Intestinal Enterococcus faecium Colonization Improves Host Defense during Polymicrobial Peritonitis

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(See the editorial commentary by Bertics and Wiepz on pages 679–81)

Background. Vancomycin-resistant (VR) Enterococcus faecium is increasingly found to colonize and infect hospitalized patients. Enterococci are frequently isolated from polymicrobial infections originating from the intestines. The impact of VR E. faecium on these infections and vice versa is not clear.

Methods. Mice were intestinally colonized with VR E. faecium during oral vancomycin treatment; control mice received oral vancomycin only. Fourteen days later, cecal ligation and puncture (CLP) was performed in all mice to induce polymicrobial peritonitis in the presence or absence of VR E. faecium colonization.

Results. VR E. faecium colonization per se was not associated with systemic dissemination of VR E. faecium. CLP resulted in systemic VR E. faecium infection in all VR E. faecium–colonized mice, with high VR E. faecium loads in peritoneal lavage fluid, blood, liver, and lungs. Forty-eight hours after CLP, mice infected with VR E. faecium had significantly lower bacterial loads in all organs tested than mice not infected with VR E. faecium. Additionally, lower inflammatory parameters were measured in VR E. faecium–infected mice. CLP induced transient liver and kidney damage, with a faster recovery in VR E. faecium–colonized mice.

Conclusions. VR E. faecium 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.

Since the first isolation of vancomycin-resistant (VR) Enterococcus faecium in the mid-1980s, enterococci have emerged from being a physiological commensal of the gastrointestinal tract to being an important drug-resistant pathogen, with increasing rates of colonization and infection worldwide [1–3]. In the United States, enterococci currently represent the third-leading cause of health care–associated infections, and ~33% of Enterococcus infections are caused by VR E. faecium [4]. The origin of infections, including bacteremia, is usually the gastrointestinal tract, colonized by hospital-acquired strains of VR E. faecium [5, 6]. Enterococci isolated from secondary peritonitis most commonly are part of a mixed flora; their pathogenicity during these infections is still not clear [7–10]. The origin of infections, including bacteremia, is usually the gastrointestinal tract, colonized by hospital-acquired strains of VR E. faecium [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, *E. faecalis* has been partially replaced by multiresistant *E. faecium* in European and US hospitals [14, 15]. To investigate the pathogenicity of VR *E. faecium* during mixed flora intra-abdominal infection, we used the model of cecal ligation and puncture (CLP) in mice first colonized by VR *E. faecium*. We induced polymicrobial peritonitis and subsequent systemic infection with a mixed endogenous microbial flora that did or did not contain VR *E. faecium*. This resembles the clinical situation of patients colonized by hospital strains of *E. faecium* and suffering subsequent intestinal damage, such as postsurgical intestinal leakage [9, 16].

**MATERIALS AND METHODS**

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

**Bacterial strain.** VR *E. faecium* strain E155 was used in all experiments. This clinical isolate from Cook County Hospital, Chicago, Illinois, belongs to a genetic subpopulation of hospital-associated *E. faecium* responsible for the worldwide emergence of nosocomial multidrug-resistant *E. faecium*, characterized by high-level quinolone and ampicillin resistance, a pathogenicity island containing the variant *esp* gene, and the presence of 5 cell surface protein genes [6, 17]. For all experiments, the bacteria were grown overnight on sheep blood agar (BA) plates and then grown to midlogarithmic phase for ~3.5 h in Todd-Hewitt broth (THB; Difco) at 37°C, with shaking.

**Colonization model.** A mouse model of VR *E. faecium* gastrointestinal colonization was used, as described elsewhere [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 a gastric inoculation of 10⁷ cfu 

For quantification of intestinal VR *E. faecium*, fresh stool was plated on Slanetz-Bartley (SB) agar plates (Oxoid), supplemented with vancomycin (6 μg/mL; SBv). After 14 days of VR *E. faecium* colonization, 8 mice per group were killed to determine cecal bacterial flora and VR *E. faecium* counts, respectively.

**CLP procedure.** Fourteen days after VR *E. faecium* colonization or inoculation with THB, CLP was performed, as described elsewhere [19]. Two hours after the procedure and every 8 h thereafter, mice received subcutaneous imipenem–cilastatin (0.5 mg/0.5 mL; Tienam; Merck Sharp & Dohme) [20]. Imipenem-cilastatin has no activity against the VR *E. faecium* strain used. Nine mice per group were included for each time point, and they were killed 24 or 48 h after the CLP procedure.

