Antimicrobial resistance surveillance in Australian isolates of *Clostridium difficile*

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BACKGROUND AND AIMS

Clostridium difficile infection (CDI) and its life-threatening sequelae present a major clinical and economic burden to global healthcare systems [1-2]. The key antimicrobial agents for the treatment of CDI include metronidazole (MTZ), vancomycin (VAN) and, most recently, fidaxomicin (FDX).

Currently, hypervirulent strains of *C. difficile* (PCR ribotypes (RTs) 078 and 027) are not endemic to Australia. Despite this absence, and the implementation of new infection control and patient management practices, rates of CDI in all states in Australia have increased significantly since mid-2011 possibly reflecting the emergence of new RTs such as RT244 and a significant proportion of cases (26%) from sources outside the hospital setting [3-4].

Despite this increase in incidence, most clinical microbiology laboratories in Australia do not routinely culture diarrhoeal stools for *C. difficile* or perform antimicrobial susceptibility testing on recovered strains. In this study, we sought to determine the activity of FDX and comparator antimicrobials against *C. difficile* isolated from patients with CDI in Australian hospitals and in the community.

METHODS

Sample collection Ten diagnostic microbiology laboratories across five states in Australia were selected to participate in this study. Half of these laboratories were based in large tertiary care medical centres (public hospital sites) and the other half were private pathology laboratories (community sites). A total of 309 isolates or PCR positive stool samples were sent during two collection periods in winter/spring (August-September, phase I) 2013 and summer/autumn (February-March, phase II) 2014 to the central testing laboratory (The University of Western Australia).

***C. difficile* culture and PCR characterisation** PCR positive stool samples and isolates of *C. difficile* were cultured directly on *C. difficile* ChromID™ agar and identified as previously described [5].

MIC determination using agar incorporation The MICs of FDX, amoxicillin-clavulanate (AUG), ceftriaxone (CTX), clindamycin (CLI), meropenem (MER), MTZ, moxifloxacin (MXF), rifaximin (RFX) and VAN were determined by agar incorporation methodology as described by the Clinical and Laboratory Standards Institute (CLSI, M11-A7) [6]. Clinical breakpoints used for AUG, CTX, MER, MXF, MET were those recommended by CLSI document M100-S23 [7]. Vancomycin breakpoints (susceptible, ≤2 mg/L; resistant, ≥2 mg/L) breakpoints were those recommended by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org) [8]. Currently no breakpoints exist for FDX.

Epidemiological typing One-third of all isolates from each state (n=99) underwent PCR for the presence of the toxin A (*tcdA*), toxin B (*tcdB*) and ADP-ribosyltransferase (binary toxin) genes (*cdtA* and *cdtB*), and PCR ribotyping, as previously described [9].

RESULTS AND DISCUSSION

Sample collection

A total of 309 eligible patient samples were received during two collection periods and, from these, 290 (93.3%) isolates of *C. difficile* were recovered (Table 1).

TABLE 1 Summary of sample collection and *C. difficile* recovery.

Phase	Site type	N specimens/Isolates						*C. difficile* recovery N (%)
		NSW	QLD	SA	VIC	WA	Total	
I	Private	19	21	13	15	9	77	67 (87.0)
	Public	31	5	21	22	19	98	89 (90.8)
	Total	**50**	**26**	**34**	**37**	**28**	**175**	**156 (89.1)**
II	Private	15	10	14	15	14	68	68 (100.0)
	Public	15	6	15	15	15	66	66 (100.0)
	Total	**30**	**16**	**29**	**30**	**29**	**134**	**134 (100.0)**
Total		**80**	**42**	**63**	**67**	**57**	**309**	**290 (93.9)**

PCR ribotyping and toxin profiling

Overall, 25 different RTs were identified and 85.6% (85/99) were assigned one of 13 internationally recognised RTs (Fig. 1). No RT 027, RT 078 or RT 244 were detected. RT 014 was the most common RT found overall representing 34.3% (34/99) of isolates, followed by RT 002 comprising 19.2% of isolates (19/99) (Fig.1), the former being significantly higher in prevalence than previously reported [10] and also recently found to be prominent in neonatal pigs in Australia [9]. A number of isolates (n=14) were not able to be identified with the available reference library and were designated with internal typing nomenclature, prefixed with QX. Of the 99 isolates of *C. difficile* genotyped, 99% of isolates (n=98/99) were positive for *tcdA* and *tcdB* (A^+B^+), of which 2 (2%) were also positive for binary toxin genes *cdtA/B* (CDT^+). A single isolate of *C. difficile* identified as RT 010 was non-toxigenic ($A^-B^-CDT^-$).

