QUANTITATIVE CHARACTERIZATION OF *Clostridioides difficile* POPULATION IN THE GUT MICROBIOME OF PATIENTS WITH *C. difficile* INFECTION AND THEIR ASSOCIATION WITH CLINICAL FACTORS

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Abstract

Background: Objective was to analyse bacterial composition and abundance of *C. difficile* in gut microbiome of patients with *C. difficile* infection (CDI) in association with clinical characteristics.

Methods: Whole metagenome sequencing of gut microbiome of 26 CDI patients was performed, and the relative abundance of *C. difficile* and its toxin genes was measured Clinical characteristics of the patients were obtained through medical records.

Results: A strong correlation between the abundance of *C. difficile* and *tcdB* genes in CDI patients was found. The relative abundance of *C. difficile* in the gut microbiome ranged from undetectable to 2.8% (median 0.089) . Patients with fever exhibited low abundance of *C. difficile* in their gut, and patients with fewer *C. difficile* organisms required long-term anti-CDI treatment. Abundance of *Bifidobacterium* and *Bacteroides* negatively correlated with that of *C. difficile* at the genus level. CDI patients were clustered using the bacterial composition of the gut: one with high population of *Enterococcus* (cluster 1, n=12) and another of *Bacteroides* or *Lactobacillus* (cluster 2, n=14). Cluster1 showed significantly lower bacterial diversity and clinical cure at the end of treatment.Additionally, patients with CDI exhibited increased ARGs notably, bla_{TEM}, bla_{SHV} and bla_{CTX-M} were enriched.

Conclusion: *C. difficile* existed in variable proportion of the gut microbiome in CDI patients. CDI patients with *Enterococcus*-rich microbiome in the gut had lower bacterial diversity and poorer clinical cure.

Table 1. Demographic data and clinical characteristics of patients of *Clostridioides difficile* infection

Demographics & underlying diseases		
Age	median (1Q, 3Q)	66.5 (59.8, 76.3)
Gender	female - N (%)	13 (50)
Hospital day	median (1Q, 3Q)	18.5 (7.8, 33.5)
Charlson comorbidity index	median (1Q, 3Q)	3 (1, 5)
Chronic obstructive pulmonary diseases	N (%)	4 (15.4)
Asthma	N (%)	1 (3.8)
Malignancy	N (%)	7 (26.9)
Diabetes mellitus	N (%)	7 (26.9)
Chronic kidney disease	N (%)	6 (23.1)
Haemodialysis	N (%)	4 (15.4)
History within 2 months - Yes		
Admission	N (%)	16 (61.5)
Use of antibiotics	N (%)	24 (92.3)
Use of proton pump inhibitor	N (%)	13 (50)
Use of probiotic	N (%)	5 (19.2)
Clinical findings		
WBC	median (1Q, 3Q)	11000 (7600, 16350)
Albumin	median (1Q, 3Q)	2.7 (2.3, 3.1)
Body temperature	median (1Q, 3Q)	37.2 (37, 38)
Leukocytosis[1]	N (%)	7 (26.9)
Hypoalbuminemia[2]	N (%)	10 (38.5)
Fever[3]	N (%)	3 (11.5)
Acute kidney injury[4]	N (%)	2 (7.7)
Severity score		
2 factors[5]	median (1Q, 3Q)	0 (0, 1)
Severe CDI by 2 factors	N (%)	8 (30.8)
4 factors[6]	median (1Q, 3Q)	1 (1, 2)
Severe CDI by 4	N (%)	12 (46.2)
Toxin assay A&B	positive equivocal negative	22 (84.6) 3 (11.5) 1 (3.8)
Medication - initial	N (%)	N=26
Metronidazole		20 (76.9)

Table 2. Clinical findings associated with relative abundance of *C. difficile* in gut microbiome

	rho	*p*-value
Demographics & underlying diseases		
Age	0.11	0.592
Charlson comorbidity index	0.155	0.45
Chronic obstructive pulmonary diseases	-0.078	0.704
Malignancy	0.318	0.113
Diabetes mellitus	-0.023	0.911
Chronic kidney disease	-0.104	0.615
History within 2 months - Yes		
Admission	-0.026	0.898
Use of antibiotics	0	1
Use of proton pump inhibitor	-0.144	0.484
Use of probiotics	0.137	0.505
Clinical findings		
Leukocytosis[1]	0.012	0.955
Hypoalbuminemia[2]	0.084	0.682
Fever[3]	-0.41	0.038
Acute kidney injury[4]	-0.173	0.397
Severity score		
Severe CDI by 2 factors[5]	-0.006	0.978
Severe CDI by 4 factors[6]	-0.031	0.881
Toxin test		
Ct value of real time PCR for *tcdB*	-0.605	0.002
Toxin assay A&B	0.18	0.379
Treatment duration for CDI	-0.405	0.05
Clinical response		
Mortality	0.246	0.247
Recurrence	-0.093	0.666
Global cure	0.073	0.76

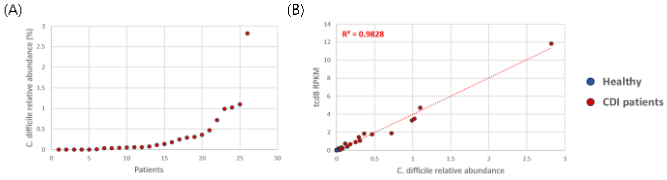

Figure 2. Relative abundance of *Clostridioides difficile* in the gut microbiome of 26 patients with CDI. (A) Distribution of *tcdB* abundance in the gut microbiota of 26 CDI patients; it ranges from 0% to 2.82%. (B) Correlation between *tcdB* abundance and abundance of *C. difficile* based on clade-specific marker genes.