**Preparation of blood samples and homogenates.** Mice were anesthetized by inhalation of isoflurane (2%; Abbott Laboratories) with O₂ (2 L). Peritoneal lavage was performed with 5 mL of sterile phosphate-buffered saline and a 18-gauge needle, and peritoneal lavage fluid (PLF) was collected in polypropylene tubes (BD). Blood was obtained by cardiac puncture and transferred to heparin gel Vacutainer tubes. Livers and lungs were harvested, along with the intestines in control experiments. Differences in organ weight were corrected for by the addition of 4 times the weight (in milligrams) in microliters of sterile saline. The organs were homogenized at 4°C with a tissue homogenizer (BioSpec Products). For intestinal cytokine measurements, homogenates were lysed in 1 volume of lysis buffer (300 mmol/L sodium chloride, 15 mmol/L Tris, 2 mmol/L MgCl₂, 2 mmol/L Triton X-100, pepstatin A, leupeptin, and aprotinin (20 ng/mL; pH 7.4) on ice for 30 min and spun down. Supernatants and plasma were frozen at −20°C until assayed.

**Determination of bacterial outgrowth.** Bacterial counts were determined in PLF, blood, and liver and lung homogenates. Serial 10-fold dilutions were made of each sample of the homogenates, PLF and blood in sterile saline, and 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 and 5% CO₂, and colony-forming units were counted after 20 h (BA and McC plates) or 44 h (CNA and SBv plates).

**Typing bacteria by multiple-locus variable-number tandem repeat analysis (MLVA).** MLVA was used to identify VR *E. faecium* from stool as *E. faecium* strain E155. The MLVA typing was performed as described elsewhere [21].

**Cell counts and differentials.** Total cell counts were counted for each PLF sample using a hemocytometer (Beckman Coulter). Differential cell counts were performed on cytospin preparations, stained with a modified Giemsa stain (Diff-Quik; Dade Behring). PLF supernatants were stored at −20°C until determination of cytokine levels.

**Assays.** Macrophage inflammatory protein 2 (MIP-2), cytokine-induced neutrophil chemoattractant (KC), and lipopolysaccharide-induced CXC chemokine (LLX) were measured in PLF by enzyme-linked immunosorbent assays (ELISAs; R&D Systems). Tumor necrosis factor (TNF)–α, interleukin (IL)–6, IL-10, and monocyte chemoattractant protein 1 (MCP-1) were measured in PLF and plasma by means of a cytometric bead array multiplex assay (BD Biosciences). Serum amyloid A was measured by ELISA (BioSource International). All tests were performed according to the manufacturers’ instructions. C3 was detected by sandwich ELISA [22]. Aspartate aminotransferase (AST) and creatinine levels were determined.
casionally available kits (Sigma-Aldrich), using a Hitachi analyzer (Boehringer Mannheim).

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

**Statistical analysis.** All data are expressed as means ± standard errors of the mean. Differences between groups were calculated by Mann-Whitney U test. For all analysis, GraphPad Prism software was used (version 4; GraphPad Software). Differences were considered statistically significant at \( P < .05 \).

**RESULTS**

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

**Intestinal colonization with VR \( E. faecium \) and systemic VR \( E. faecium \) infection after CLP.** CLP caused systemic VR \( E. faecium \) infection in all mice colonized with this pathogen (Figure 1). At 24 h after CLP, high VR \( E. faecium \) loads were recovered from all body sites examined (PLF, blood, liver, and lungs). VR \( E. faecium \) loads remained high thereafter, although at 48 h after CLP the numbers of VR \( E. faecium \) cultured from PLF and lungs were lower than at 24 h. In addition, whereas 8 of 9 colonized mice had blood cultures positive for VR \( E. faecium \) at 24 h, only 4 of 9 had positive blood culture results at 48 h. VR \( E. faecium \) could not be recovered from any body site in control mice subjected to CLP.

**VR \( E. faecium \) accelerates bacterial clearance after CLP.** To determine the influence of VR \( E. faecium \) 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 VR \( E. faecium \)-colonized and control mice 24 or 48 h after CLP (Figure 2). At 24 and 48 h after CLP, all mice had high polymicrobial outgrowth in PLF, blood, liver, and lungs. Forty-eight hours after the CLP procedure, mice colonized with VR \( E. faecium \) had significantly lower total aerobic bacterial loads than control mice in all cultured organs; a trend toward this difference was seen after 24 h (Figure 2). Similar differences between VR \( E. faecium \)-colonized and control mice were seen with regard to total gram-negative and gram-positive bacterial loads (data not shown). Furthermore, 48 h after CLP 2 of the 9 control mice had died, whereas all VR \( E. faecium \)-positive mice were still alive.

