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EXPERIMENTAL THERAPEUTICS

Enterococcus faecium Clade Competition in the Presence of β-Lactam Antibiotics in a Mouse GI Tract Colonization Model

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ABSTRACT Previously, we showed that *Enterococcus faecium* clade B strains outcompeted health care-associated clade A1 strains in murine gastrointestinal colonization. Here, parenterally administered piperacillin-tazobactam and ceftriaxone significantly promoted colonization by clade A1 over clade B strains except that ceftriaxone, at the dose used, did not favor the least β-lactam-resistant A1 strain. The advantage that β-lactam administration gives to more highly ampicillin-resistant *E. faecium* over ampicillin-susceptible strains mirrors what occurs in hospitalized patients administered these antibiotics.

KEYWORDS ceftriaxone, piperacillin-tazobactam, *E. faecium*, mouse GI colonization, ampicillin resistant

Genome analyses have indicated a split of *Enterococcus faecium* into 2 clades (clades A and B), with branching of clade A (1, 2). While clade B is the predominant gut *E. faecium* clade in humans in the community and is generally antibiotic susceptible, gut colonization with clade A1 strains, which are more antibiotic resistant, including to vancomycin and ampicillin (AMP), is very common in hospitalized patients, replacing *Enterococcus faecalis* and clade B strains (1, 2). The different levels of AMP resistance are due, in large part, to differences in the *pbp5* sequence (3, 4) and to different levels of expression of PBP5 (5).

Using a preconditioned gastrointestinal tract (GIT) mouse model, we previously showed that clade B strains significantly outcompeted clade A strains (6) in GIT colonization. This provided, experimentally, a basis for the observation that hospital-associated vancomycin-resistant *Enterococcus* (VRE) generally decreases and even disappears once patients are no longer on antibiotics (7–9). Using the same strain pairs, we report here results of GIT colonization and competition between clade A1 and clade B during systemic β-lactam administration.

E. faecium strains representing clade A1 ($C68_{A1}$, $TX82_{A1}$, and $TX16_{A1}$) and clade B ($COM15_{B}$ and $E980_{B}$), used previously (6), and MICs (per CLSI [10]) are in Table 1. Our established mouse GIT model and methods were used with preconditioning with gentamicin and clindamycin (6). Piperacillin-tazobactam (TZP) (3.37 mg/kg of body weight, every 12 h [Q12h]) and ceftriaxone (CRO) (0.5 mg/kg, Q12h) (both from Sigma-Aldrich Chemicals) were given subcutaneously (s.c.) as in reference 11 for 14 days starting 1 h postinoculation. Dosing was designed for proof of principle with minimal injection repeats (11). Approximately 10^9 CFU/mL (confirmed by subsequently plating) was given by oral gavage as previously described (6). Cages, animals, and fecal pellets were handled as in reference 6 under AWC-19-0139 of the University of Texas Health Science Center at Houston.

Statistical analyses were performed as previously described (6).

Figure 1A shows strain $TX82_{A1}$ coinoculated with $COM15_{B}$. Both s.c. CRO (top left) and s.c. TZP (top right) promoted a highly significant increase in the percentage of the

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TABLE 1 *In vitro* MICs for *E. faecium* strains

Bacterial strain and description	MIC (μg/mL)[a]		
	AMP	CRO	TZP
E. faecium TX82 AMP^r, ERY^r *erm*(B), TET^r, VAN^r	64	512	256
E. faecium C68 AMP^r, LZD^{ns}, TET^r, VAN^r	128	>1,024	512
E. faecium TX16 ERY^r *erm*(B), TET^r	8–16	512	64
E. faecium E980	0.75–1	256	16
E. faecium COM15	0.12–0.19	128	4

[a]CLSI breakpoints (10) are as follows: CLSI breakpoints for enterococci are ≤8 μg/mL (susceptible) and ≥16 μg/mL (resistant) with AMP results used to predict susceptibility to piperacillin-tazobactam. There are no CLSI breakpoints for *E. faecium*.

