In Vitro Antienterococcal Activity Explains Associations between Exposures to Antimicrobial Agents and Risk of Colonization by Multiresistant Enterococci

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We compared ceftriaxone and piperacillin-tazobactam at doses ranging from 0.1 to 2 times the human equivalent daily dose (HEDD), to determine their impact on gastrointestinal colonization by ampicillin- and vancomycin-resistant Enterococcus faecium C68 in a mouse model. Ceftriaxone failed to promote colonization at doses up to 0.25 times the HEDD, whereas piperacillin-tazobactam promoted colonization at doses up to 0.5 times the HEDD. Ceftriaxone promoted colonization at doses at least 0.5 times the HEDD, whereas piperacillin-tazobactam inhibited colonization at doses at least 0.75 times the HEDD. Both piperacillin-tazobactam and ceftriaxone inhibited colonization by an enterococcal strain devoid of low-affinity penicillin-binding protein–5 (significantly increasing its susceptibility to these agents), at doses that promoted colonization by E. faecium C68. These results support a model in which the impact that different β-lactam agents have on colonization by VRE is related to the level of the β-lactam agent’s intrinsic antienterococcal activity against the colonizing strain.

Gastrointestinal colonization by vancomycin-resistant enterococci (VRE) is an important risk factor for subsequent infection with and nosocomial spread of these pathogens [1, 2]. Data from a large number of clinical studies implicate the administration of vancomycin, extended-spectrum cephalosporins, and agents with potent activity against anaerobic bacteria in colonization by and infection with VRE [3–5]. For β-lactam antibiotics, animal studies suggest that biliary excretion and intrinsic activity against Enterococcus faecium are important factors in promoting gastrointestinal colonization by VRE, whereas potency of activity against anaerobic bacteria correlates best with persistence of high levels of colonization [6–8].

Studies from our laboratory have consistently shown that, in a mouse model, exposure to parenteral ceftriaxone promotes colonization by VRE, whereas exposure to piperacillin-tazobactam appears to prevent colonization at high levels [6–8]. One potential explanation for this difference is the intrinsic activity that these antimicrobial agents have against VRE, which are typically resistant to β-lactam antibiotics. For many VRE strains, the MICs of ampicillin range from ∼128 to 512 μg/mL, with the MICs of piperacillin-tazobactam being approximately double these (256 to 1024 μg/mL) [9]; the MICs of ceftriaxone are considerably higher, often >10,000 μg/mL [9]. If standard doses of piperacillin-tazobactam yield biliary concentrations that exceed the MIC for the (presumably) few VRE that initially pass through the upper gastrointestinal tract, they may inhibit growth of VRE enough to prevent the establishment of significant colonization in the lower gastrointestinal tract. Because, after standard dosing, biliary concentrations of piperacillin range from 1000 to 2000 μg/mL [10], such inhibition is within the realm of pos-
sibility; in contrast, even the higher levels of ceftriaxone (i.e., biliary concentrations as high as 5000 μg/mL) [11] would not exceed the MIC for the typical VRE.

If the intrinsic activity that piperacillin-tazobactam has against VRE explains its protective effect in our mouse model of gastrointestinal colonization, we should be able to identify a dose low enough that, because the biliary concentration would fall below the MIC of the VRE strain, this protective effect would be diminished or eliminated. Similarly, we may also be able to lower the dose of ceftriaxone enough to reduce its selectivity for colonization by VRE. Furthermore, if the intrinsic activity of these antibiotics explains this differential selection, then both of them should inhibit colonization by an E. faecium strain devoid of PBP5 and therefore highly susceptible to these agents. The present study was designed to test these hypotheses.

MATERIALS AND METHODS

Bacterial strain. We used E. faecium strain C68 [12], an ampicillin- and vancomycin-resistant (VanB-type) clinical strain, for the dose-ranging studies. This strain has been described in detail and was used in our previous animal studies [6, 7]. We used E. faecium D344SRF (pLRM23) for the studies designed to test whether ceftriaxone and piperacillin-tazobactam would inhibit colonization by a susceptible strain of E. faecium. E. faecium D344SRF [13] is an ampicillin- and vancomycin-susceptible spontaneous derivative of E. faecium D344R from which the chromosomal region containing pbp5 has been deleted. To promote colonization by this strain, a conjugation experiment was performed with E. faecium C68, to select for transfer of a hyaluronidase plasmid (pLRM23) [14] that, in this model, promoted colonization after selection with the non-β-lactam antibiotic clindamycin (data not shown). The transconjugant selected for further studies was tested for susceptibility to piperacillin-tazobactam (MIC, 4 μg/mL for both D344SRF and the transconjugant) and ceftriaxone (MIC, 4 μg/mL for both D344SRF and the transconjugant), to guarantee that no β-lactam–resistance phenotypes had been acquired during the transfer. It was also shown to be devoid of pbp5 and the VanB operon (MIC of vancomycin, 2 μg/mL), by both polymerase chain reaction amplification and Southern hybridization (data not shown). For this strain, the MIC of clindamycin was very high (>20,000 μg/mL).