**Inhibition of local and systemic inflammatory responses by VR \( E. faecium \).** All mice responded to induction of polymicrobial peritonitis with a strong neutrophil influx into the peritoneal cavity, with highest counts after 24 h; no differences were found between VR \( E. faecium \)-colonized and control mice (Figure 3A). In addition, no differences were found for peritoneal macrophage or lymphocyte counts (data not shown). The murine CXC chemokines KC, MIP-2, and LIX are known neutrophil-attracting and -activating mediators. Consistent with the similar peritoneal neutrophil counts after 24 h in the 2 groups, there were no differences in KC (Figure 3B), MIP-2 (Figure 3C), or LIX (Figure 3D) levels at this time point. Interestingly, 48 h after CLP, peritoneal neutrophil counts were similar in the 2 groups, yet peritoneal levels of KC, MIP-2, and LIX were significantly reduced in mice with VR \( E. faecium \)-positive polymicrobial peritonitis (\( P < .01 \)–.001 for comparison with control mice). As with the chemokine levels, peritoneal and plasma levels of TNF-\( \alpha \), IL-6, IL-10, and MCP-1 were similar in the 2 groups after 24 h, but after 48 h, both peritoneal

### Table 1. Cecal Vancomycin-Resistant (VR) \( E. faecium \) and Total Aerobic Bacterial Outgrowth

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>VR ( E. faecium ) count, colony-forming units</th>
<th>Total aerobic count, colony-forming units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin and VR ( E. faecium )</td>
<td>9.6 × 10⁸ ± 1.0 × 10⁸</td>
<td>1.5 × 10⁹ ± 0.3 × 10⁹</td>
</tr>
<tr>
<td>Vancomycin and THB</td>
<td>0</td>
<td>1.1 × 10⁹ ± 0.2 × 10⁹</td>
</tr>
</tbody>
</table>

**NOTE.** Mice were treated with vancomycin (250 \( \mu \)g/mL) in drinking water for 19 days; 10⁷ cfu of vancomycin-resistant \( E. faecium \) or sterile Todd-Hewitt broth (THB) was administered by gastric instillation 5 days after the initiation of vancomycin treatment. Specimens were obtained 14 days after VR \( E. faecium \) instillation. Counts are numbers per gram of cecal contents and represent means ± standard errors of the mean for 8 mice per group.

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Figure 1. Systemic vancomycin-resistant (VR) Enterococcus faecium infection after cecal ligation and puncture (CLP) in VR E. faecium–colonized mice. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 cfu of vancomycin-resistant E. faecium was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed after 14 days of VR E. faecium colonization, and mice were killed 24 or 48 h thereafter. Data are colony-forming units of VR E. faecium (means ± standard errors) in peritoneal lavage fluid (PLF), blood, liver, and lung (9 mice per group per time point). Numbers above bars in B indicate positive blood cultures. *P < .05. **P < .01.

and plasma levels of TNF-α, IL-6, and MCP-1 were significantly reduced in mice with VR E. faecium–positive peritonitis (Figure 4). IL-10 levels were reduced in plasma but not significantly reduced in PLF. The diminished inflammatory response was further illustrated by reduced levels of plasma acute-phase proteins C3 and serum amyloid A in VR E. faecium–positive mice 48 h after CLP (Figure 5).

VR E. faecium infection and CLP-induced organ damage.

Consistent with the low mortality in the model of CLP-induced sepsis used in this study, only mild inflammatory changes were seen in liver and lungs on histopathological examination. Pathology scores did not differ between VR E. faecium–colonized and control mice at either 24 or 48 h after CLP (data not shown). CLP was associated with transient hepatocellular injury and renal dysfunction, as reflected by elevated plasma concentrations of AST and creatinine, respectively, especially 24 h after the surgical procedure (Figure 6). VR E. faecium–colonized mice displayed less hepatocellular injury at 48 h after CLP, with plasma AST concentrations that were lower than in control mice (P < .01).

DISCUSSION

In this study we show that intestinal colonization with a hospital-acquired strain of VR E. faecium causes systemic VR E. faecium infection after intestinal perforation induced by CLP. Mice colonized with VR E. faecium had improved infectious and inflammatory outcomes after CLP. Whereas no significant differences were found between VR E. faecium–colonized and control mice 24 h after CLP, after 48 h VR E. faecium–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 VR E. faecium–colonized mice and a faster recovery from liver damage.

