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Susceptibility of common *Clostridium difficile* PCR ribotypes to delafloxacin and seven comparator antimicrobials

J Vernon,[1] J Freeman,[1] A Marra,[2] MH Wilcox[1]*

1. Leeds Teaching Hospitals & Univ. of Leeds. UK, 2. Melinta Therapeutics, New Haven, CT, USA

*Corresponding author: Mark.Wilcox@nhs.net

Leeds General Infirmary and University of Leeds
Old Medical School
Thorseby Place
Leeds, UK
0113 3926818
0113 3928782

Abstract

Background
Fluoroquinolone (FQ) use is a risk factor for *Clostridium difficile* (CD) infection and particularly associated with epidemic PCR ribotypes (RT). FQ resistance is conferred by mutations in the quinolone resistance determining region (QRDR). Delafloxacin (DLX) is ~~a~~ currently being assessed for the treatment of acute bacterial skin infections. We investigated the activity of DLX and 7 comparators against prevalent CD RTs.

Method
A panel of prevalent PCR RTs (n=30) were selected from the *Clostridium difficile* Ribotype Network Reference Laboratory, Leeds, UK. RT027 (n=10), 001, 014, 002, 020, 015, 005, 078, 126, 018, 023 & 017 (all n=2).
Minimum inhibitory concentrations (MIC) of delafloxacin (DLX), levofloxacin (L), moxifloxacin (MXF), clindamycin (CLIN), vancomycin (V), metronidazole (M), meropenem (MER) and tigecycline (TIG) were determined by a Wilkins Chalgren agar incorporation method.

Results

	mg/L							
	M	V	DLX	MXF	L	MER	CLIN	TIG
Range	<0.125-2	0.5-4	0.06-4	1-32	2->64	1-4	0.5->64	0.03-0.06
Geometric mean MIC (All)	0.4	0.8	0.4	3.3	14.4	1.9	10.6	0.05
Geometric mean MIC (RT027)	1.9	0.8	4.0	19.7	128	3.3	10.6	0.05
Geometric mean MIC (non-RT027	0.2	0.9	0.1	1.5	5.3	1.5	10.6	0.05

DLX was the most active FQ: Geometric mean (GM) DLX, MXF and L MICs were 0.4, 3.3 and 14.4mg/L respectively. GM DLX MICs for RT027 were uniformly elevated (4 mg/L) and 31-fold higher than non-RT027 isolates (0.06-2 mg/L). Similarly, GM MXF and L MICs were 13 and 21-fold higher in RT027 than non-RT027 (19.7 & 1.5 mg/L for RT027 vs non-RT027, respectively).
All isolates were susceptible to M, but GM MICs were 10-fold higher for RT027 strains than non-RT027.
All CD were susceptible to MER (range 1-4 mg/L), but GM MER MIC were greater in RT027 vs non-RT027 (3.3 vs 1.5 mg/L).
GM V, CLIN and TIG MICs for RT027 vs non-RT027 were very similar (see table).
High level CLIN resistance (>64 mg/L) was observed singly in RT027, 078, 126 and 017.

Conclusions
DLX was the most active FQ tested (DLX>MXF>L).
GM FQ MICs for RT027 were higher than those for non-RT027.
Reduced susceptibility (RS) to DLX corresponded with RS in MXF and L.
Existing QRDR mutations may confer RS to DLX.
Potential contribution of elevated DLX MICs in RT027 to selection pressure (as in other FQs) is unclear.

Introduction

Clostridium difficile infection (CDI) is a significant cause of nosocomial diarrhoea and remains a major burden on healthcare resources.[1] CDI is linked to depletion of gut microflora by antimicrobial action, allowing the organism to proliferate and cause symptomatic disease. Most antimicrobials have been associated with CDI at some point, including those used to treat the disease, such as vancomycin and metronidazole. Fluoroquinolone use has been identified as a risk factor for CDI development, and fluoroquinolone resistance is associated with certain PCR ribotypes, particularly PCR ribotype 027 (NAP1/BI).[2,3,4]
Delafloxacin is an investigational fluoroquinolone currently being assessed for the treatment of acute bacterial skin and skin structure infections due to Gram-positive and Gram-negative bacteria.