Microbiologic techniques. Broth macrodilution MICs were performed in brain-heart infusion broth. Overnight cultures grown without antibiotic selection were diluted to achieve an inoculum of $1 \times 10^8$ cfu/mL. Cultures were incubated overnight at 37°C, and the MIC was determined to be that in the tube containing the lowest antibiotic concentration for which there was no detectable turbidity after 24 h of incubation. Standard 2-fold dilutions of antibiotics were used.

Mouse model. The mouse model of colonization by VRE was essentially the same as that described in our previous study [7]. In brief, ~25–30-g female CF1 mice (Harlan Sprague-Dawley) were administered subcutaneous (sc) injections of antibiotics 2 times per day for 2 days before gavage with enterococci and for the duration of the experiment. The dose of antibiotics chosen reflected the usual daily dose for a 70-kg human, adjusted for the weight of the mouse, and was divided into 2 doses administered 12 h apart. The total standard daily dose of ceftriaxone was 2.4 mg/day, and that of piperacillin-tazobactam was 8 mg/day. For experiments in which E. faecium D344SRF (pLRM23) was used, positive-control mice were pretreated with clindamycin (1.4 mg/day), which, in this model, was found to promote colonization at high levels (data not shown). In all experiments, negative-control mice were administered normal saline (NS) in a volume equivalent to that used in the administration of antibiotics. After 2 days of sc administration of either an antibiotic or NS, mice were administered, by use of a stainless-steel feeding tube (Perfektum; Popper and Sons), an inoculum of $\sim 2 \times 10^9$ cfu of either E. faecium C68 or E. faecium D344SRF (pLRM23), both derived by NS dilution of a frozen stock of known inoculum. Fresh stool samples were collected from all mice on days 1, 3, and 6 after gavage. Stool samples were weighed, homogenized in sterile NS, and serially diluted. Dilutions were plated onto Enterococcosel agar containing either vancomycin (20 μg/mL), for E. faecium C68, or fusidic acid (25 μg/mL) and rifampin (100 μg/mL), for E. faecium D344SRF (pLRM23). Colonies that hydrolyzed esculin in the agar were counted after 48 h of incubation. When no VRE were detectable on initial screening, a larger aliquot (100 μL) of homogenized stool was plated, to

![Figure 1](https://academic.oup.com/jid/article-abstract/190/12/2162/864058/10.1093/jid/190.12.2162)
increase detection of small numbers of VRE (lower limit of detection, ∼2.0 log_{10} CFU/g of stool); if no VRE were detected in these samples, a number equal to the lower limit of detection was assigned. Results are reported as the average ± SD of all determinations during the 3 days of collection. The bedding in the cages was changed daily to prevent coprophagia, which had been shown, in a previous experiment, to complicate the analysis [8]. These experiments were reviewed and approved by the Louis Stokes Cleveland VA Animal Care and Use Committee.

**Statistical analysis.** Statistical analyses were performed by use of STATA (version 5.0; StataCorp). The mean log_{10} colony-forming units were determined across the 3 data-collection time points for each mouse and were then examined by use of a 1-way analysis of variance, for each type of treatment. Overall and pairwise differences were examined, with P values being adjusted, by use of the Scheffe test, for multiple comparisons. Statistical significance was defined as P<.05.

**RESULTS**

The doses of ceftriaxone and of piperacillin-tazobactam that were used in this study were 0.1, 0.25, 0.5, 0.75, 1, and 2 times the defined standard dose. Seventeen mice in total received NS. For experiments using *E. faecium* C68, 4 mice were included in each dosing group, and each experiment was repeated. Results obtained with the different doses of ceftriaxone are shown in figure 1. At a dose equivalent to 0.1 times the standard dose, ceftriaxone did not promote colonization by VRE; at 0.25 times the standard dose, colonization was minimal (but more variable from mouse to mouse); and, at 0.5 times the standard dose and at all higher doses, colonization was strongly promoted. At the higher doses, the differences between the levels of colonization by VRE were statistically significantly different from those in the group treated with NS. These data are consistent with a model in which ceftriaxone, by its activity against competing microorganisms in the upper gastrointestinal tract, promotes colonization by VRE. Once a concentration of ceftriaxone sufficient to inhibit these other microorganisms is achieved (presumably at ∼0.5 times the standard dose, according to our experiments), ceftriaxone promotes a high level of colonization. That we were unable to administer a dose of this antibiotic sufficient to inhibit colonization by VRE is consistent with an inability to achieve biliary concentrations of ceftriaxone that are sufficient to exceed the very high MIC for *E. faecium* C68.

