P0805

In vitro activity of surotomycin (formerly CB-183,315) against 126 clinical isolates of *Clostridium difficile*

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Background

Clostridium difficile is the most common cause of healthcare related diarrhea, usually associated with prior antimicrobial agents. Treatment of *C. difficile* infections (CDI) remains sub-optimal because of the unsatisfactory efficacy of current therapeutics and the high rate of recurrences.
Surotomycin (formerly CB-183,315) is a novel cyclic lipopeptide antibiotic. This new antibiotic is currently in phase 3 clinical development for CDI.

Objectives

The objective of the present study was to assess the antimicrobial susceptibility of *C. difficile* strains isolated in a period of time spanning from 2005 to 2013 to surotomycin, metronidazole, vancomycin, rifampin, clindamycin and fidaxomicin.

Materials and Methods

Strains

- 116 epidemiologically unrelated and non-repetitive toxigenic strains of *C. difficile* isolated from patients with *C. difficile* infection (CDI) were selected in order to be representative of the genotypes circulating in France (Eckert *et al.,* 2013)
- 10 non-toxigenic isolates with decreased susceptibility to metronidazole (DSM) were also included.
- Strains were characterized by PCR ribotyping and toxinotyping

Method

- Susceptibility testing was performed by agar dilution method following CLSI recommendations (CLSI M11 A7)
- **Medium** : Brucella agar supplemented with 0.01% Hemin, 0.05% vitamin K1, 5% lysed sheep blood. All assay media for testing surotomycin were supplemented with a final concentration of 50 mg /liter calcium.
- **Inoculum** : 0.5-1 McFarland standard
- **Application** : Uridot (1µL/spot) in duplicate
- *Clostridium difficile* ATCC 700057, *Bacteroides thetaiotamicron* ATCC 29741, *Eubacterium lentum* ATCC 43055 were used as QC strains
- **Incubation** : 48h in anaerobic atmosphere at 35-37°C.
- **Antibiotics** (see Table 1)

Table 1 : Origin of antibiotics and preparation of stock solution

Antibiotics (ATB)	Laboratory	Concentration of stock solution (g/L)	Diluant	ATB presentation	Range of tested concentration (mg/L)
Vancomycin (VAN)	Mylan	0.64	Water	Powder	0.016 – 64
Metronidazole (MTZ)	Farchemia	12.8	DMSO	Powder	0.016 – 32
Clindamycin (CLI)	Mylan	5.12	-	Solution	0.016 – 256
Rifampicin (RIF)	Sanofi	0.64	Water	Powder	0.002 – 32
Fidaxomicin (FDX)	Astellas	6.4	DMSO	Powder	0.002 – 32
Surotomycin (SUR)	Cubist	3.2	Water	Powder	0.016 – 16

Results

Table 2: Characterization of the strains

Strains	No. of strains	PCR-Ribotyping	Binary toxin	Toxinotype
Toxigenic strains	10	001	T	0
	11	002	T	0
	10	003	T	0
	10	005	T	0
	10	015	T	0
	10	017	N	VIII
	10	023	Y	IV
	12	027	Y	III
	7	106	T	0
	14	014/020/077	T	0
	12	78/126	Y	V
DSM Strains	10	010	N	-

T = truncated binary toxin (pseudogenes) Y= Yes N = No

Table 3: Distribution of MICs to the different antibiotics.

	PCR-ribotype	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Breakpoints ((mg/L)	%R
VAN	All strains	0.062 – 2	0.25	1	>2*	0
	023	0.062 – 1	0.5	1		0
	078/126	0.25 – 2	0.5	0.5		0
	Other	0.062 - 2	0.25	1		0
MTZ	All strains	0.062 – 8	0.5	1	>2*	1.8
	001	0.5 - 8	0.5	1		10
	027	0.25 - 4	0.5	2		8.3
	Other	0.062 - 2	0.25	0.5		0
CLI	All strains	8 - ≥ 256	≥ 256	≥256	>4**	100
	027	256 - ≥ 256	≥ 256	≥256		100
	014/020/077	8 - 256	128	≥256		100
	Other	8 - 256	256	≥256		100
RIF	All strains	≤ 0.002 - 32	≤ 0.002	0.0078	>16**	3.7
	001	0.002 – 32	≤ 0.002	0.002		10
	017	0.002 – 32	0.0039	≥ 32		30
	Other	0.002 - 2	0.002	0.0039		0
FDX	All strains	0.062 – 1	0.5	0.5		
	023	025 - 1	1	1		
	001	0.062 – 0.25	0.25	0.25		
	Other	0.125 - 1	0.5	0.5		
SUR	All strains	0.125 - 2	0.5	1		
	106	1 - 2	1	2		
	078	0.5-2	1	2		
	017	0.125 – 0.5	0.5	0.5		
	023	0.25-1	0.5	0.5		
	Other	0.125 - 2	0.5	1		

* According to EUCAST ** According to CA-SFM

- Characterization of the strains are described in **Table 2** and MIC_{50}, MIC_{90} and MIC range of each antimicrobial agent are described in **Table 3.**
- All clinical toxigenic *C. difficile* isolates were highly susceptible to surotomycin and the MIC distribution was unimodal. Isolates belonging to PCR-ribotypes 106 and 078 had four folds higher surotomycin MIC_{90} values than isolates belonging to ribotypes 017 and 023.
- Overall, 1.8 % of isolates exhibited a decreased susceptibility to metronidazole (MIC >2 mg/L), all from ribotypes 027 or 001.
- All isolates were resistant to clindamycin and 3.7% were resistant to rifampicin (from ribotypes 001 and 017).
- Non-toxigenic isolates with reduced susceptibility to metronidazole had MICs ranging from 4 to 16 mg/L. The activity of surotomycin against these isolates was the same as against any of the other isolates tested with a MIC_{50} and a MIC_{90} of 0.5 mg/L and 1 mg/L, respectively (**Table 4**).

Table 4: Distribution of MICs of strains with decreased susceptibility to metronidzole (DSM) to the different antimicrobial agents.

	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
CLI	256 – 256	256	256
RIF	≤0.002 – 0.0078	0.0039	0.0078
FDX	0.25 – 0.5	0.5	0.5
SUR	0.125 – 2	0.5	1

Conclusions

There was no evidence of *in vitro* resistance of *Clostridium difficile* to surotomycin tested against 116 clinical isolates in this study. Surotomycin maintained low MICs (including on strains with decreased susceptibility to metronidazole) regardless of the PCR-ribotype.

References

CLSI. Methods for antimicrobial suseptibility testing of anaerobic bacteria. 7ed. Approved standard M11-A7. Clinical Laboratory Standards Institute. Wayne. PA

Eckert C., et al. Médecine et Maladies Infectieuses, 2013 Feb;43(2):67-74

This study was funded by Cubist Pharmaceuticals