Microbiota–based markers predictive of development of *Clostridioides difficile* infection

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***Clostridioides difficile* infection (CDI) is associated with considerable morbidity, mortality, and healthcare costs globally. CDI is preceded by asymptomatic *C. difficile* carriage and antibiotic-induced modulation of the intestinal microbiota is thought to initiate infection. However, there are currently no predictive markers for CDI development. Using high-resolution 16S rRNA gene-profiling we characterized longitudinally collected fecal samples from 1,007 hospitalized patients receiving broad-spectrum antibiotic treatment and identified microbiota-based markers associated with a 5-fold higher risk of CDI development. These markers may be used to develop microbiota-based diagnostics for clinical management of patients at risk of CDI.**

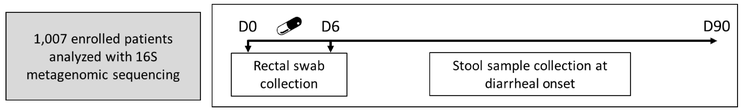
METHODS

1,007 patients receiving treatment with broad-spectrum antibiotics were enrolled in this study, where 1,002 provided fecal samples at baseline (D1), 848 after antibiotic treatment (D6), and 33 at occurrence of a first diarrheal episode (S1). All samples underwent targeted 16S rRNA gene paired-end sequencing on a MiSeq instrument (Illumina Inc., USA). Data analysis with OCToPUS (1) rendered 945, 775, and 32 successfully sequenced samples at D1, D6, and S1, respectively.

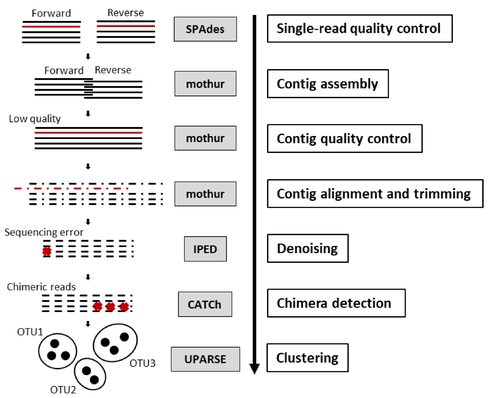
Definitions:

AAD: Patients with *C. difficile*-negative antibiotic-associated diarrhea
CDI: Patients with clinically confirmed CDI
ND: Non-diarrheic patients

Sample collection:



Data analysis:



RESULTS

Microbiota-based biomarkers predictive of CDI were identified by comparing microbial diversity and composition of the human intestinal microbiota at D1 in patients who later during the 90-day study period developed CDI and AAD with ND patients. Among patients who developed diarrhea 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 diarrhea (n=669, 198 lost to follow-up). 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 (**Fig. 1**).

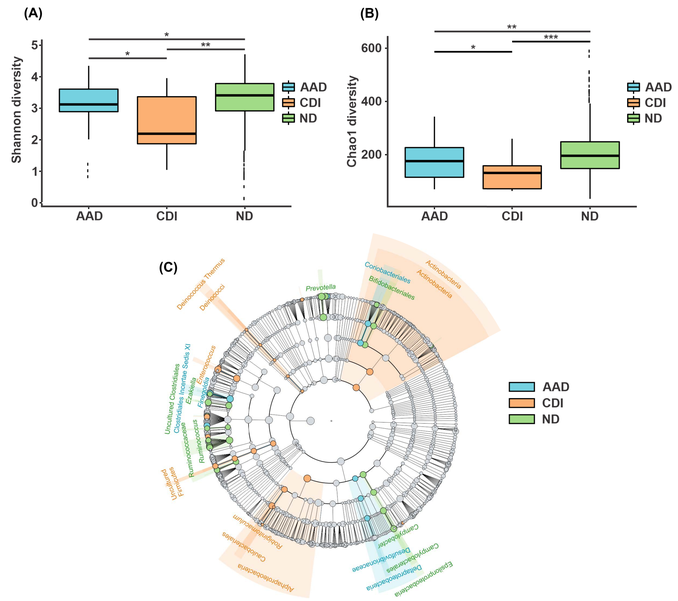


Figure 1: **Characterization of microbial diversity in baseline (D1) samples.** Microbial diversity and composition was compared in patients who later developed CDI (n = 14, blue), AAD (n = 64, green), and ND patients (n = 669, 198 patients excluded due to loss of follow-up, death, or lack of CDI testing). Patients who later developed CDI harbored a microbiota with distinctly lower alpha diversity described by the Shannon **(A)** and Chao1 indices **(B)**, compared to AAD, and ND patients at D1. Microbial composition, described by the Jaccard beta diversity index, was compared between CDI, AAD, and ND patients at D1 using LEfSe (2), where an LDA score > 2.0 and p < 0.05 was considered distinct **(C)**. *: p<0.05. **: p<0.01. ***: p<0.001.

Microbial composition was compared a baseline (D1), after treatment with broad-spectrum antibiotics (D6), and at occurrence of the first diarrheal episode (S1) in patients developing AAD (n = 26). A severely disrupted microbiota is observed in patients with diarrhea (**Fig. 2**, AMOVA, p<0.001).

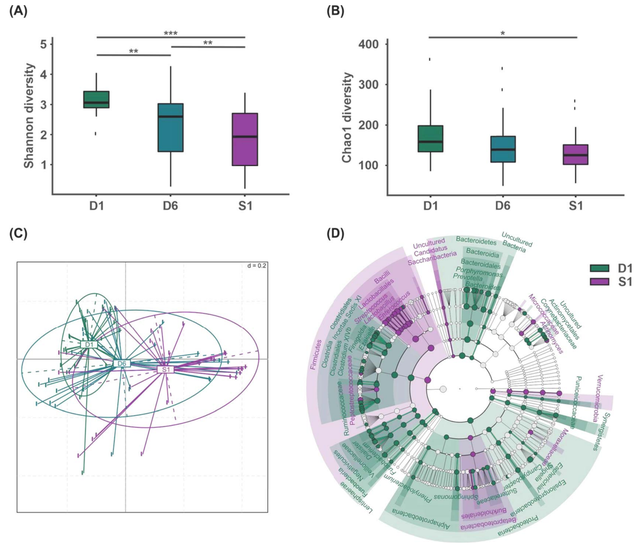


Figure 2: **Longitudinal analysis of microbial diversity and dysbiosis in patients developing AAD.** A gradual decrease in Shannon diversity **(A)** is observed for AAD patients (n = 26) between all timepoints. A similar trend is observed in Chao1 diversity between D1 and S1 in patients who develop AAD **(B)**. Multi-dimensional scaling (MDS) shows a shift in microbial composition between timepoints in patients who developed AAD **(C)**, and LEfSe revealed a microbiota with large rearrangements in the Firmicutes and Proteobacteria phyla during AAD. Proteobacteria members are depleted, and a shift is observed from Clostridia to Bacilli members at occurrence of AAD. **(D)**. *: p<0.05. **: p<0.01. ***: p<0.001.

CONCLUSIONS

Here, we have identified biomarkers predictive of the development of CDI. Future applications include enrichment of high-risk patients in prospective clinical trials, development of predictive, microbiota-based diagnostics to tailor antibiotic therapy or biobanking stools from high-risk patients prior to antibiotic therapy, exemplifying a precision medicine approach.

REFERENCES

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