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Cadazolid Activity against Contemporary Antibiotic-resistant *Clostridium difficile* Isolates and Prevalent Ribotypes from Europe

IHMA Europe Sàrl
Rte de l'Ile-au-Bois 1A
1870 Monthey
Switzerland
imorrissey@ihmainc.com

Ian Morrissey[1], Mark Wilcox[2], Cyntia De Piano[1], Sophie Magnet[1], Stephen Hawser[1], Timothy W. Morris[3], Hans H. Locher[3]
[1]IHMA Europe Sàrl, Monthey, Switzerland; [2]Clostridium difficile Ribotyping Network for England and Northern Ireland (CDRN) Reference Laboratory, Leeds, UK; [3]Actelion Pharmaceuticals Ltd, Allschwil, Switzerland.

INTRODUCTION

- *Clostridium difficile* has become a leading cause of nosocomial diarrhoea worldwide.
- Hypervirulent *C. difficile* strains, such as ribotypes 027 and 078 have emerged which display increased resistance to a number of antibiotics and produce binary toxin in addition to toxin A and B (1). Infection caused by these strains has been associated with increased disease severity and mortality (2).
- Cadazolid is a novel antibiotic which combines quinolone and oxazolidinone moieties into a new class of antibacterial agents referred to here as quinoxolidinones and is in Phase 3 clinical development for the treatment of *C. difficile* associated diarrhoea (CDAD), also known as *C. difficile* infection (CDI).
- In the Phase 2 trial for treatment of CDAD, cadazolid clinical cure rates were similar to vancomycin with lower recurrence rates, resulting in higher sustained cure rates (3).
- This study evaluated the activity of cadazolid against recent clinical isolates of *C. difficile* from Europe based on resistance to other antibiotics and prevalent ribotypes.

METHODS

- A total of 652 clinical isolates of *C. difficile* were collected in 2014/2015 from European hospitals.
- The hospitals were located in Belgium (1 site, 20 isolates), Czech Republic (2, 41), France (9, 127), Germany (3, 166), Hungary (1, 29), Poland (1, 20), Romania (3, 59), Spain (5, 115), Sweden (1, 21) and the United Kingdom (3, 54)
- Minimum inhibitory concentrations (MICs) for cadazolid and antibiotic comparators were determined by agar dilution following Clinical and Laboratory Standards Institute (CLSI) guidelines (4). MIC_{50} and MIC_{90} (concentrations to inhibit 50% & 90% of isolates, respectively) were calculated.
- Susceptibility was evaluated using CLSI breakpoints for anaerobes (5), except for vancomycin and metronidazole where The European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for *C. difficile* derived from epidemiological cut-off values were used (6). Breakpoints for *C. difficile* are not based on clinical efficacy in treatment of CDAD.
- Isolates defined as having 'higher' linezolid MICs were those with MIC ≥8 mg/L (based on the CLSI breakpoint for Gram-positive aerobes).
- All isolates were ribotyped by PCR amplification of the 16S-23S intergenic spacer regions and size of fragments determined, essentially as described previously (7), with modifications. PCR-ribotypes were assigned by comparison with those in the CDRN UK PCR-ribotype library.

References

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Table 1. Antibiotic breakpoints used in this study.

Antibiotic	Breakpoint (mg/L):			Source
	Susceptible	Intermediate	Resistant	
Cadazolid	NB	NB	NB	-
Vancomycin	≤2	-	≥4	EUCAST (6)
Metronidazole	≤2	-	≥4	EUCAST (6)
Fidaxomicin	NB	NB	NB	-
Rifaximin	NB	NB	NB	-
Tigecycline	NB	NB	NB	-
Clindamycin	≤2	4	≥8	CLSI (5)
Imipenem	≤4	8	≥16	CLSI (5)
Linezolid	NB	NB	NB	-
Moxifloxacin	≤2	4	≥8	CLSI (5)

NB, no breakpoint available.

RESULTS

- Cadazolid was very active with a narrow range of MICs between 0.12 and 1 mg/L against all isolates (Table 2). Only one isolate from Spain (ribotype 010) had an MIC of 1 mg/L.
- Resistance was very high to imipenem (92.0%, 600 isolates) and clindamycin (76.4%, 498 isolates) and also high to moxifloxacin at 39.7% (259 isolates). Only 38 (5.8%) isolates had 'higher' linezolid MICs (MIC ≥8 mg/L).
- Cadazolid MIC distributions were not affected by resistance to other antibiotics (Figures 1-4)
- Overall, 107 different ribotypes were found with the hypervirulent ribotype 027 being the most common (100 isolates, 15.3%). The two next most common ribotypes were 014 (53 isolates, 8.1%) and 001 (49 isolates, 7.5%). Ribotype prevalence (where N≥10) is shown in Figure 5.
- Cadazolid MIC_{50} and MIC_{90} against the hypervirulent 027 ribotype (Table 3) was the same as against the collection as a whole (Table 2). Based on MIC_{90} cadazolid was more active than vancomycin, metronidazole and fidaxomicin against this ribotype (Table 3).
- Virtually all the 027 ribotype isolates (97%) were resistant moxifloxacin.
- The activity of cadazolid was very consistent against other ribotypes with MIC_{50} of 0.25 or 0.5 mg/L and MIC_{90} of 0.5 mg/L (data not shown).

Figure 5. Prevalence of *C. difficile* ribotype in European isolates.