Results with piperacillin-tazobactam dosing are shown in figure 2 and are essentially the reverse of those observed with ceftriaxone. At a dose equivalent to 0.1 times the standard dose, piperacillin-tazobactam promoted high levels of colonization by VRE. At 0.25 and 0.5 times the standard dose, colonization was also strongly promoted, although the results were more variable from mouse to mouse. At all these doses, the differences between the levels of colonization by VRE were statistically significantly different from those of the NS group. When a dose of 0.75 times the standard dose was administered, however, piperacillin-tazobactam strongly inhibited colonization. These data are consistent with a model in which piperacillin's intrinsic activity against *E. faecium* C68 is the important characteristic for the inhibition of colonization. Because *E. faecium* C68 expresses some resistance to piperacillin (MIC, ∼512 μg/mL), to inhibit colonization a dose large enough to achieve high concentrations in the bile and, ultimately, in the upper gastrointestinal tract needs to be administered.
Results of experiments using β-lactam–susceptible E. faecium D344SRF (pLRM23) are shown in figure 3. This strain is not as effective in colonizing the mouse gastrointestinal tract as is E. faecium C68; overall, it successfully colonized only 67% (8/12) of the mice administered clindamycin, and the average log_{10} cfu/g of stool for the clindamycin group, as shown in figure 3, is lower than that for the E. faecium C68 group, as shown in figures 1 and 2, and the SD is greater. The doses of piperacillin-tazobactam and ceftriaxone that were used in the present study’s experiments were chosen to correspond to doses that promoted high levels of colonization by VRE in the other experiments. Piperacillin-tazobactam was administered at 0.1 times the standard dose (4 mice) and 0.25 times the standard dose (4 mice), whereas ceftriaxone was administered at 1 times the standard dose (8 mice). None of the mice pretreated with these antibiotics was colonized by VRE to a detectable extent (figure 3). These data are supportive of the supposition that some β-lactam antibiotics directly inhibit gastrointestinal colonization by VRE.

**DISCUSSION**

The data presented in the present study add to the growing understanding of the mechanisms by which antimicrobial agents promote colonization by multiresistant enterococci. With regard to multiresistant enterococci, the primary focus during the last decade has been, understandably, on the emergence and spread of vancomycin resistance; and this focus has been appropriate not only because vancomycin had been considered the last line of defense against resistant enterococci but also because of fears that the vancomycin-resistance determinant would spread to staphylococci, fears that subsequently have become justified [15, 16].

One unfortunate accompaniment to the emergence and spread of VRE has been the almost universal association between vancomycin-resistance determinants (in E. faecium) and the expression of low-affinity PBP5 [9, 17], which confers resistance to ampicillin and other β-lactams [13]. The implications that this association has for treatment of VRE infections in humans are obvious. The present study indicates that this association also has implications for the spread of VRE between patients in a hospital. The Hospital Infection Control Practices Advisory Committee initially advised that the use of vancomycin should be minimized, to avoid promoting the spread of VRE [18]. Clinical studies and the results of animal studies such as the present one now make it clear that many different classes of antimicrobial agents affect the level of colonization by—and, hence, the potential for spread of—VRE strains.

The human gastrointestinal tract is a dynamic environment in which the resident microbial flora are subjected to a variety of external influences. Among the most important human-derived influences is the administration of potent antimicrobial agents. The impact that an antibiotic has on the resident microbial flora depends on several factors, including the intrinsic activity of the antibiotic, the likelihood that the antibiotic will be concentrated in the gastrointestinal tract, the susceptibility of the antibiotic to inactivating enzymes produced by resident microbial flora, and the characteristics of microorganisms existing in the immediate external environment. The interactions between these factors can be complex, but they will be better understood if we appreciate the effects of each factor.

The impact that cephalosporins at high concentrations in the gastrointestinal tract have on the spread of VRE can now be understood as a side effect of the association between the acquired vancomycin-resistance determinants and the mutated, low-affinity pbp5 genes that are present, even before vancomycin-resistance operons, in E. faecium strains involved in nosocomial infections [19]. In fact, clinical studies conducted before the discovery of vancomycin resistance implicated the administration of third-generation cephalosporins as a risk factor for gastrointestinal colonization by ampicillin-resistant E. faecium [20]. We should therefore not be entirely surprised by the association, observed in clinical studies, between VRE and cephalosporins.

**References**

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