$TX82_{A1}$ versus $COM15_B$ at all time points despite less $TX82_{A1}$ (10 to 22%) in the inoculated mixture. Most time points had no colonies of clade B recovered except for TZP at 14 days.

Similarly, for $C68_{A1}$ coinoculated with $COM15_B$, CRO (Fig. 1B, top left) resulted in highly significantly more of the clade A1 strain at 2 days, 4 days, and 7 days, despite having a lower percentage of $C68_{A1}$ in the inoculum (46 to 48%). At 14 days, CRO resulted in a nonsignificant (NS) increase in $C68_{A1}$. TZP (Fig. 1B, top right) also resulted in a highly significant increase in $C68_{A1}$ versus $COM15_B$ at day 2, 4, and 14, while the difference at day 7 was NS.

Figure 1C shows strain $TX16_{A1}$ (the least-β-lactam-resistant A1 strain) (Table 1) coinoculated with $E980_B$ (the more-β-lactam-resistant strain of the clade B strains used). Unlike the pairs above, CRO (top left) resulted in a significantly higher percentage of the commensal strain $E980_B$ on day 2. At 4 days, 7 days, and 14 days, the differences were nonsignificant. However, TZP (top right) resulted in highly significantly more $TX16_{A1}$ than $E980_B$ at all time points, similar to results above with other strain pairs.

Many factors influence a bacterium's ability to successfully colonize the GIT. These include various interactions with host components/host products, interactions with the cohabitating GI flora/its products, as well as the ability to utilize or withstand substances ingested, such as antibiotics (2, 7, 12).

Our previous study found that monoinoculation of *E. faecium* strains of clade A1, A2, or B into mice pretreated with antibiotics, but with no antibiotics after inoculation, resulted in the anticipated high density of each strain (10^9 CFU/g) on day 2, presumably related to a decrease/elimination of much of the flora by the preconditioning antibiotics. There was then a decrease to circa 10^4 CFU/g by day 14, reminiscent of reports that clade A1 VRE decrease or disappear once patients are off antibiotics (13), likely due to an effect of the return of other bacteria (7, 14). We also found that, when inoculated together without continuing antibiotics, clade B strains gradually outnumbered clade A strains after day 2 for most strain pairs, indicating that clade A1 strains were less "fit" than clade B *E. faecium* under the conditions used.

Here, we investigated the effect of the s.c. administration of two β-lactams on GIT colonization after coinoculation of strain pairs of *E. faecium*. As before, a high density (10^9 CFU/g) of *E. faecium* was achieved at day 2 by the antibiotic preconditioning. Unlike our previous study, however, under the continued presence of s.c. CRO or TZP, this high density was maintained through day 14, likely related to the continuing suppression of the normal GIT flora.

CRO lacks clinically relevant antienterococcal activity, but its primary excretion is into human bile, resulting in very high concentrations when given parenterally (15–17), and is known to support persistently high levels of stool VRE in mice. In the current study, we found that the more-β-lactam-resistant (Table 1) strains $C68_{A1}$ and $TX82_{A1}$ significantly outcompeted the more-susceptible community-associated *E. faecium* $COM15_B$ at 7/8 time points. The fold differences in CRO MICs between $TX82_{A1}$ and $COM15_B$ and between $C68_{A1}$ and $COM15_B$ are 4- and >8-fold, respectively. Although

