Abstract 9817

Increased enterococcal abundance and low microbial diversity are early predictive markers of a microbiota primed for development of *Clostridioides difficile* infection

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Background: *Clostridioides difficile* infection (CDI) is the most common form of infectious antibiotic-associated diarrhoea (AAD) causing considerable morbidity and mortality in acute-care facilities. Identification of early markers predictive of CDI in hospitalized patients could substantially contribute to decreasing the CDI burden.

Materials/methods: In this European, prospective, longitudinal cohort-study including 1,007 patients aged ≥50 years receiving broad-spectrum antibiotic treatment with penicillins + beta-lactamase inhibitors, other beta-lactam antibiotics, or fluoroquinolones during hospital stay, we characterized faecal samples using high-resolution, single-nucleotide 16S rRNA gene profiling before (D1, n = 945) and after antibiotic treatment (D6, n = 737). CDI was defined according to the ESCMID diagnostic guidelines.

Results: *C. difficile* carriage was observed in 51/945 (5.4%, D1) and 50/737 (6.8%, D6) patients. Among patients who developed diarrhoea within 90 days, those with CDI (n=14) exhibited significantly lower diversity (p≤0.016) and a distinctly different microbial composition at D1 compared to those with non-*C. difficile* AAD (n=64) and no diarrhoea (n=669, 198 lost to follow-up, Figure). At D1, the microbiota was enriched for *Enterococcus* spp. in patients who later developed CDI, for Clostridiales Incertae Sedis XI, *Blautia* and *Ruminococcus* spp. in patients developing non-*C. difficile* AAD, and for *Blautia luti*, *Porphyromonas*, *Prevotella*, and *Bifidobacterium* spp. in non-diarrheic patients. Antibiotic treatment reduced microbial diversity and induced class-specific dysbiosis; beta-lactam treatment specifically increased enterococcal abundance, and fluoroquinolone treatment depleted *Prevotella* spp.

Conclusions: Our findings of a distinct, low-diversity CDI-associated microbiota can be exploited for enriching high-risk patients in prospective clinical trials and for the development of predictive, microbiota-based diagnostics for clinical management of patients at high risk of CDI.

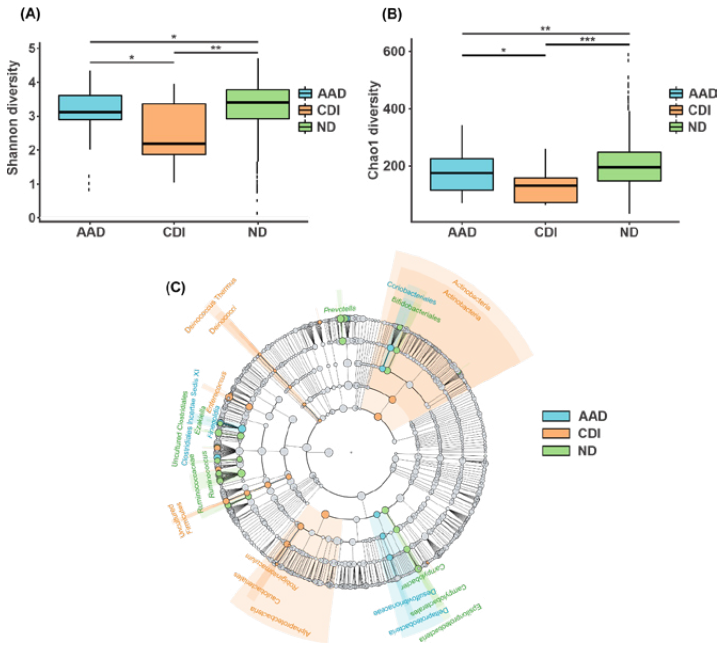

Figure. **Characterization of microbial diversity in baseline (D1) samples**. CDI patients (blue) display significantly lower alpha diversity in terms of Shannon **(A)** and Chao1 indices **(B)**, compared to AAD (light blue), and ND patients (green) at D1. Cladogram demonstrating significantly higher abundances of Actinobacteria, Alphaproteobacteria and *Enterococcus* spp. in the gut microbiota of CDI patients at baseline (D1) compared to AAD and ND patients **(C)**. AAD: patients with antibiotic-associated diarrhoea. CDI: patients with confirmed *C. difficile* infection. ND: non-diarrheic patients. *: p<0.05. **: p<0.01. ***:p<0.001.

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