Methods

Isolates
A panel of 32 *C. difficile* isolates were selected from the collection held by the *Clostridium difficile* Ribotype Network (CDRN) Reference Laboratory at Leeds, UK. Isolates were selected to reflect commonly circulating PCR ribotypes in Europe (Table 1)

Control organisms
C. difficile ATCC700057, *C. difficile* E4 (PCR Ribotype 010), *Bacteroides fragilis* ATCC25285, *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212

PCR ribotype (RT)	N=
027	10
001	2
014	2
002	2
020	2
015	2
005	2
078	2
126	2
018	2
023	2
017	2

Table 1: List of C. difficile isolates tested

Methods

Agar incorporation analysis [5]
•Wilkins-Chalgren agar plates containing a range of metronidazole, vancomycin, moxifloxacin, levofloxacin, clindamycin, meropenem, tigecycline and delafloxacin concentrations from 0.125-64 mg/L were prepared.
• *C. difficile* isolates were cultured anaerobically for 24h at 37ºC in Schaedlers anaerobic broths.
• *C. difficile* test strains were diluted in pre-reduced saline and multipoint inoculated onto the antibiotic-containing agar plates.
• Plates were incubated anaerobically for 48h at 37ºC and the MIC determined as the lowest concentration of antibiotic that completely inhibited *C. difficile* growth.

Results

	M	V	DLX	MXF	L	MER	CLIN	TIG
Range	<0.125-2	0.5-4	0.06-4	1-32	2->64	1-4	0.5->64	0.03-0.06
Geometric mean MIC (ALL) (mg/L)	0.38	0.84	0.37	3.29	14.36	1.92	10.60	0.05
Geometric mean MIC (RT027) (mg/L)	1.87	0.76	4.00	19.70	128.00	3.25	10.56	0.05
Geometric mean MIC (non-RT027) (mg/L)	0.18	0.88	0.13	1.46	5.31	1.51	10.62	0.05
-fold difference in geometric mean	10.23	0.86	31.41	13.50	24.10	2.16	0.99	0.86

Table 2: Geometric mean MICs of delafloxacin and 7 comparator antibiotics for RT027 and non-RT027 *C. difficile* isolates. Elevated GM mean differences are highlighted.

Delafloxacin was the most active fluoroquinolone tested (Tables 2 and 3):
- GM delafloxacin MIC = 0.4 mg/L (range 0.06-4 mg/L)
- GM moxifloxacin MIC = 3.3 mg/L (range 1-16 mg/L)
- GM levofloxacin MIC = 14.4 mg/L (range 2-16 mg/L).

Eighteen of the thirty *C. difficile* isolates tested were susceptible to delafloxacin at a breakpoint concentration <0.125 mg/L.

RT027 strains had uniformly elevated fluoroquinolone MICs *vs* non RT027 strains (Table 3):
- For **delafloxacin,** GM MICs were **31-fold higher** in RT027 (GM =4 mg/L) compared to non-RT 027 isolates (GM=0.1 mg/L:
- For **moxifloxacin** GM MICs were **13-fold higher** in RT027 (GM=19.7 mg/L) compared to non-RT027 isolates (GM1.5 mg/L)
- For **levofloxacin**, GM MICs were **24-fold higher** in RT027 (GM=128 mg/L) compared to non-RT027 isolates (GM=5.3 mg/L)

Single isolates of RT001 and RT126 also had elevated MICs of delafloxacin (2 mg/L), moxifloxacin (16 mg/L) and levofloxacin (<62 mg/L).

RT	M	V	DLX	MXF	L	MER	CLIN	TIG
027	1	0.5	4	16	>64	4	8	0.06
027	2	1	4	16	>64	4	8	0.03
027	2	1	4	16	>64	4	8	0.03
027	2	0.5	4	16	>64	2	8	0.03
027	2	1	4	16	>64	4	8	0.03
027	2	0.5	4	16	>64	4	16	0.06
027	2	1	4	32	>64	2	>64	0.06
027	2	1	4	16	>64	2	4	0.06
027	2	0.5	4	32	>64	4	8	0.06
027	2	1	4	32	>64	4	8	0.06
001	1	4	2	16	>64	2	8	0.06
001	0.25	0.5	0.06	1	4	1	8	0.06
014	≤0.125	1	0.125	1	4	1	32	0.06
014	0.25	0.5	0.125	2	4	1	8	0.06
002	0.25	1	0.125	2	4	2	16	0.03
002	0.25	0.5	0.06	1	4	1	8	0.06
020	0.5	0.5	0.125	2	4	1	16	0.06
020	0.25	0.5	0.125	2	4	2	16	0.06
015	0.25	1	0.125	1	4	2	8	0.06
015	≤0.125	1	0.125	1	4	1	1	0.03
005	0.25	2	0.125	1	4	2	8	0.06
005	≤0.125	0.5	0.06	1	4	2	4	0.03
078	≤0.125	1	0.06	1	4	2	>64	0.06
078	≤0.125	1	0.06	1	4	1	8	0.06
126	≤0.125	1	2	16	>64	2	16	0.06
126	≤0.125	0.5	0.06	1	2	1	>64	0.06
018	≤0.125	1	0.06	1	4	4	8	0.06
018	≤0.125	2	0.125	1	4	2	8	0.03
023	≤0.125	1	0.125	1	4	2	2	0.06
023	≤0.125	1	0.125	1	4	2	0.5	0.06
017	≤0.125	1	0.125	1	4	1	>64	0.06
017	≤0.125	0.5	0.125	1	4	1	16	0.06

Table 3: MICs of delafloxacin and 7 comparator antibiotics for 32 *C. difficile* solates. Highlighting indicates elevated MICs.