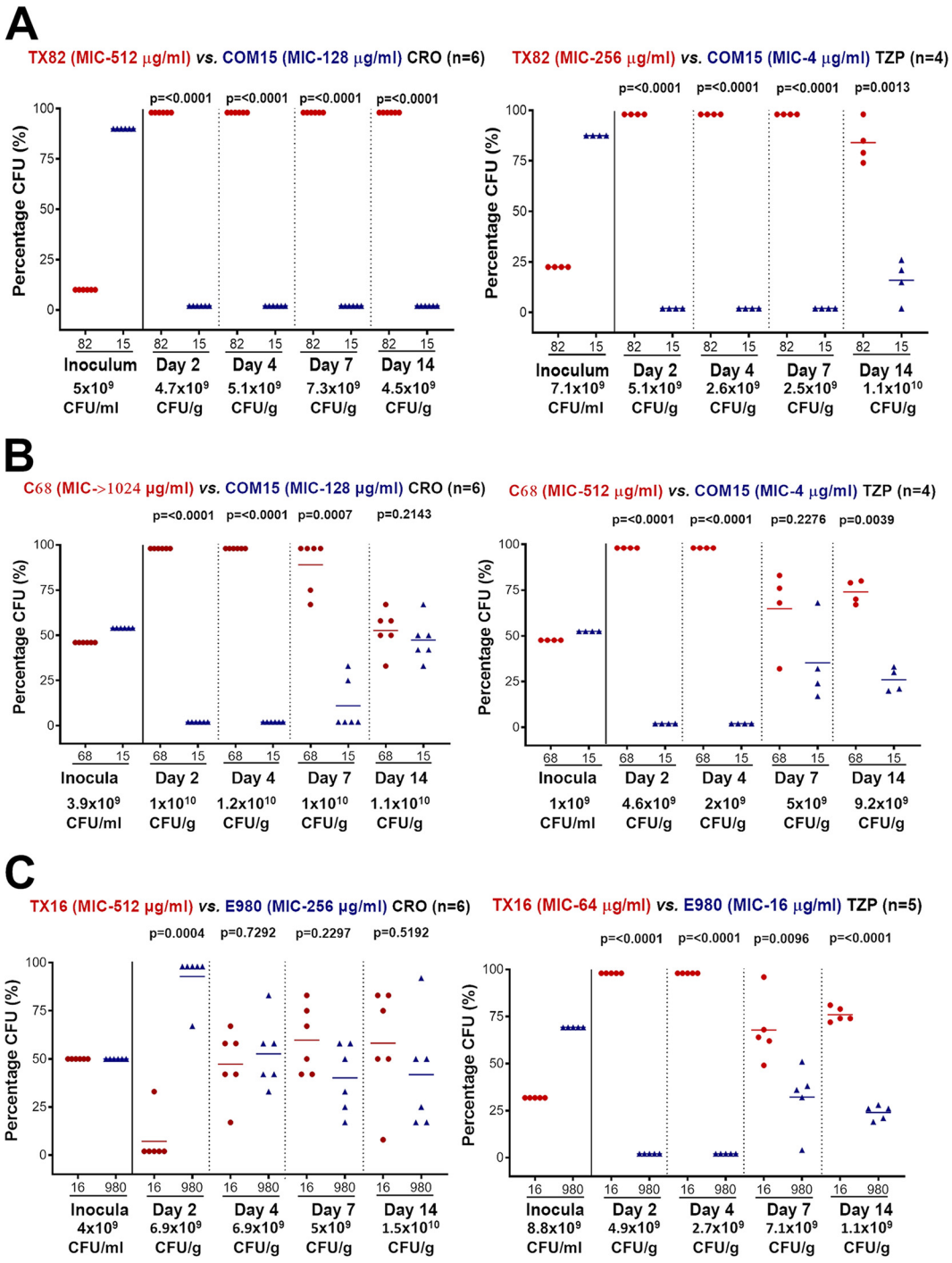

FIG 1 Mouse gastrointestinal tract (GIT) colonization by *E. faecium* clade A1 versus clade B strains in a mixed inoculation (1:1) competition assay; shown are the percentages (%) of total *E. faecium* CFU of clade A1 versus that of clade B strains in the inoculum mix and from fecal pellets recovered 2, 4, 7, and 14 days after oral inoculation. The horizontal lines indicate the means. A paired *t* test was used to calculate *P* values for the percentage of bacteria recovered in the fecal pellets versus that of the inoculum mix. The total *E. faecium* CFU per milliliter (inoculum) or CFU per gram (pellets) is given below the day. (A) Top left panel shows the effect of s.c. CRO administration ($n = 6$) after inoculation of a mix of strains $TX82_{A1}$ and $Com15_{B}$. Top right panel shows the effect of s.c. TZP administration ($n = 4$) after oral inoculation of mice with the same mixture. Solid red circles represent $TX82_{A1}$, and solid blue triangles represent $Com15_{B}$. (B) Top left panel shows the effect of s.c. CRO administration ($n = 6$) after inoculation of a mix of strains $C68_{A1}$ and $Com15_{B}$. Top right panel shows the effect of s.c. TZP administration ($n = 4$) after oral inoculation of mice with the same mixture. Solid red circles represent $C68_{A1}$, and solid blue triangles represent $Com15_{B}$. (C) Top left panel shows results with s.c. CRO administration ($n = 6$) after inoculation of a mix of strains $TX16_{A1}$ versus $E980_{B}$. Top right panel shows the effect of s.c. TZP antibiotic treatment ($n = 4$) after inoculation of a mix of the same strains. Solid red circles represent $TX16_{A1}$, and solid blue triangles represent $E980_{B}$.

we do not know the CRO concentrations in the gut, our results indicate that there was sufficient CRO not only to promote a high density of *E. faecium* but also to favor the more-β-lactam-resistant A1 strains over the clade B strain, a result opposite to our earlier observations without β-lactam use.

In contrast, when the least β-lactam resistant (Table 1) of the A1 strains, $TX16_{A1}$, was paired with $E980_B$, a clade B strain with higher β-lactam MICs then $COM15_B$ used above, parenteral CRO favored $E980_B$ over $TX16_{A1}$ at day 2 and at no time favored the A1 strain, unlike what was seen with other strain pairs. While MICs are an imprecise measure of susceptibility, the smaller difference in CRO MICs (2-fold) between these two strains versus those for the other strain pairs (4- and >8-fold) supports the concept that the degree of resistance would likely be important in determining a selective advantage.

