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Antimicrobial Susceptibility Pattern of *Clostridium difficile* and Its Relation to PCR Ribotypes in a Swedish University Hospital

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All 238 *Clostridium difficile* isolates were susceptible to metronidazole and vancomycin, whereas 84% and 1% were resistant to clindamycin and fusidic acid. Etest MICs for metronidazole were lower than agar dilution MICs ($P < 0.01$) but without difference in susceptible-intermediate-resistant categorization. No particular PCR ribotype was associated with clindamycin or fusidic acid resistance.

Clostridium difficile-associated diarrhea (CDAD) is common in hospitals (2, 3, 11, 14, 21, 22, 27, 29) and usually treated with metronidazole or vancomycin, which have comparable response rates (80% to 90%) and relapse rates (5% to 25%) (13, 23). Metronidazole is currently the first choice agent due to its lower potential for selecting vancomycin-resistant enterococci and for economical reasons, and fusidic acid has been suggested as an alternative drug (17, 30).

As several studies have reported full susceptibility of *C. difficile* isolates for metronidazole and vancomycin, clinical laboratories do not routinely perform culture and susceptibility testing of the organism (5, 8, 10, 12). However, resistance to metronidazole in up to 9% of isolates (1, 4, 6, 18), intermediate resistance to vancomycin in 3% of isolates (1), and "relatively poor" outcome of metronidazole therapy in CDAD (15, 19, 28) was recently reported. This emphasizes the need for periodic monitoring of any emergence of drug resistance in *C. difficile*. The aim of the present study was to investigate its susceptibility to metronidazole, vancomycin, fusidic acid, and clindamycin in a Swedish tertiary care hospital by using the Etest.

The first *C. difficile* isolate from each of 238 consecutive CDAD patients at Huddinge University Hospital, Stockholm, Sweden, from 10 February 2000 through 10 February 2001, were studied. The definition of CDAD, culture method, and epidemiological data for these patients were recently described (25). All *C. difficile* isolates were also subject to PCR ribotyping according to the method of Stubbs et al. (24) but with an improved method previously correlated with serotypes (25). Isolates were subcultured three times on horse blood agar prior to susceptibility testing to ensure purity. Bacteria were suspended in tryptic soy broth to a McFarland standard of 1 to 1.5 for testing against clindamycin, fusidic acid, metronidazole, and vancomycin by Etest on *Brucella* blood agar according to the manufacturer's instructions (AB-Biodisk, Solna, Sweden). Plates were scored after anaerobic incubation at 36°C for 48 h.

A random subset of 34 isolates was also tested using agar dilution to determine metronidazole MICs for comparison with Etest data. Agar dilution was performed on Wilkins-Chalgren agar (1) incubated as described above. *Eubacterium lentum* ATCC 43055 and *Clostridium perfringens* ATCC 13124 were used for quality control.

MICs for the drugs tested are shown in Table 1. Using the pharmacological breakpoints (Table 1) recommended by the Swedish Reference Group for Antibiotics (SRGA) (http://www.srga.org), all isolates were susceptible to metronidazole and vancomycin, and 99% were susceptible to fusidic acid. In contrast, only 16% of the isolates were susceptible to clindamycin and 11% were highly resistant to this agent. When using CLSI (formerly NCCLS) breakpoints for clindamycin (susceptible, ≤2; intermediate, 4; resistant, ≥8) 56.3%, 31.1%, and 12.6% were classified as susceptible, intermediate, and resistant, respectively. Interpretation for metronidazole was the same when using CLSI breakpoints as when using SRGA breakpoints. CLSI does not recommend any breakpoints for fusidic acid or vancomycin (7). MIC results for metronidazole from

TABLE 1. Antibiotic susceptibility of *C. difficile* isolates[a]

Vancomycin (n = 236)		Metronidazole (n = 238)		Clindamycin (n = 238)		Fusidic acid (n = 238)	
MIC (mg/liter)	n (%)	MIC (mg/liter)	n (%)	MIC (mg/liter)	n (%)	MIC (mg/liter)	n (%)
0.5	78 (33.1)	0.032	5 (2.1)	1	5 (2.1)	0.032	2 (0.8)
1	154 (65.3)	0.064	40 (16.8)	2	32 (13.4)	0.064	7 (2.9)
2	4 (1.7)	0.125	111 (46.6)	4	97 (**40.8**)	0.125	81 (34.0)
4	0 (0.0)	0.25	64 (26.9)	8	74 (**31.1**)	0.25	117 (49.2)
8	0 (**0.0**)	0.5	15 (6.3)	16	4 (**1.7**)	0.5	28 (11.8)
		1	3 (1.3)	32	0 (**0.0**)	1	3 (**1.3**)
		2	0 (0.0)	64	0 (**0.0**)		
		4	0 (0.0)	128	0 (**0.0**)		
		8	0 (**0.0**)	>256	26 (**10.9**)		

[a] The breakpoints according to the SRGA (http://www.srga.org) used were as follows: vancomycin susceptible, ≤4.0 mg/liter; vancomycin resistant, >4.0 mg/liter; metronidazole susceptible, ≤4.0 mg/liter; metronidazole resistant, >4.0 mg/liter; clindamycin susceptible, ≤2.0 mg/liter; clindamycin resistant, >2.0 mg/liter; fusidic acid susceptible, ≤0.5 mg/liter; fusidic acid resistant, >0.5 mg/liter. Percentages in boldface type indicate resistant isolates.

