± SEM. Differences between groups were calculated by Mann-Whitney U test. For all analysis GraphPad Prism version 4 (GraphPad Software, San Diego, CA) was used. A p-value < 0.05 was considered statistically significant.

Results
Intestinal VRE colonization
Prior to any intervention no VRE could be cultured from fecal pellets. Mice treated with oral vancomycin in combination with oral VRE were successfully colonized with VRE, as reflected by positive VRE cultures from fresh stool during the 14 days of the experiment (data not shown). In accordance, the cecums of these mice were colonized with high VRE loads (Table 1), as tested 14 days after VRE inoculation. These colonies were confirmed E. faecium strain E155 by MLVA typing. VRE could not be cultured from the cecal contents of control mice treated with oral vancomycin and TH broth. The cecums of VRE colonized and control mice contained similar amounts of total aerobic and gram-negative bacteria (shown for aerobic bacteria in Table 1). Systemic dissemination of bacteria was not detected in any of the mice. Furthermore, no histopathological changes of the intestinal epithelial cell lining were observed and no increases in cytokine levels were measured in the intestinal homogenates or in plasma (data not shown).

Table 1. Cecal VRE and total aerobic bacterial outgrowth (per gram of cecal contents).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>VRE counts</th>
<th>Total aerobic count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin and VRE</td>
<td>9.6 ± 1.0 x 10⁸</td>
<td>1.5 ± 0.3 x 10⁹</td>
</tr>
<tr>
<td>Vancomycin and TH</td>
<td>0</td>
<td>1.1 ± 0.2 x 10⁹</td>
</tr>
</tbody>
</table>

Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10⁷ CFU of VRE or sterile TH broth were administered by gastric inoculation 5 days after the initiation of vancomycin treatment. Specimens were obtained 14 days after VRE instillation. Data are means ± SEM of 8 mice per group.
Intestinal colonization with VRE results in systemic VRE infection after CLP

CLP caused systemic VRE infection in all mice colonized with this pathogen (Fig. 1). At 24 hours after CLP high VRE loads were recovered from all body sites examined, i.e. PLF, blood, liver and lungs. VRE loads remained high thereafter, although at 48 hours after CLP the numbers of VRE cultured from PLF and lungs were lower than those cultured from these body sites at 24 hours after CLP. In addition, whereas at 24 hours 8 out of 9 colonized mice had positive blood cultures for VRE, at 48 hours only 4 out of 9 colonized mice had positive blood cultures for VRE. VRE could not be recovered from any body site in control mice subjected to CLP.

Figure 1. Systemic VRE infection after CLP in VRE colonized mice. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 CFU of VRE was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. After 14 days of VRE colonization CLP was performed and mice were sacrificed 24 or 48 hours thereafter. Bars show mean (± SEM) VRE CFU in peritoneal fluid PLF, blood, liver and lung (n = 9 mice per group per time point). Numbers above the bars in panel B indicate blood culture positivity. * p<0.05; ** p<0.01.
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VRE accelerates bacterial clearance after CLP
To determine the influence of VRE on the course of polymicrobial peritonitis, counts of total aerobic, gram-negative and gram-positive bacteria were determined in PLF, blood, liver and lungs harvested from VRE colonized and control mice 24 and 48 hours after CLP (Fig. 2). Twenty-four and 48 hours after CLP all mice had high polymicrobial outgrowth in PLF, blood, liver and lungs. Forty-eight hours after the CLP procedure, mice colonized with VRE had significantly lower total aerobic bacterial loads in all cultured organs when compared with control mice; a trend of this difference was seen after 24 hours (Fig. 2). Similar differences between VRE colonized and control mice were seen with regard to total gram-negative and total gram-positive bacterial loads (data not shown). Furthermore, 48 hours after CLP 2 of the 9 control mice had died, whereas all VRE positive mice were still alive.

