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Gut microbiota in recurrent *Clostridium difficile*-associated diarrhea

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ARROW DIAGNOSTICS

Background and Aim

Most cases of *Clostridium difficile* infection (CDI) respond initially, recurrence (rCDI) after the discontinuation treatment can occur (1). Old age, use of antibiotics, gastric acid suppression, and infection with a hypervirulent strain are currently regarded as the major risk factors for rCDI (2). The composition of gut microbiota is affected by antibiotic treatments especially with metronizadole and vancomycin, promoting CDI (2). In adults, healthy microbiota is composed mostly by Bacteroidetes and Firmicutes usually, whereas Actinobacteria, Proteobacteria and Verrucomicrobia are generally minor constituents (3).

This study aimed to compare the gut microbiota of: - patients with rCDI and patients with a single CDI episode at the first episode (T_0);

- patients with rCDI during the first and second episode.

Materials and Methods

The faeces of 11 patients, 4 with a single CDI episode and 7 with a rCDI, were collected. In all samples the presence of *Clostridium difficile* and its toxins was searched through a three-step algorithm (Figure 1). The V1-V3 regions of 16S rRNA gene were amplified, sequenced and analysed by Arrow Diagnostics service using Microbiota Solution A kit and SmartSeq software.

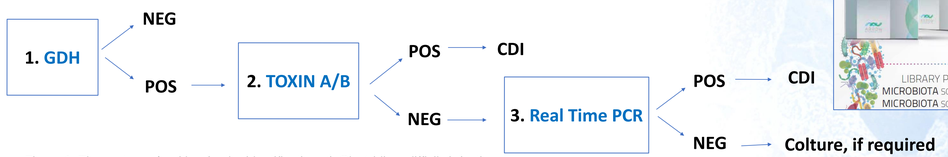
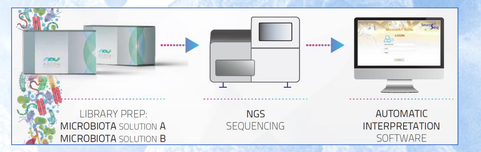


Figure 1. Three-steps algorithm for the identification of *Clostridium difficile* infections.



Population characteristic

PATIENT	EPISODE	AGE	DAYS FROM THE ADMISSION	ΔT BETWEEN 1ST AND 2ND EPISODES	HOSPITAL WARD	GDH (BIOHIT Health Care)	Tox (Meridian Bioscience Inc.)	PCR (Gene Xpert Cepheid)	TYPE OF STOOL	REASON FOR THE HOSPITALIZATION	THERAPY BEFORE DIARRHEA	THERAPY AFTER DIARRHEA
1	I	54	1	45	General Medicine	POS	Negative	POS	FSL	Fever > 38 °C	MEM, PIP/TAZ, VAN	MTZ, VAN
	II	54	48		General Medicine	POS	POS	-	FSL	Fever > 38°C	PIP/TAZ	PIP/TAZ, VAN
2	I	64	3	44	General Medicine	POS	POS	-	FSL	Sepsis	MEM, PIP/TAZ	MEM, VAN
	II	64	8		Infectious Diseases	POS	POS	-	FSL	Asthenia, anorexia	MEM, PIP/TAZ, VAN	MI, VAN
3	I	80	14	66	General Medicine	POS	POS	-	FL	Respiratory failure	-	VAN, Fidaxomicin
	II	80	NA		Lab Analysis	POS	POS	-	FSL		Not available	Not available
4	I	75	0	57	Emergency Medicine	POS	POS	-	FSL	Diarrhea	LEV	AZM, PIP/TAZ, VAN
	II	75	0		Emergency Medicine	POS	POS	-	Fecal Swab			
5	I	71	59	39	Anesthesiology and Reanimation	POS	POS	-	FSL	Heart failure	CN, CL, MEM	VAN, FLU
	II	71	96		Anesthesiology and Reanimation	POS	POS	-	FSL	Heart failure		
6	I	83	NA	48	Lab Analysis	POS	POS	-	FSW		Not available	Not available
	II	83	NA		Lab Analysis	POS	POS	-	FSW		Not available	Not available
7	I	82	1	29	General Medicine	POS	POS	-	FSL	Fever	PIP/TAZ	VAN
	II	82	2		Infectious Diseases	POS	POS	-	SL	Fever	PIP/TAZ	MEM, SXT, VAN
8	I	56	14	NA	Urology	POS	POS	-	LIQ	Sepsis		VAN
9	I	76	30	NA	Infectious Diseases	POS	Negative	POS	SS	Cirrhosis	PIP/TAZ	CIP, CRO, TEC, VAN
10	I	52	0	NA	Obstetrics – Gynaecology	POS	POS	-	FSW	Fever >38°C, diarrhea	LEV	MTZ, VAN
11	I	93	0	NA	General Medicine	POS	Negative	POS	SL	Thighbone fracture	PIP/TAZ	VAN

AZM=Azithromycin, CIP=Ciprofloxacin, CL= Colystin, CN=Gentamycin, CRO= Ceftriaxone, FLU= Fluconazol LEV= Levofloxacin, MEM=Meropenem, MI=Minocycline, MTZ= Metronidazole, PIP/TAZ=Piperacillin/tazobactam, SXT= Trimethoprim/sulfamethoxazole, TEC=Teicoplanin, VAN= Vancomycin

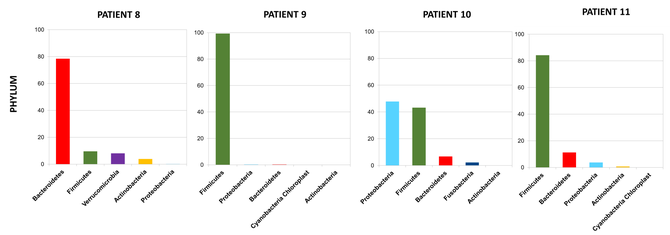
8/11 were male, 10 were hospitalized.

