Comparative In Vitro Killing Kinetics of Ibezapolstat and Comparator Antibiotics in *Clostridioides difficile* and *Enterococcus* Co-Cultures

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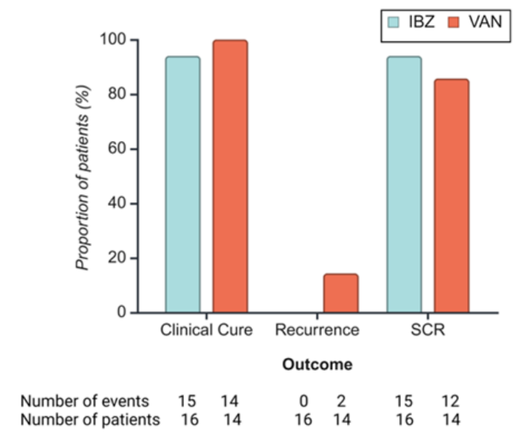
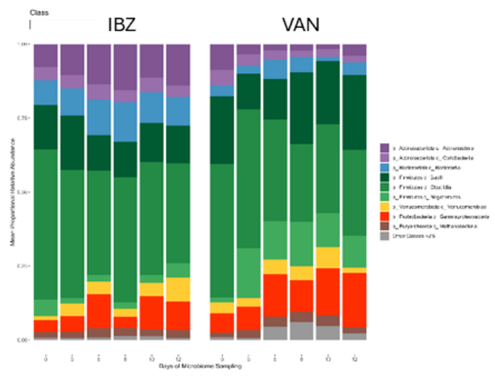
BACKGROUND

- Ibezapolstat (IBZ) is a Gram-positive selective spectrum (GPSS®) oral antibiotic that inhibits the bacterial DNA polymerase IIIC currently in clinical trial development for the treatment of *C. difficile* infection (CDI) in adults

A. No CDI Recurrence in clinical trials

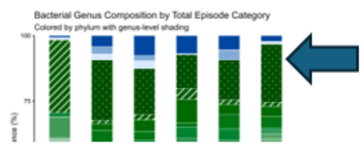


B. Favorable microbiome results

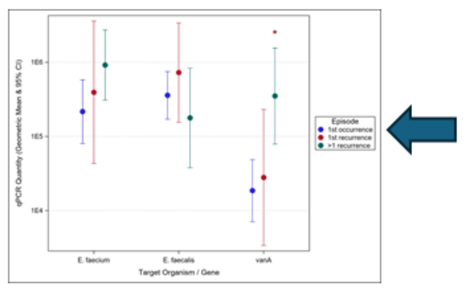


- Expansion of Actinobacteria (Bifidobacteria)
- Preservation of Clostridiales (bile acid metabolizers)

- Enterococcus dominate in patients with recurrent CDI



Enterococcus dominates in recurrent CDI patients



vanA gene is significantly higher in recurrent CDI patients

Clinical trials of IBZ focused on patients with multiple recurrent CDI is in progress (NCT07513285))

OBJECTIVE

The objective of this study was to evaluate the *in vitro* bactericidal and molecular efficacy of IBZ, vancomycin (VAN), and fidaxomicin (FDX) against *C. difficile* and *Enterococcus* in standalone and complex co-culture environments.