VRE inhibits local and systemic inflammatory responses
All mice responded to induction of polymicrobial peritonitis with a strong neutrophil influx into the peritoneal cavity, with highest numbers after 24 hours; no differences were found between VRE colonized or control mice (Fig. 3A). In addition, no differences were found for peritoneal macrophage or lymphocyte numbers (data not shown). The murine CXC chemokines KC, MIP-2 and LIX are known neutrophil attracting and activating mediators. In line with the similar peritoneal neutrophil numbers found after 24 hours in both groups, no differences were found in KC (Fig. 3B), MIP-2 (Fig. 3C) and LIX (Fig. 3D) levels at this time point. Interestingly, 48 hours after CLP comparable peritoneal neutrophil numbers were counted in both groups, yet highly significantly reduced peritoneal levels of KC, MIP-2 and LIX were measured in mice with VRE positive polymicrobial peritonitis (p < 0.01-0.001 versus control mice). Analogous to the chemokine levels, peritoneal and plasma levels of TNF-α, IL-6, IL-10 and MCP-1 were comparable in both groups after 24 hours, but significantly reduced levels of TNF-α, IL-6, and MCP-1 were found in mice with VRE positive peritonitis in both PLF and in plasma after 48 hours (Fig. 4). IL-10 levels were reduced in plasma, but not significantly in PLF. The diminished inflammatory response was further illustrated by reduced levels of plasma acute phase proteins C3 and SAA, 48 hours after CLP in VRE positive mice (Fig. 5).
Figure 2. Increased clearance of polymicrobial infection after CLP in VRE colonized mice. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 CFU of VRE (black bars) or sterile TH broth (white bars) was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed and all mice were treated with imipenem/cilastatin. Mice were sacrificed 24 or 48h after CLP. Total aerobic bacterial load was determined in PLF, blood, liver and lung. Data are means ± SEM, n = 7-9 mice per group per time point. * p< 0.05; ** p< 0.01; *** p< 0.001.
Figure 3. VRE colonized mice have reduced chemokine levels 48h after CLP. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 CFU of VRE (black bars) or sterile TH broth was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed on all mice and mice were sacrificed 24 or 48h thereafter. Peritoneal neutrophils (A) were counted and KC (B), MIP-2 (C) and LIX (D) were measured. Data are means ± SEM of n = 7-9 mice per group per time point. ** p < 0.01; *** p < 0.001.
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Figure 4. VRE colonized mice demonstrate reduced cytokine responses 48h after CLP. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 CFU of VRE (black bars) or sterile TH broth (white bars) was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed on all mice and mice were sacrificed 24 or 48h thereafter. Peritoneal (A-D) and plasma (E-H) levels of TNF-α (A + E), IL-6 (B + F), IL-10 (C + G) and MCP-1 (D + H) were measured. Data are means ± SEM of n = 7-9 mice per group per time point. * p < 0.05; ** p < 0.01; *** p < 0.001.
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VRE infection does not influence CLP-induced organ damage

Consistent with the low mortality in the model of CLP-induced sepsis used here only mild inflammatory changes were seen in liver and lungs upon histopathological examination. The pathology scores did not differ between VRE colonized and control mice at either 24 or 48 hours after CLP (data not shown). CLP was associated with transient hepatocellular injury and renal dysfunction, as reflected by elevated plasma concentrations of ASAT and creatinine, respectively, especially 24 hours after the surgical procedure (Fig. 6). VRE colonized mice displayed less hepatocellular injury at 48 hours after CLP, as indicated by plasma ASAT concentrations that were lower than in control mice ($p < 0.01$).
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Figure 6. Impact of VRE infection on CLP-induced organ damage Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 CFU of VRE (black bars) or sterile TH broth (white bars) was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed on all mice and mice were sacrificed 24 or 48h thereafter. Hepatocellular and kidney damage are shown by plasma ASAT (A) and creatinine (B). Data are means ± SEM of n = 7-9 mice per group per time point. Dotted horizontal lines represent values in healthy mice (n = 5 mice). ** p< 0.01.

Discussion

In this study we show that intestinal colonization with a hospital acquired strain of VRE causes systemic VRE infection after intestinal perforation induced by CLP. Mice colonized with VRE had improved infectious and inflammatory outcomes after CLP: whereas 24 hours after CLP no significant differences were found between VRE colonized and control mice, 48 hours after CLP VRE colonized mice had significantly less polymicrobial outgrowth in all cultured body-compartments. This improved antibacterial defense was accompanied by attenuated peritoneal and plasma inflammatory responses in the VRE colonized mice and a faster recovery of liver damage.