The median time between the first episode and the rCDI was 44 days (range 29-66).

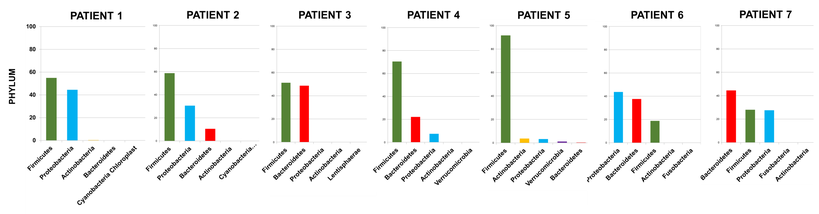
6/7 patients with rCDI were treated with vancomycin for 10 days after the first episode.

Microbiota 1° episode of CDI

PATIENTS WITH A SINGLE EPISODE:



PATIENTS WITH rCDI:



3/4 (75%) patients with a single episode and 5/7 (71.4%) with rCDI showed scarce biodiversity with a high prevalence of Bacteroidetes and Firmicutes especially belonging to Bacteroidales and Clostridiales orders up to 90% at T_0. High level of Proteobacteria (>20%) are observed in 4/7 patients with rCDI and in 1/4 patient with a single episode. Dysbiosis is commonly associated with the presence of Proteobacteria that usually increase in gut diseases (celiac disease). No correlation was found in the microbiota composition between the CDI and rCDI groups (p>0.05).

GENUS	%	Number of patients
Enterococcus	55-70	1
	40-55	1
	15-25	1
Clostridium XI	40-55	1
	15-25	1
	5-15	1
Bacteroides	40-55	1
	5-15	1
Escherichia/Shigella	40-55	1
Alistipes	15-25	1
Akkermansia	5-15	1
Rikenella	5-15	1
Parabacteroides	0-5	1
Streptococcus	0-5	2
Enterobacter	0-5	1
Anaerococcus	0-5	1
Butyricimonas	0-5	1
Delftia	0-5	1
Lactobacillus	0-5	1

Figure 2. The most frequently isolated genera for patients with CDI.

GENUS	%	Number of patients
Enterococcus	40-55	2
	5-15	2
Bacteroides	40-55	3
	15-25	1
Klebsiella	25-40	2
	15-25	1
	5-15	1
Clostridium XI	25-40	1
	5-15	1
	0-5	1
Veillonella	25-40	1
	5-15	1
Lactobacillus	15-25	1
	5-15	1
Alistipes	5-15	2
Clostridium XIVa	5-15	2
ClostridiumXVIII	0-15	2
Blautia	15-25	1
Faecalibacterium	15-25	1
Hafnia	15-25	1
Proteus	5-15	1
Clostridium IV	0-5	1
Eggerthella	0-5	1
Enterobacter	0-5	1
Escherichia/Shigella	0-5	1
Turicibacter	0-5	1

Figure 3. The most frequently isolated genera for patients with rCDI.

Microbiota 1° episode vs. 2° episode in patients with rCDI

PATIENT 1:

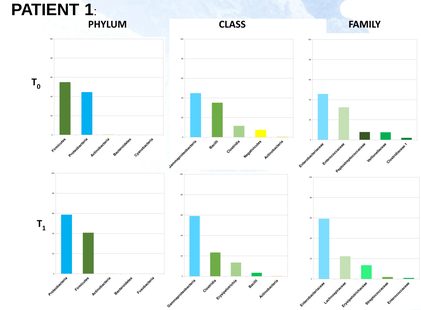


Figure 4. The prevalence of the 5 most frequently isolated phyla, classes and families for patient 1.

3/7(43%) patients showed an increase of Proteobacteria and Clostridium XIVa at T_1, and a decrease of Firmicutes in particular Clostridium XI. In 1 of these patients also Bacteroidetes decreased.

In 1/7 patient the microbiota remained stable throughout the two episodes.

In 3/7 (43%) patients Firmicutes increased up to the double and Proteobacteria decreased. In 2 of these patients (57.1%) Bacteroidetes decreased, while in only 1/7 patient remained stable.

Conclusion

Our patients with CDI and rCDI presented low level of microbial diversity. In particular the reduction in the genera *Faecalibacterium, Roseburia* and *Eubacterium* decreased short chain fatty acids that are necessary to regulate the gut tropism. Moreover, the dysbiosis can contribute to the loss of colonization resistance against *C. difficile*.

References

(1) Seekatz et al., 2016
(2) Song et al., 2018
(3) Lozupone et al., 2012

Acknoledgement

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