VR E. faecium is increasingly found colonizing the intestines of hospitalized patients, especially in 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...
Figure 2. Increased clearance of polymicrobial infection after cecal ligation and puncture (CLP) in mice colonized with vancomycin-resistant (VR) Enterococcus faecium. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 cfu of VR E. faecium (VRE) (black bars) or sterile Todd-Hewitt 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 killed 24 or 48 h after CLP. The total aerobic bacterial load was determined in peritoneal lavage fluid (PLF), blood, liver, and lung. Data are means ± standard errors of the mean for 7–9 mice per group per time point. *P < .05. **P < .01. ***P < .001.

by VR E. faecium is paralleled by an increase in prevalence of VR E. faecium infections [1–3, 5, 24]. Surgical treatment has been associated with the development of enterococcal bacteremia in other studies [9]. In 1 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 that patients with prior surgical operations in the gastrointestinal, genital, or urinary tract had a 2–4-fold higher frequency of enterococcal infections than patients with nosocomial infections caused by other organisms [26].

Most commonly, enterococci are isolated from polymicrobial intra-abdominal infections originating from a gastrointestinal tract 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 postoperative complication rate but does not affect the overall mortality [27, 28]. Others showed increased mortality in the presence of enterococci [10, 29]. However, findings in the majority of investigations are inconclusive, suggesting the importance of severe underlying illness when enterococci are isolated [8, 30]. In animal studies E. faecalis was shown to increase postoperative 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 of the clinical and experimental data discussed above concern E. faecalis. Because infections with multiresistant E. faecium are emerging, more knowledge is needed on the pathogenesis of infections with this particular enterococcal species. In the current study, we used the well-established model of CLP in mice with or without prior intestinal colonization with a VR E. faecium strain of the genetic complex responsible for most hospital-acquired E. faecium infections. Postoperative polymicrobial peritonitis with endogenous intestinal flora was induced, in a model resembling the clinical scenario of a patient with polymicrobial peritonitis, with or without concurrent VR E. faecium infection. At 24 h after the CLP procedure, VR E. faecium could be isolated from all cultured body sites, and 8
of 9 mice had VR *E. faecium*–positive blood cultures, indicating that we successfully caused systemic VR *E. faecium* infection in mice previously colonized with this pathogen. Notably, quantitative VR *E. faecium* cultures 48 h after CLP showed modest but statistically significant decreases in most organs tested, and the number of positive blood cultures for VR *E. faecium* had decreased to 4 of 9.

These data suggest that, even in the presence of polymicrobial peritonitis treated with an antibiotic not active against VR *E. faecium* (imipenem-cilastatin), VR *E. faecium* does not continue to grow and disseminate. It should be noted that healthy mice rapidly clear VR *E. faecium* after intraperitoneal injection [33]. Complete clearance of VR *E. faecium* is unlikely to occur in the current model, given that the source of VR *E. faecium* (the perforated colonized gut) remains present, in contrast to the situation produced in our earlier study after a single intraperitoneal injection of VR *E. faecium* [34]. Nonetheless, together these findings indicate that the host, even when compromised as after CLP, has several defense mechanisms that limit the growth of VR *E. faecium*.

Our study also provides insight into the impact of VR *E. faecium* infection on the host response against polymicrobial peritonitis; mice with concurrent VR *E. faecium* infection demonstrated reduced polymicrobial bacterial loads in all body sites tested. Indeed, although a trend toward reduced bacterial loads in PLF, blood, liver, and lungs was already seen 24 h after CLP, the differences in bacterial loads between VR *E. faecium*–infected and –uninfected mice became statistically significant after 48 h. In parallel, many inflammatory responses were attenuated in VR *E. faecium*–infected mice 48 h after CLP. The attenuated inflammatory response was detected both locally at the site of the primary infection, as demonstrated by diminished chemokine and cytokine concentrations in PLF, and systemically, as demonstrated by lower plasma cytokine and acute-phase protein levels.