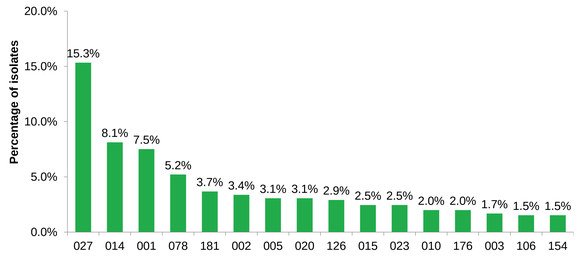

Figure 1. Cadazolid MIC distribution against **imipenem-resistant** (MIC ≥16 mg/L) versus imipenem-non-resistant (MIC ≤8 mg/L) *C. difficile*.

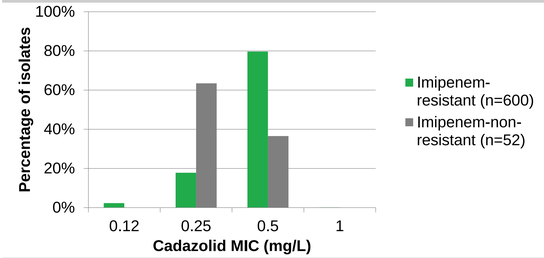

Figure 2. Cadazolid MIC distribution against **clindamycin-resistant** (MIC ≥8 mg/L) versus clindamycin-non-resistant (MIC ≤4 mg/L) *C. difficile*.

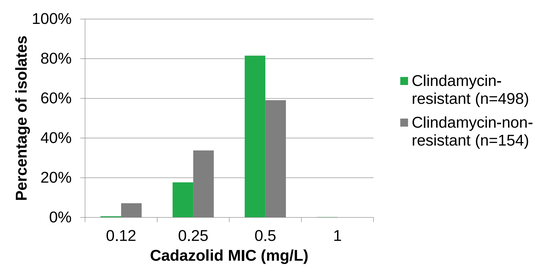

Figure 3. Cadazolid MIC distribution against **moxifloxacin-resistant** (MIC ≥8 mg/L) versus moxifloxacin-non-resistant (MIC ≤4 mg/L) *C. difficile*.

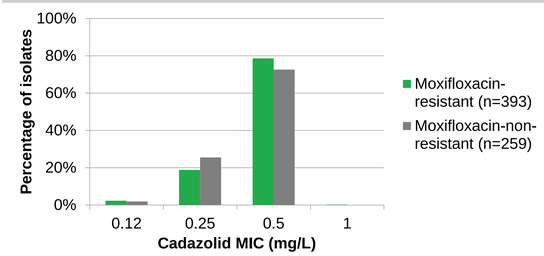

Figure 4. Cadazolid MIC distribution against **'higher' linezolid MIC** (≥8 mg/L) versus 'lower' linezolid MIC (≤4 mg/L) *C. difficile* .

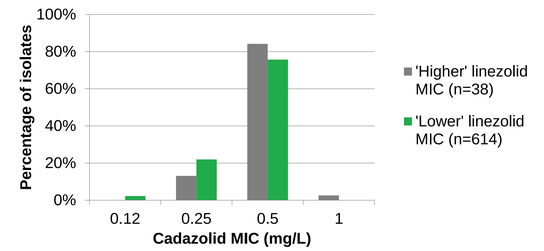

Table 2. Activity of cadazolid and comparators against all clinical isolates of *C. difficile* (n=652).

Compound	MIC (mg/L):				Percentage		
	MIC_{50}	MIC_{90}	Min	Max	Sus	Int	Res
Cadazolid	**0.5**	**0.5**	**0.12**	**1**	-	-	-
Vancomycin	1	2	≤0.25	4	97.2	-	2.8
Metronidazole	0.5	1	≤ 0.06	4	99.9	0.0	0.2
Fidaxomicin	0.25	0.5	0.03	1	-	-	-
Rifaximin	0.015	> 16	≤0.002	> 16	-	-	-
Tigecycline	0.12	0.12	≤0.015	2	-	-	-
Clindamycin	8	> 32	≤0.06	> 32	7.2	16.4	76.4
Imipenem	> 16	> 16	4	> 16	0.6	7.4	92.0
Linezolid	2	4	≤0.25	32	-	-	-
Moxifloxacin	2	32	≤0.5	> 32	59.8	0.5	39.7

Table 3. Activity of cadazolid and comparators against ribotype 027 *C. difficile* (n=100).

Compound	MIC (mg/L):				Percentage		
	MIC_{50}	MIC_{90}	Min	Max	Sus	Int	Res
Cadazolid	**0.5**	**0.5**	**0.12**	**0.5**	-	-	-
Vancomycin	1	2	0.5	4	91.0	-	9.0
Metronidazole	1	2	0.25	2	100	0.0	0.0
Fidaxomicin	0.5	1	0.06	1	-	-	-
Rifaximin	> 16	> 16	0.015	> 16	-	-	-
Tigecycline	0.12	0.12	0.03	0.25	-	-	-
Clindamycin	8	> 32	4	> 32	0.0	17.0	83.0
Imipenem	> 16	> 16	8	> 16	0.0	2.0	98.0
Linezolid	2	8	1	16	-	-	-
Moxifloxacin	16	32	2	> 32	3.0	0.0	97.0

Sus, susceptible; Int, intermediate; Res, resistant.

CONCLUSIONS

- **Cadazolid showed consistent activity against European *C. difficile* isolates irrespective of country of origin or ribotype, including the hypervirulent ribotypes 027 and 078.**
- **Cadazolid activity was not affected by resistance to imipenem, clindamycin or moxifloxacin; or by raised linezolid MIC.**
- **The consistent activity of cadazolid against 2014/2015 European isolates supports its continued investigation as a new therapy for *C. difficile*-associated diarrhoea.**

Acknowledgments

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