All *C. difficile* isolates tested were susceptible to metronidazole but isolates belonging to RT027 showed a 10-fold increase in geometric mean MICs over geometric means over non RT027 isolates (Tables 2 and 3).

C. difficile isolates were susceptible to meropenem in the range of 1-4 mg/L, with a marginally increased geometric mean meropenem MIC among RT027 isolates *vs* non-RT027 isolates (3.25 vs 1.51 mg/L, respectively; Tables 2 and 3).

Geometric mean vancomycin, clindamycin and tigecycline MICs for RT027s *vs* non-RT027s were similar (Tables 2 and 3).

One RT001 isolate showed reduced susceptibility to vancomycin at 4 mg/L, while high level resistance to clindamycin (>64 mg/L) was observed singly in RT027, 078, 126 and 017 (Table 3).

Discussion

Delafloxacin was the most active fluoroquinolone agent tested in this study.
•Fluoroquinolones have been implicated in outbreaks of CDI associated with epidemic PCR ribotype 027 (NAP1/BI) [2,3,4] and resistance among these *C. difficile* strains has been well documented.[6,7] Previous studies have noted high level resistance among *C. difficile* to older fluoroquinolones such as ciprofloxacin, while newer fluoroquinolones, such as moxifloxacin showed superior activity.[8] However, moxifloxacin resistance (>8 mg/L) is not uncommon and in a recent pan-European study of antimicrobial susceptibility among *C. difficile* PCR ribotypes, only 51% of isolates were susceptible (<2 mg/L), with considerable variation in levels of resistance among commonly isolated PCR ribotypes.[9]
Reduced susceptiblity to delafloxacin corresponded with reduced susceptibility to moxifloxacin and levofloxacin and may be associated with existing QRDR mutations.
•These isolates were predominantly RT027 (which had uniformly elevated delafloxacin MICs), and single RT001 and 126 isolates. Geometric mean delafloxacin MICs for RT027 were 31-fold higher than those of non-RT027 isolates, and 13- and 21-fold higher for moxifloxacin and levofloxacin, respectively. Previous studies of fluoroquinolone resistance in *C. difficile* have described a number of mutations in *gyrA* and *gyrB*. In particular an amino acid substitution Thr82 to Ile in GyrA has been implicated in fluoroquinolone resistance in RT027.[10] Although we did not undertake molecular characterization of the isolates showing reduced susceptibility, it seems likely that existing mutations in QRDR that confer resistance to older fluoroquinolones may confer reduced susceptibility to delafloxacin.

MIC results for metronidazole, vancomycin, meropenem, clindamycin and tigecycline reflected those previously reported.[9]
•There was no evidence of metronidazole resistance, but geometric mean metronidazole MICs for RT027 isolates were 10-fold higher than for non-RT027 isolates, reflecting previous results.[5,9]
•Despite the increasing identification of prior carbapenem use as a risk factor for CDI, data available on *C. difficile* susceptibility to carbapenems are lacking. Buchler et al. examined meropenem susceptibility as part of a study of changing antimicrobial susceptibility patterns in *C. difficile*. They reported all isolates (n=86) as susceptible to meropenem <4 mg/L .[11] However, Freeman et al found elevated geometric mean imipenem MICs among RT027 and 106, possibly as a result of greater sample size (n=918).[9] The isolates tested in the present study showed MICs in the same order as those reported by Buchler et al, although geometric mean MICs were 2-fold higher for RT027 isolates than for non-RT027 isolates (3.25 mg/L vs 1.51 mg/L).
***C. difficile* RT027 showed the most evidence of antimicrobial resistance among the panel of isolates selected for testing in this study.**
•This reflects previous observations describing this ribotype as multi-drug resistant.[7,9] However, ribotype comparisons are difficult to make in this study due to the high representation of RT027 strains (n=10) relative to other, prevalent ribotypes (n=2).

Conclusion

In summary, delafloxacin was more active than moxifloxacin and levofloxacin against the panel of *C. difficile* isolates tested here. Delafloxacin susceptibility was lower for the tested *C. difficile* isolates exhibiting reduced susceptibility/resistance to moxifloxacin and levofloxacin, in particular RT027. This likely indicates that mutations in the QRDR, already associated with fluoroquinolone resistance, may confer reduced susceptibility to delafloxacin. It remains unclear whether the elevated MICs of delafloxacin seen for some *C. difficile* isolates (notably RT027) will confer a selection pressure for this fluoroquinolone that is similar to or less than that seen with other members of this class. This will depend in part on the relative bioactive concentrations of delafloxacin in the gut.

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