METHODS

Figure 1. Study Schematic

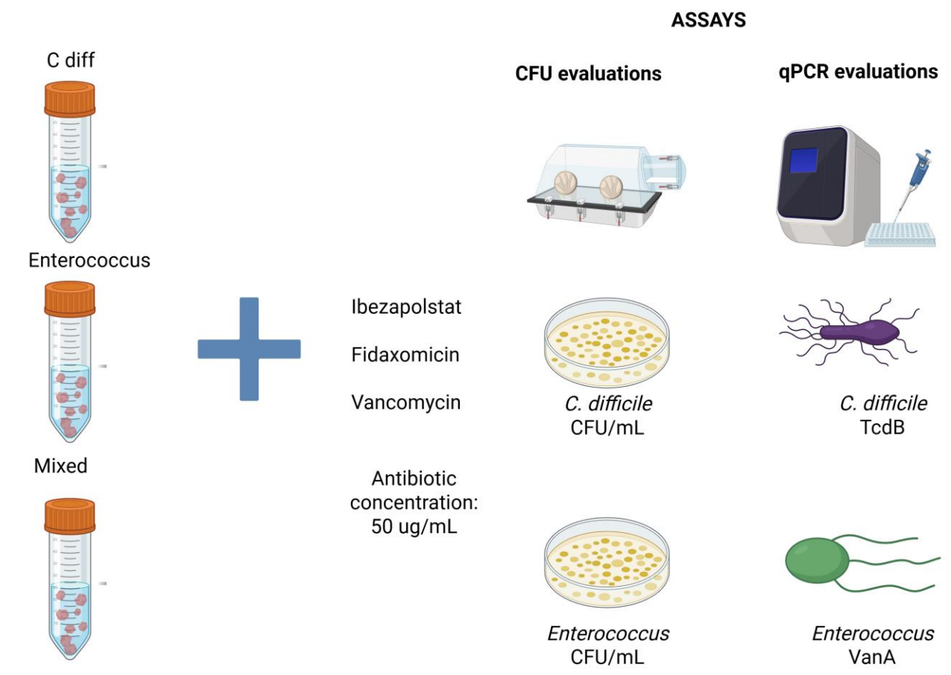


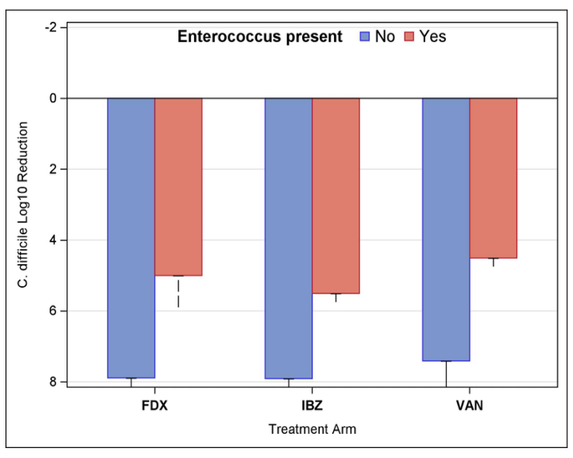
Figure 2. Primers and Probes

Primer ID	Sequence (5'-3')	Target	Referance
tcdB	tcdB-1F: GAAGGTGGTTCAGGTCATAC tcdB-1R: CATTTTCTAAGCTTCTTAAACCTG	tcdB	Lance R et al.,
vanA	vanA3-FP; CTGTGAGGTCGGTTGTGCG vanA3-RP; TTTGGTCCACCTCGCCA vanA3-FAM; FAM-CAACTAACGCGGCACTGTTTCCCAAT- BHQ1	vanA	Volkmann H et al.

RESULTS

Figure 3. CFU Determinations

Antibiotic Effect on *C. difficile* Co-cultured with *Enterococcus*



Antibiotic Effect on *Enterococcus* Co-cultured with *C. difficile*

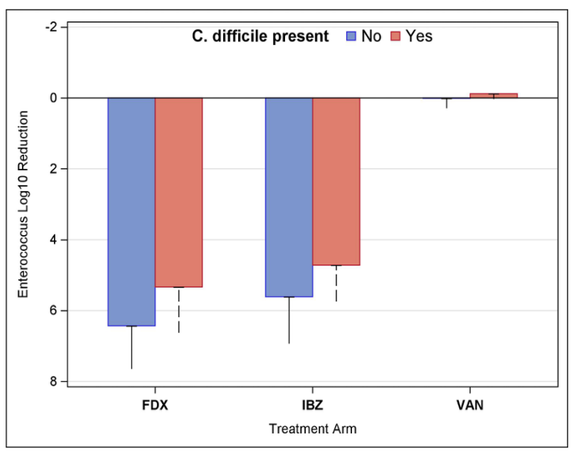
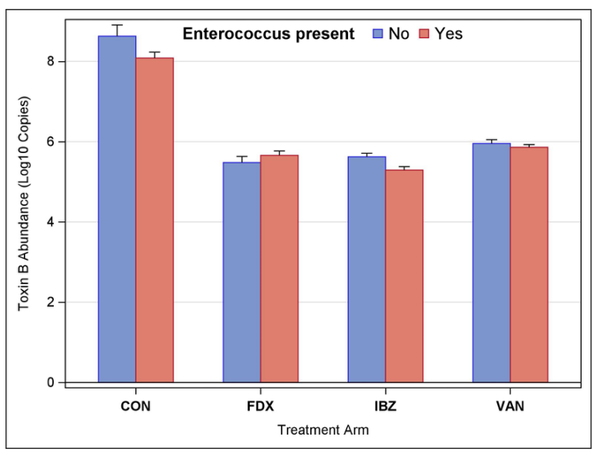
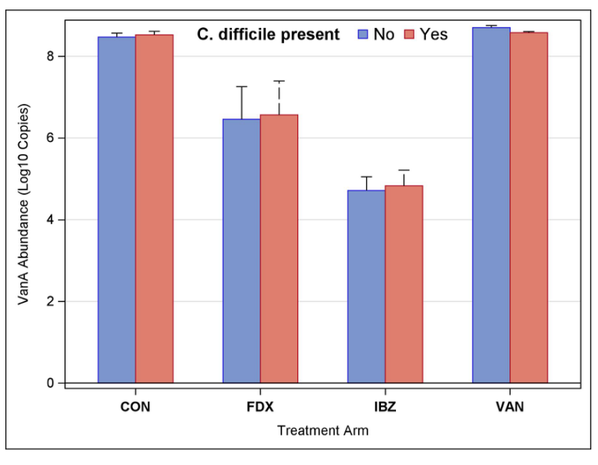


Figure 4. **Molecular qPCR Determinations**

Antibiotic Effect on TcdB Co-cultured with *Enterococcus*





IBZ: ibezapolstat; FDX: fidaxomicin; VAN: vancomycin; CON: control

In monoculture, IBZ displayed potent bactericidal activity non-inferior to FDX ($p \geq 0.2381$). *Enterococcus* co-culture with *C. difficile* created microbial interference that impaired *C. difficile* killing across all antibiotic arms ($p = 0.0054$), decreasing killing by up to 2.90 logs; however, IBZ maintained the highest residual anti-*C. difficile* performance (5.51-log reduction), significantly outperforming VAN ($p = 0.0112$)

At the transcriptional level, all treatments significantly suppressed *tcdB* abundance compared to control ($p < .0001$). Notably, co-culture with *Enterococcus* improved IBZ effect on TcdB with a 0.33-log additional reduction ($p=0.0082$). IBZ decreased VanA by 3.87-logs compared to VAN ($p<.0001$) and 1.74 logs improvement compared to FDX ($p<0.0001$).

CONCLUSIONS

- These findings demonstrate that *Enterococcus* creates a protective microenvironment that actively impairs *C. difficile* killing using standard antibiotic regimens.
- IBZ maintained superior bactericidal activity under co-culture conditions while simultaneously eradicating vancomycin-resistant reservoirs.
- If validated in clinical practice in the treatment of patients with CDI, IBZ's activity has the potential to reduce the nosocomial emergence of the critical pathogen VRE while treating CDI.

FUNDING

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