VRE is increasingly found colonizing the intestines of hospitalized patients, especially on intensive care, nephrology, oncology, transplantation and long-stay wards. Intestinal microbes are a major source of systemic infection in immunocompromised, postsurgical and trauma patients [23]. Correspondingly, the increasing prevalence of intestinal colonization by VRE is paralleled by an increase in prevalence of infections by this pathogen [1-3, 5, 24]. Surgical treatment has been associated with the development
of enterococcal bacteremia in prior studies [9]. In one study, 48% of patients with enterococcal bacteremia had undergone recent major surgery or had sustained full-thickness burns or multiple traumatic injuries [25]. Another study showed a two- to four-fold higher frequency of enterococcal infections amongst patients with prior surgical operation of the gastrointestinal, genital or urinary tract compared to patients with nosocomial infections caused by other organisms [26].

Most commonly, enterococci are isolated from polymicrobial intra-abdominal infections originating from the gastrointestinal tract that was previously colonized by hospital strains of enterococci [5-9]. The pathogenicity of enterococci isolated from these polymicrobial infections is controversial. Some authors have suggested that the presence of concurrent enterococcal infection increases the infectious post-operative complication rate, but does not affect the overall mortality [27, 28]. Others showed increased mortality in the presence of enterococci [10, 29]. However, the majority of investigations is inconclusive and addresses the importance of severe underlying illness when enterococci are isolated [8, 30]. In animal studies E. faecalis was shown to increase post-operative morbidity [31, 32]. Experimental data have revealed that E. faecalis can develop a synergistic relationship with other bacteria, leading to abscess formation and inhibition of phagocytosis and killing of other pathogens, with subsequent increased morbidity and mortality [11-13].

Most clinical and experimental data discussed above concern E. faecalis. Since infections with multiresistant E. faecium are emerging, more knowledge on the pathogenesis of infections with specifically this enterococcal species is needed. In the current study we used the well established model of CLP in mice with or without prior intestinal colonization with a VRE strain belonging to the genetic complex that is responsible for most hospital-acquired E. faecium infections. As such, a postoperative polymicrobial peritonitis with endogenous intestinal flora was induced resembling the clinical scenario of a patient with polymicrobial peritonitis with (or without) concurrent VRE infection. Twenty-four hours after the CLP procedure VRE could be isolated from all cultured body sites and 8 out of 9 mice had VRE positive blood cultures, indicating that we successfully caused systemic VRE infection in mice previously colonized with this pathogen. Notably, quantitative VRE cultures 48 hours after CLP showed modest but statistically significant decreases in most organs tested and the number of positive blood cultures for VRE had declined to 4/9. These data suggest that even in the presence of polymicrobial peritonitis treated with an antibiotic (imipenem/cilastin) not active against VRE, VRE does not further grow and disseminate. In this respect it should be noted that healthy mice rapidly clear VRE after intraperitoneal injection [33]. Complete clearance of VRE is unlikely to occur in the model used in the current investigation, considering that the source of
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VRE (the perforated colonized gut) remains present, which contrasts with the situation produced after a single intraperitoneal injection of VRE used in our earlier study [34]. Nonetheless, together these findings indicate that the host, even when compromised such as after CLP, has several defense mechanisms that limit the growth of VRE. Moreover, our study provides insight in the impact of VRE infection on the host response against polymicrobial peritonitis: mice with concurrent VRE infection demonstrated reduced polymicrobial bacterial loads in all body sites tested. Indeed, whereas a trend toward reduced bacterial loads in PLF, blood, liver and lungs was already seen 24 hours after CLP, the differences in bacterial loads between VRE infected and VRE not-infected mice became statistically significant 48 hours after CLP. In parallel, many inflammatory responses were attenuated in VRE infected mice 48 hours after CLP. The attenuated inflammatory response was detected both locally at the site of the primary infection, as illustrated by diminished chemokine and cytokine concentrations in PLF, and systemically, as reflected by lower plasma cytokine and acute phase protein levels. These data suggest that the polymicrobial infection induced by CLP (and not VRE) drives the inflammatory response in this model and that most likely the attenuated inflammatory response in VRE infected mice was caused by the reduced polymicrobial loads in multiple body sites, providing a diminished proinflammatory stimulus to cytokine producing cells. Of note, concurrent VRE infection did not influence the recruitment of cells to the peritoneal cavity, making an effect on the number of cytokine producing leukocytes at the primary site of infection an unlikely cause of the diminished cytokine response in VRE infected mice. In addition, VRE infected mice recovered faster from CLP-induced hepatocellular injury, as shown by lower plasma ASAT levels when compared with mice not infected with VRE 48 hours after CLP. These data indicate that VRE does not worsen the outcome of polymicrobial peritonitis, but rather facilitates bacterial clearance and attenuates the associated inflammatory response. Moreover, these results further establish that VRE infection by itself does not lead to a strong proinflammatory response in vivo, an observation previously documented in healthy mice [33]. Our study did not directly examine the impact of concurrent VRE infection on CLP-induced mortality, although the fact that 22% of CLP control mice had died 48 hours after the surgical procedure versus none of the CLP VRE mice, together with the finding that VRE infection attenuated the inflammatory response to CLP, suggests that VRE exerts protective effects during CLP-induced polymicrobial sepsis.

