Investigation of *Enterococcus* Colonisation Impact on *Clostridioides difficile* Disease Severity

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BACKGROUND AND AIMS

Enterococcus colonisation with *Enterococcus faecalis* and *Enterococcus faecium* is a known risk factor for *Clostridioides difficile* infection (CDI).[1-3] *Enterococcus* co-colonisation increases *C. difficile* virulence and toxin production through cross-feeding.[4] However, current disease severity definitions do not take into account the patients' microbiota. This study aimed to investigate the impact of *Enterococcus* colonisation on disease severity.

MATERIALS AND METHODS

This was a case-control study of adult patients hospitalised with CDI from two health systems (14 hospitals) in Houston, Texas, USA (2016–2025). Patients with severe CDI were matched to non-severe patients (1:1) on age ±10 years and immunocompromised status. Stool samples were collected from hospitalised patients with CDI, and stool underwent DNA extraction for quantitative PCR of *E. faecalis* and *E. faecium*. Metabolomics were completed by liquid chromatography-tandem mass spectrometry. Disease severity and CDI classification were defined according to the 2017 Infectious Diseases Society of America (IDSA)/Society for Healthcare Epidemiology of America (SHEA) clinical guidelines.[5]

RESULTS

A total of 190 patients (95 matches) with CDI were included (female: 54.7%; age >65 years: 60%; hospital-acquired CDI: 42%; CDI initial episode: 87%). Patients with *Enterococcus* spp. quantity $>10^6$ were designated as high colonisation; 61% of patients were highly colonised. The group with severe disease had a higher percentage of patients categorised as having high *Enterococcus* spp. colonisation, though not statistically significant (67.5% versus 58.7%; p=0.07). While high *Enterococcus* spp. colonisation trends with IDSA disease severity, it is highly possible that other microbiota members cross-feed with *C. difficile*. Metabolomics completed on 84 matches with sufficient stool showed that patients with severe disease had significantly higher amounts of

ornithine in the stool than patients with non-severe disease (6,739 ng/mL versus 4,270 ng/mL; p=0.02).

CONCLUSION

The gut microbiota is a diverse environment with many inter-species interactions. While a trend is observed between *Enterococcus* spp. colonisation and CDI disease severity, it is highly likely that other microorganisms contribute to this association. Future metagenomic and metabolomic analysis is warranted.

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