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Evaluation of Clinical and Laboratory Standard Institute (CLSI) Quality Control (QC) Ranges for Cadazolid.

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INTRODUCTION

- Cadazolid is a novel quinolonyl-oxazolidinone antibiotic (Fig. 1) currently in phase 3 clinical development for treatment of *Clostridium difficile*-associated diarrhoeae (CDAD).
- Cadazolid is also active against other Gram-positive bacteria, including quinolone and linezolid-resistant strains.
- In previous studies cadazolid showed potent *in vitro* activity against *C. difficile* clinical isolates (1-6), and in a human gut model of CDAD, while having only a very limited impact on bacteria of the normal gut microflora (5).
- In Phase 1 studies this compound was well tolerated with very low systemic exposures resulting in high concentration in the colon (7).
- Recently, a Phase 2 trial in CDAD showed clinical cure rates similar to vancomycin while having lower recurrence rates, resulting in higher sustained cure rates (8).
- Here we present Tier 2 QC data for *C. difficile* ATCC 700057, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213.

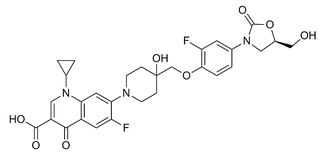

Figure 1. Chemical structure of cadazolid

METHODS

- A multi-laboratory (9 laboratories) study to evaluate QC parameters for cadazolid MIC testing was carried out according to CLSI guidelines (9).
- QC organism *C. difficile* ATCC 700057 was tested by CLSI agar dilution and by broth microdilution in *Brucella* agar/broth (supplemented with 5 µg/ml haemin, 1 µg/ml vitamin K1 and 5% (v/v) laked sheep blood) under anaerobic conditions (10).
- QC organisms *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were tested by broth microdilution in Mueller Hinton broth under aerobic conditions (11).
- Linezolid was used as the control antibiotic for all experiments except for anaerobic broth microdilution where ceftaroline was used [note: ceftaroline was only used for QC and is not a therapy for *C. difficile*].
- Each evaluation included 3 batches of each test medium from separate manufacturers as indicated in Table 1.
- Each participating laboratory provided 10 replicate cadazolid MIC values for each QC organism with 3 separate lots of test medium (30 replicates per lab; 270 replicates in total).

Table 1. Source of test media used in the study

Medium	Manufacturer		
	1	2	3
Brucella agar (5 µg haemin and 1 µg vitamin K1 per ml)	Anaerobe Systems (Morgan Hill, CA, USA) Cat #AS-810	Becton Dickinson and Co. (Sparks, MD, USA) Cat # 211086	Remel (Lenexa, KS, USA) Cat #R452652
Brucella broth (5 µg haemin and 1 µg vitamin K1 per ml)	Hardy Diagnostics (Santa Maria, CA, USA) Cat #C5311	Becton Dickinson and Co. (Sparks, MD, USA) Cat # 211088	Remel (Lenexa, KS, USA) Cat #R452662
Cation-adjusted Mueller Hinton broth	Teknova (Hollister, CA, USA) Cat #M5887	Fluka (St. Louis, MI, USA) Cat #90922	Becton Dickinson and Co. (Sparks, MD, USA) - Cat # 212322

RESULTS

C. difficile ATCC 700057 agar dilution

- By agar dilution all linezolid QC data were in range, but one lab had low inoculum counts; these data were omitted – data not shown.
- Nevertheless, sufficient data (240 MICs from 8 labs) were available for analysis [CLSI required minimum is 210 MICs from 7 labs].
- Cadazolid MIC distributions against *C. difficile* ATCC 700057 by agar dilution for three medium manufacturers (M-1; M-2 and M-3) are shown in Figure 2.
- Approved CLSI ranges are summarised in Table 2.

C. difficile ATCC 700057 broth microdilution

- By broth microdilution ceftaroline QC was out of range for one laboratory so these data were omitted – data not shown.
- Nevertheless, sufficient data (240 MICs from 8 labs) were available for analysis [CLSI required minimum is 210 MICs from 7 labs].
- Cadazolid MIC distributions against *C. difficile* ATCC 700057 by broth microdilution for three medium manufacturers (M-1; M-2 and M-3) are shown in Figure 3.
- Approved CLSI ranges are summarised in Table 2.

E. faecalis ATCC 29212 broth microdilution

- All linezolid QC MIC data were in range and all inoculum sizes were satisfactory so analysis was performed on all data (270 MICs from 9 labs).
- Cadazolid MIC distributions against *E. faecalis* ATCC 29212 by broth microdilution for three medium manufacturers (M-1; M-2 and M-3) are shown in Figure 4.
- Approved CLSI ranges are summarised in Table 2.