For TZP, although some is secreted into the bile (1,125.3/13.9 μg/mL) (17–22), its primary excretion is via the kidneys; 68% and 80% of the unchanged piperacillin and tazobactam, respectively, of the administered dose gets excreted in the urine (23). Conversely, it is more potent than CRO against enterococci (Table 1). We noted that there was a bigger difference in TZP MICs (Table 1) between the A1 and B strain pairs (Fig. 1A to C, 64-fold, 128-fold, and 4-fold, respectively) than for CRO MICs. Here, with all 3 strain pairs, TZP conferred a highly significant selective advantage to the A1 versus the clade B strains ($P < 0.005$) at 11/12 time points. This indicates that, under these conditions, enough TZP gets into the GIT to sustain a high density of *E. faecium* as well as to select for the more-TZP-resistant *E. faecium* strain (the A1 strain) in each pair. Additionally, Fig. 1B data (day 14) show the narrowing of the colonization differences between clade A1 and clade B strains (left), which could be due to other transmissible genetic factors, e.g., $hyaluronidase_{Efm}$, that promote colonization of the mouse GI tract (24) and were not studied.

As was pointed out above, the GIT concentrations of TZP and CRO in this study are not known; however, published literature in mouse model has documented these levels (25, 26). Further, few if any recent hospital-associated clade A1 strains display the low β-lactam resistance (e.g., AMP MIC of 8 to 16 μg/mL) seen with $TX16_{A1}$, a strain isolated in 1992. Thus, the difference in the effect of CRO and TZP on the outcome of competition between this clade A1 strain and normal flora clade B strains should not be extrapolated to humans or to current isolates.

In summary, we showed conditional predominance of clade A1 *E. faecium* strains over clade B strains in an *in vivo* competition model for mouse GIT colonization at 18/24 time points, with the "condition" being the parenteral administration of a β-lactam; predominance also appeared to correlate with the degree of β-lactam resistance. These results are consistent with the observation that AMP-resistant VRE (i.e., clade A1 *E. faecium*) can become the predominant flora in patients during hospitalization and receipt of antibiotics (27, 28), which typically includes a β-lactam, helping the more resistant *E. faecium* clade A1 strains overcome their relative lack of fitness when present with clade B strains (6) in the absence of antibiotics.

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