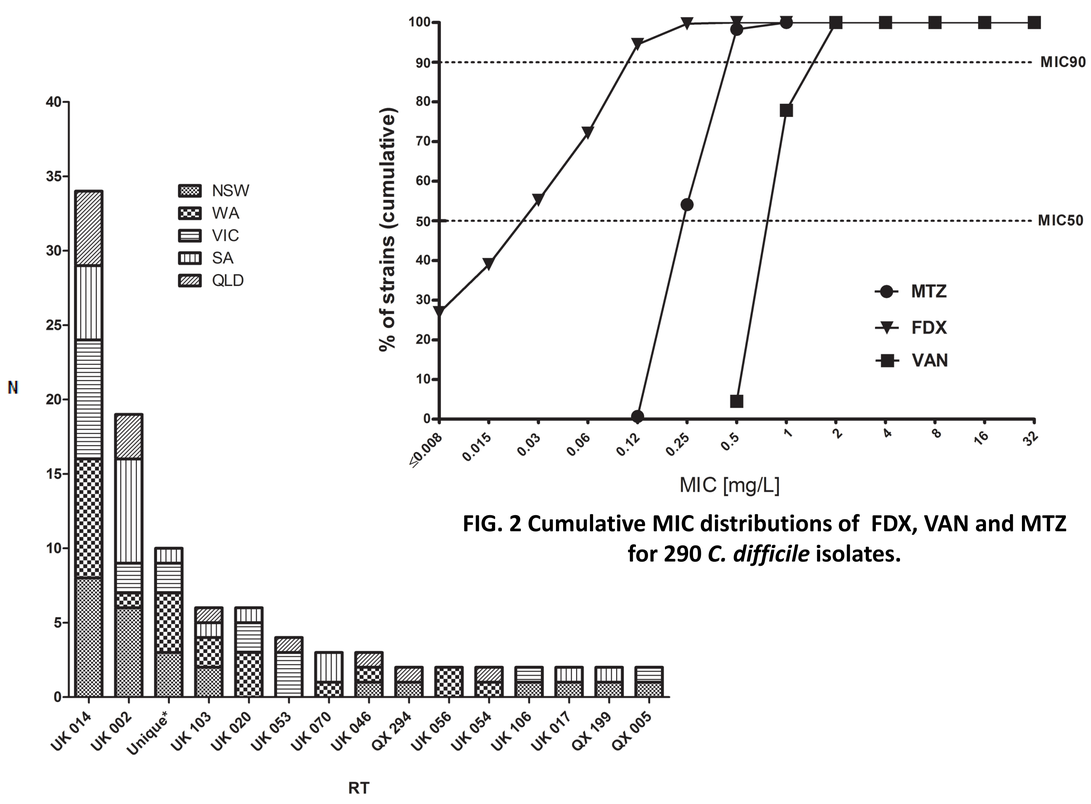

FIG. 1 Summary of prevalence and national distribution of *C. difficile* PCR ribotypes (n=99).

*Unique: UK 010, QX 011, QX 076, QX 137, QX 150, QX 209, QX 244, QX 250, QX 294, QX 412. NSW - New South Wales, SA - South Australia, VIC - Victoria, WA - Western Australia and QLD - Queensland.

FIG. 2 Cumulative MIC distributions of FDX, VAN and MTZ for 290 *C. difficile* isolates.

in vitro activity of FDX and comparators

FDX showed potent *in vitro* activity against all *C. difficile* isolates with an MIC50 3-fold and 5-fold lower than MTZ and VAN (0.03 mg/L, 0.25 and 1 mg/L, respectively) (Table 2 and Fig.2). A comparable trend and MIC distribution for these agents was observed in all five states (data not shown) and is in accordance with data from other studies [11-12]. VAN and MTZ resistance were not detected and the proportion of isolates resistant to MXF (MIC >4 mg/L) was low (n=10/290, 3.4%).

TABLE 2 Susceptibility and summary MIC data for FDX and comparators against *C. difficile*.

PCR Ribotype	Agent	S		I		R		MIC Range [mg/L]	MIC50 [mg/L]	MIC90 [mg/L]
		n	%	n	%	n	%			
All (23 RTs, n=290)	FDX	290	100	-	-	-	-	≤0.008 - 0.5	0.03	0.12
	VAN	290	100	-	-	-	-	0.5 - 2	1	2
	MTZ	290	100	-	-	-	-	0.12 - 1	0.25	0.5
	RFX	290	100	-	-	-	-	0.004 - 0.015	0.008	0.015
	MXF	278	95.9	2	0.7	10	3.4	0.5 - 32	2	2
	CLI	15	5.2	33	11.4	242	83.4	1 - >32	8	>32
	AUG	290	100	-	-	-	-	0.25 - 2	0.5	1
	CTX	40	13.8	189	65.2	61	21	16 - >128	32	128
	MER	288	99.3	2	0.7	-	-	1 - 8	2	4
RT 014 (n=34/99, 34.3%)	FDX	34	100.0	-	-	-	-	≤0.008 - 0.12	0.015	0.12
	VAN	34	100.0	-	-	-	-	1 - 2	1	2
	MTZ	34	100.0	-	-	-	-	0.12 - 0.5	0.25	0.5
	RFX	34	100.0	-	-	-	-	0.004 - 0.015	0.008	0.015
	MXF	31	91.2	1	2.9	2	5.9	0.5 - 8	2	2
	CLI	3	8.8	6	17.6	25	73.5	1 - >32	8	16
	AUG	34	100.0	-	-	-	-	0.5 - 2	0.5	1
	CTX	7	20.6	22	64.7	5	14.7	16 - >128	32	64
	MER	34	100.0	-	-	-	-	2 - 4	2	2
RT 002 (n=19/99, 19.2%)	FDX	19	100.0	-	-	-	-	≤0.008 - 0.25	0.03	0.12
	VAN	19	100.0	-	-	-	-	0.5 - 2	1	2
	MTZ	19	100.0	-	-	-	-	0.25 - 0.5	0.5	0.5
	RFX	19	100.0	-	-	-	-	0.004 - 0.015	0.008	0.015
	MFX	19	100.0	-	-	-	-	2	2	2
	CLI	1	5.3	-	-	18	94.7	2 - >32	8	>32
	AUG	19	100.0	-	-	-	-	0.5 - 1	0.5	1
	CTX	0	0.0	10	52.6	9	47.4	32 - >128	32	>128
	MER	19	100.0	-	-	-	-	2 - 4	2	4

CONCLUSIONS

Resistance to agents used to treat CDI (VAN, MTZ and FDX) was not detected and resistance to fluoroquinolones was very low.

FDX showed superior *in vitro* activity compared to VAN and MTZ further supporting the use of FDX for treatment of CDI in Australia.

A heterogeneous strain population was identified, dominated by RT 014 - the most common *C. difficile* strain type in humans in Australia and overseas.

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