S. aureus ATCC 29213 broth microdilution

- All linezolid QC MIC data were in range and all inoculum sizes were satisfactory so analysis was performed on all data (270 MICs from 9 labs).
- Cadazolid MIC distributions against *S. aureus* ATCC 29213 by broth microdilution for three medium manufacturers (M-1; M-2 and M-3) are shown in Figure 5.
- Approved CLSI ranges are summarised in Table 2.

Table 2. Summary of CLSI approved QC ranges for cadazolid

	C. difficile ATCC 700057		*E. faecalis* ATCC 29212	*S. aureus* ATCC 29213
Method	Agar	Broth	Broth	Broth
Approved MIC QC Range for cadazolid (µg/ml)	0.12 to 0.5	0.06 to 0.25	0.06 to 0.25	0.06 to 0.5
# dilutions	3	3	3	4
% Replica data captured	100%	97.9%	99.3%	100%

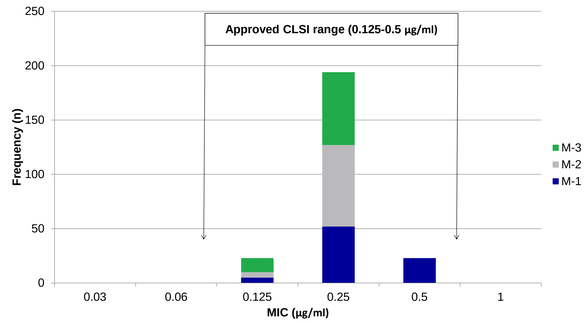

Figure 2. MIC distribution for Cadazolid MIC by agar dilution against C. difficile ATCC 700057.

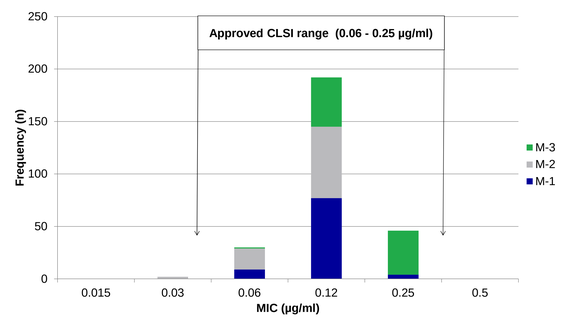

Figure 4. MIC distribution for Cadazolid MIC by broth microdilution against E. faecalis ATCC 29212.

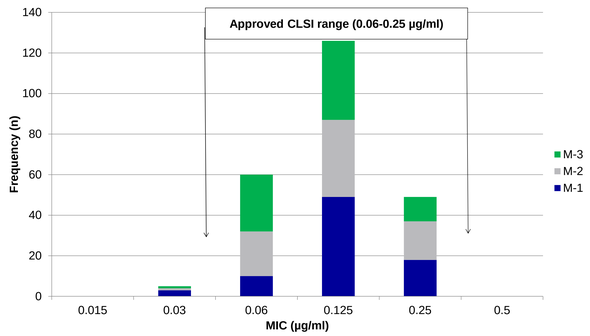

Figure 3. MIC distribution for Cadazolid MIC by broth microdilution against C. difficile ATCC 700057.

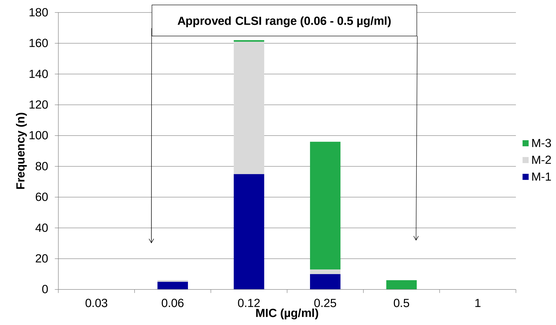

Figure 5. MIC distribution for Cadazolid MIC by broth microdilution against S. aureus ATCC 29213.

CONCLUSIONS

- **Highly reproducible MICs were obtained for cadazolid against *C. difficile* ATCC 700057 by agar dilution and broth microdilution, as demonstrated previously (6).**
- **Cadazolid agar dilution MIC ranges were one dilution higher than broth microdilution ranges against *C. difficile* ATCC 700057, which is typical of antimicrobial agents when tested under anaerobic conditions (12).**
- **Highly reproducible MICs were also obtained for cadazolid against *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 by broth microdilution.**
- **These MIC ranges, as approved by CLSI in June 2015 will be published in January 2016 (CLSI M-100-S26).**

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Presented at the Joint Interscience Conference on Antimicrobials Agents & Chemotherapy/International Congress of Chemotherapy, 17-21 September, 2015, San Diego, CA, USA.