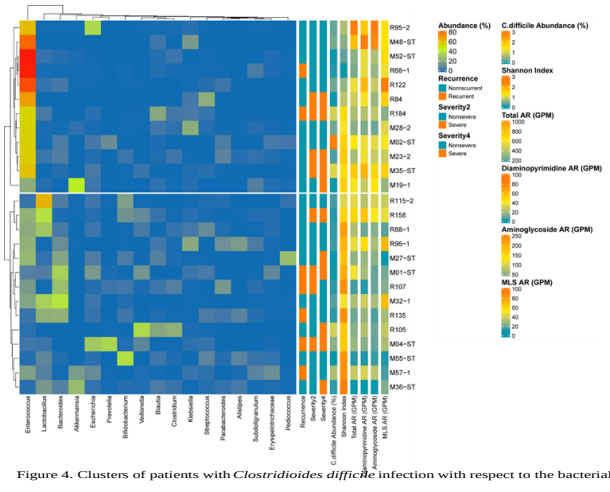

Figure 4. Clusters of patients with *Clostridioides difficile* infection with respect to the bacterial composition.

Table 3. Comparison of clinical characteristics between the cluster with high abundance of *Enterococcus* (cluster 1, n=12) and the cluster with high abundance of *Bacteroides* or *Lactobacillus* (cluster 2, n=14) among *C. difficile* infections

Clinical characteristics		*Enterococcus* N=12	*Bacteroides* N=14	*p* value
Age	Median (1Q, 3Q)	67 (59.3, 76.8)	66.5 (60.5, 76.8)	1*
Female	N (%)	4 (33.3)	9 (64.3)	0.116
Charson comorbidity index	Median (1Q, 3Q)	1.5 (1, 4.5)	3 (2, 5.3)	0.13*
Malignancy	N (%)	1 (8.3)	6 (42.9)	0.081
Diabetes mellitus	N (%)	3 (25)	4 (28.6)	1
Chronic kidney disease	N (%)	2 (16.7)	4 (28.6)	0.652
Leukocytosis[1]	N (%)	4 (33.3)	3 (21.4)	0.665
Hypoalbuminemia[2]	N (%)	6 (50)	4 (28.6)	0.422
Fever[3]	N (%)	0	3 (21.4)	0.225
Acute kidney injury[4]	N (%)	1 (8.3)	1 (7.1)	1
Hemodialysis	N (%)	0	4 (28.6)	0.1
Severe CDI	N (%)			
by 2 factors[5]		4 (33.3)	4 (28.6)	1
by 4 factors[6]		5 (41.7)	7 (50)	0.713
Treatment duration	Median (1Q, 3Q)	10 (7, 15)	14 (10, 16.5)	0.268*
Toxin assay A&B-positive	N (%)	10 (83.3)	12 (85.7)	0.579*
Relative abundance of *C. difficile* (RPKM)	Median (1Q, 3Q)	0.25549 (0.025646, 0.79769)	0.05952 (0.00629, 0.26057)	0.129*
Medical history				
Admission history within 2 months	N (%)	7 (58.3)	9 (64.3)	1
Proton pump inhibitor intake	N (%)	3 (25)	10 (71.4)	0.018
Probiotics intake	N (%)	3 (25)	2 (14.3)	0.635
History of antibiotics usage within 2 months	N (%)	12 (100)	12 (85.7)	0.483
Exposed days of antibiotics	Median (1Q, 3Q)	18 (8.5, 41.3)	8.5 (3.8, 23)	0.143*
Total days of antibiotics (sum of days for each antibiotics)	Median (1Q, 3Q)	22.5 (10.5, 66.3)	11 (3.8, 30.8)	0.089*
Class of antibiotics	N (%)			
Broad spectrum cephalosporin		6 (50)	9 (64.3)	0.462
Fluoroquinolones		5 (41.7)	1 (7.1)	0.065
Carbapenem		5 (41.7)	4 (28.6)	0.683
ß-lactam/ß-lactamamase inhibitor		5 (41.7)	6 (42.9)	0.951
Glycopeptides		2 (16.7)	3 (21.4)	1
Clinical response	N (%)	N=11	N=13	
Cure at the end of treatment		7 (63.6)	13 (100)	0.031
Failure		1 (9.1)	0	