The better outcome of VRE infected mice is remarkable in light of rat experiments with E. faecalis, in which concurrent enterococcal infection was reported to negatively impact on concurrent polymicrobial infection [11-13, 31, 32]. Several differences in study design may explain the differences between these and our study. Indeed, besides the fact that
we used *E. faecium* rather than *E. faecalis* and that mice and rats may respond differently to enterococcal infection [35], our investigation is the first to induce peritonitis by the endogenous intestinal microbial flora after intestinal surgery, while in the other studies two or more bacterial species were introduced into the abdominal cavity exogenously. Importantly, in our investigation all mice underwent exactly the same treatment and had comparable loads of aerobic intestinal outgrowth, the only difference between groups being VRE inoculation and colonization. Multiple differences with regard to expression of virulence factors exist within different *E. faecium* strains and between *E. faecium* and *E. faecalis*. It would be of considerable interest to establish whether our current results can be reproduced with distinct *E. faecium* (and *E. faecalis*) strains. Moreover, it is important to mention that the mice used were healthy before going into surgery and 10 weeks of age, resembling a situation of mid- to late-adolescence in humans. Considering that patients suffering from VRE infections usually are older and almost invariably have significant comorbidity, it would be of interest to examine the impact of *E. faecium* on CLP-induced infection in older mice with diverse underlying illnesses.

The mechanism by which VRE infection influenced the host response to polymicrobial peritonitis remains to be established. *E. faecium* is one of the lactic acid bacteria (LAB) used as probiotic [36]. Previous studies have indicated that intestinal *E. faecium* colonization impacts on the inflammatory response [36, 37]. Furthermore, probiotics in the intestines can lead to inhibition of the growth of conventional organisms or potential pathogens through a variety of mechanisms. These include their capacity to decrease luminal pH, secrete bacteriocins, and inhibit bacterial adhesion to epithelial cells. In addition, there is evidence that probiotics interfere with the production of defensins in the intestinal crypts [38]. Certain *E. faecium* strains are known bacteriocin producers that can inhibit growth of, or have antibacterial activity against, other microorganisms [39, 40]. Potentially, colonization with hospital-acquired *E. faecium* alters intestinal microbial networks thereby reducing the number of pathogenic bacteria and/or creating a favourable environment for less pathogenic bacteria, with beneficial immunologic properties.

VRE are emerging pathogens in hospital-acquired infections. In light of the fact that the significance of concurrent VRE infection in settings of polymicrobial infection is controversial, we here developed a model in which VRE causes infection from a natural source, i.e. from the intestinal tract previously colonized with this pathogen. We demonstrate that VRE does not worsen the outcome of CLP-induced polymicrobial peritonitis and sepsis in mice, but rather facilitates bacterial clearance and damps host inflammatory responses. Our data therefore do not substantiate an important pathogenic role of VRE infection in the context of fecal peritonitis.
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