These data suggest that the polymicrobial infection induced by CLP (and not VR *E. faecium*) drives the inflammatory response in this model and that the attenuated inflammatory response in VR *E. faecium*–infected mice was most likely caused by the reduced polymicrobial loads in multiple body sites, providing a diminished proinflammatory stimulus to cytokine-producing cells. Concurrent VR *E. faecium* infection did not influence the recruitment of cells to the peritoneal cavity, making it unlikely that the number of cytokine-producing leukocytes at the primary site of infection caused the diminished cytokine response in VR *E. faecium*–infected mice. In addition,
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VR E. faecium–infected mice recovered faster from CLP-induced hepatocellular injury than mice not infected with VR E. faecium, as shown by lower plasma AST levels 48 h after CLP. These data indicate that VR E. faecium 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 VR E. faecium infection by itself does not lead to a strong proinflammatory response in vivo, an observation documented elsewhere in healthy mice [33]. Our study did not directly examine the impact of concurrent VR E. faecium infection on CLP-induced mortality. However, the fact that none of the VR E. faecium–

Figure 4. Reduced cytokine responses 48 h after cecal ligation and puncture (CLP) in mice colonized with vancomycin-resistant (VR) Enterococcus faecium. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10^7 cfu of VR E. faecium (VRE) (black bars) or sterile Todd-Hewitt broth (white bars) was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. CLP was performed in all mice, and mice were killed 24 or 48 h thereafter. Peritoneal (A–D) and plasma (E–H) levels of tumor necrosis factor (TNF–α) (A, E), interleukin (IL)–6 (B, F), IL–10 (C, G), and monocyte chemoattractant protein 1 (MCP–1) (D, H) were measured. Data are means ± standard errors of the mean for 7–9 mice per group per time point. * P < .05. ** P < .01. *** P < .001.
Reduced acute-phase protein response in mice colonized with vancomycin-resistant (VR) Enterococcus faecium. Mice were treated with vancomycin (250 μg/mL) in drinking water for 19 days; 10⁷ cfu of VR E. faecium (VRE) (black bars) or sterile Todd-Hewitt broth (white bars) was administered by gastric inoculation 5 days after the initiation of vancomycin treatment. Cecal ligation and puncture (CLP) was performed in all mice, and mice were killed 24 or 48 h thereafter. Plasma C3 (A) and serum amyloid A (SAA) (B) levels were measured. Data are means ± standard errors of the mean for 7–9 mice per group per time point. **P < .01. ***P < .001.

Infected mice had died 48 h after the surgical procedure, compared with 22% of the control mice, together with the finding that VR E. faecium infection attenuated the inflammatory response to CLP, suggests that VRE. faecium exerts protective effects during CLP-induced polymicrobial sepsis.

The better outcome of VR E. faecium–infected mice is remarkable in light of rat experiments with E. faecalis, in which concurrent enterococcal infection was reported to have a negative impact on concurrent polymicrobial infection [11–13, 31, 32]. Several differences in study design may explain the differences in findings between these studies and ours. Indeed, besides the facts 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; in the other studies ≥2 bacterial species were introduced into the abdominal cavity exogenously. Importantly, in our investigation all mice underwent exactly the same treatment and had similar loads of aerobic intestinal outgrowth, the only difference between groups being VR E. faecium inoculation and colonization.

There are multiple differences with regard to expression of virulence factors 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 10 weeks of age and healthy before going into surgery, resembling the condition of mid- to late adolescence in humans. Considering that patients suffering from VR E. faecium infections usually are...
older and almost invariably have significant comorbidity, it would be interesting to examine the impact of *E. faecium* on CLP-induced infection in older mice with diverse underlying illnesses.

The mechanism by which VR *E. faecium* infection influenced the host response to polymicrobial peritonitis remains to be established. *E. faecium* is one of the lactic acid bacteria used as a probiotic [36]. Previous studies have indicated that intestinal *E. faecium* colonization affects the inflammatory response [36, 37]. Furthermore, probiotics in the intestines can inhibit 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 the growth of, or have antibacterial activity against, other microorganisms [39, 40]. Colonization with hospital-acquired *E. faecium* may alter intestinal microbial networks, thereby reducing the number of pathogenic bacteria and/or creating a favorable environment for less pathogenic bacteria, with beneficial immunological properties.

VR *E. faecium* is an emerging pathogen in hospital-acquired infections. Because the significance of concurrent VR *E. faecium* infection in settings of polymicrobial infection is controversial, we developed a model in which VR *E. faecium* causes infection from a natural source, from the intestinal tract previously colonized with this pathogen. We demonstrated that VR *E. faecium* does not worsen the outcome of CLP-induced polymicrobial peritonitis and sepsis in mice but rather facilitates bacterial infection in the context of fecal peritonitis.

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