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TABLE 2. Comparison of metronidazole MICs obtained by agar dilution and Etest for 34 isolates of *C. difficile*

Agar dilution MIC (mg/liter)	No. of isolates	No. of isolates with Etest MIC (mg/liter):						
		0.032	0.064	0.125	0.25	0.5	1	2
≤0.25	13	2	3	2	2	4	0	0
0.5	12	0	1	3	2	6	0	0
1	7	0	0	0	1	5	1	0
2	2	0	0	0	0	0	2	0

comparison of Etest to agar dilution are presented in Table 2. Etest MICs were generally lower than agar dilution data. One isolate differed by 3 dilution steps, one isolate by 2 steps, and 9 isolates by 1 step (41% lower MICs), whereas only 4 isolates (12%) had 1-step-higher MICs by Etest ($P < 0.01$, chi-square test). No difference in susceptible-intermediate-resistant categorization resulted from the discrepancies.

PCR ribotyping identified 70 different types (25). The most frequent PCR ribotypes were SE20, SE21, SE21b, and SE22 (Table 3). The three isolates that were resistant to fusidic acid belonged to PCR ribotypes SE7b, SE26a, and SE44b. All isolates of several major PCR ribotypes were clindamycin-resistant isolates (SE12, SE14, SE16, SE17, and SE30), whereas other PCR ribotypes comprised 43 to 93% resistant isolates (SE3, SE19, SE20, SE21, SE21b, SE22, SE29b, SE25, and SE37). Thus, no particular PCR ribotype was associated with fusidic acid or clindamycin resistance.

Treatment of CDAD with metronidazole or vancomycin is effective in most patients (13, 23), and resistance to metronidazole or vancomycin has not been reported as a cause of therapeutic failure or recurrence of CDAD. Several studies indicate that the MICs of vancomycin and metronidazole for *C. difficile* have remained low over the years (5, 8, 10, 12).

Also among the 238 isolates of *C. difficile* analyzed here, there was no evidence of resistance to metronidazole and vancomycin, and their MIC ranges were 0.032 mg/liter to 1 mg/liter and 0.5 mg/liter to 2 mg/liter, respectively. In a previous study, comprising 57 *C. difficile* isolates from consecutive patients at our hospital during 1997, all isolates were susceptible to vancomycin, but one isolate was resistant to metronidazole (MIC ≥ 256 mg/liter) (26). Consequently, both metronidazole and vancomycin still seem to be adequate alternatives for empirical treatment of CDAD in our patients. Of these isolates, 86% were resistant to clindamycin, compared to 83% in the present investigation, despite the fact that only 8% had been treated with this agent within the 2 months prior to infection (data not shown). In contrast to some previous reports (9, 16), no predominant clones associated with clindamycin resistance were found in the present study. However, isolates for which MICs were >256 mg/liter were found only in about half of the major PCR ribotypes.

In earlier studies, metronidazole MICs for *C. difficile* determined by Etest have been lower than those obtained by agar dilution. In one study, 80% of the Etest MICs were within one dilution of the agar dilution MICs of metronidazole (20). These results were confirmed in our study. The difference between the methods, however, did not result in any discrepancy in susceptible-intermediate-resistant categorization. This suggests that Etest can be used to screen for metronidazole resistance in *C. difficile*.

TABLE 3. Response to clindamycin of major PCR ribotypes of *C. difficile* comprising ≥5 isolates each[c]

PCR ribotype[a]	Presumed serogroup[a]	*n*	Proportion (%) classified		
			Susceptible	Intermediate[b]	Resistant
SE3	D	10	10	30	60
SE12	A2	5	0	100	0
SE14		5	0	80	20
SE16	A	8	0	100	0
SE17		5	0	40	60
SE19		8	12.5	88.5	0
SE20	G	14	7.1	71.9	21
SE21	H	35	14	86	0
SE21b	A8	22	14	81.5	4.5
SE22		13	15	85	0
SE29b		8	25	50	25
SE25		7	57	43	0
SE30		5	0	100	0

[a] See reference 25.
[b] This group is also defined as resistant by the SRGA.
[c] The definitions used in this context were susceptible (MIC ≤ 2.0 mg/liter), intermediate (>2.0 to 256 mg/liter), and resistant (MIC > 256 mg/liter). The three isolates resistant to fusidic acid belonged to different ribotypes (see text).

We conclude that resistance to metronidazole and vancomycin in *C. difficile* remains rare in our hospital. As susceptibility testing of *C. difficile* is labor intensive and thus costly, although less so with Etest compared to agar dilution, routine culture for *C. difficile* and susceptibility testing of isolates still cannot be recommended. In the present situation, periodic assessment of the antimicrobial susceptibility pattern of *C. difficile* seems sufficient but remains important for detection of changes over time.

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