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Prevalence of Antimicrobial Resistance among Clinical Isolates of *Bacteroides fragilis* group in Canada in 2010-2011: CANWARD Surveillance Study

S. SEPEHRI, R. VASHISHT, B. WESHNOWESKI, J. A. KARLOWSKY, A. J. WALKTY, H. J. ADAM, D. J. HOBAN, AND G. G. ZHANEL

University of Manitoba, Diagnostic Services of Manitoba, Winnipeg, MB

Dr. George G. Zhanel
MS673 Microbiology,
Health Sciences Centre
820 Sherbrook Street
Winnipeg, Manitoba, Canada
R3A 1R9
Phone: 204 787-4902
Fax: 204 787-4699
Email: ggzhanel@pcs.mb.ca

REVISED ABSTRACT

Background: *Bacteroides fragilis* group are important human pathogens that are most frequently associated with intra-abdominal, pelvic and complicated skin and soft tissue infections. Canadian susceptibility data have not been published for clinical isolates of *B. fragilis* group in almost 20 years (Antimicrob. Agents Chemother. 1992:36;343-7).

Methods: Clinical isolates of *Bacteroides fragilis* group (n = 377) were collected from patients attending nine Canadian hospitals in 2010-2011 and tested for susceptibility to 10 antimicrobial agents using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (M11-A7, 2007). The 377 *B. fragilis* group isolates were 183 *B. fragilis* (48.5%), 67 *B. fragilis* group (17.8%), 58 *B. ovatus* (15.4%), 32 *B. thetaiotamicron* (8.5%), 22 *B. stercoris* (5.8%), 9 *Parabacteroides distasonis/merdae* (2.4%), 4 *B. vulgatus* (1.1%), and one isolate each of *B. buccae* (0.3%) and *B. caccae* (0.3%). Isolates were tested using custom designed broth microdilution panels and MICs were interpreted using M100-S21 (2011) breakpoints. Breakpoints for doripenem and tigecycline were based on the US FDA recommendations.

Results: For all isolates, percent susceptibility rates were 99.7% for metronidazole, 99.5% for piperacillin-tazobactam, 99.2% for imipenem, 97.6% for ertapenem, 91.8% for doripenem, 87.0% for amoxicillin-clavulanate, 80.9% for tigecycline, 66.1% for cefoxitin, 55.7% for moxifloxacin, and 52.5% for clindamycin. Percent susceptibility to clindamycin, cefoxitin, and moxifloxacin was lowest for *Bacteroides thetaiotaomicron* (25.0, 31.3, and 34.4%, respectively) and *Bacteroides ovatus* (27.6, 39.7, and 29.3%, respectively). One isolate (*B. thetaiotamicron*) was resistant to metronidazole, and two isolates (both *B. fragilis*) were resistant to both piperacillin-tazobactam and imipenem.

Conclusion: Metronidazole, piperacillin-tazobactam, imipenem, and ertapenem were the agents with the greatest susceptibilities (>97%) against *B. fragilis* group isolates collected from across Canada in 2010-2011. The susceptibility rates for metronidazole, piperacillin-tazobactam, and imipenem remain unchanged compared with Canadian data published in 1992. In contrast, rates of resistance for *B. fragilis* group have increased for amoxicillin-clavulanate, from 0.8% (1992) to 6.4% (2010-2011), and for clindamycin, from 9% (1992) to 33.4% (2010-2011).

INTRODUCTION

Bacteroides fragilis group are known to be important human pathogens and are most frequently associated with intra-abdominal, pelvic, complicated skin and soft tissue, and bloodstream infections. *B. fragilis* group contains >20 species and are commonly isolated from patient specimens as a part of mixed infection. The susceptibilities of isolates of *B. fragilis* group has been reported to vary for individual species and by geographic location (1), and in vitro antimicrobial susceptibility testing results have been shown to be relevant to determining patient outcome, even in the presence of mixed infections (2). Unfortunately, even in 2010-2011, anaerobic antimicrobial susceptibility testing is only performed in a minority of clinical laboratories and physicians are forced to rely on published surveys to acquire information regarding their empiric antimicrobial prescribing decisions (3).

Antimicrobial susceptibility testing data for clinical isolates of *B. fragilis* group collected from Canadian patients have not been published in almost 20 years (4). During this time *B. fragilis* group isolates have been reported to have evolved in other locations from relatively susceptible organisms (exception penicillin, most isolates produce β-lactamase) to pathogens that now demonstrate resistance to all classes of anti-anaerobic agents including carbapenems and metronidazole (3, 5). Investigators in the United States have reported resistance among *B. fragilis* group organisms to be high and increasing with agents such as cefoxitin, clindamycin, and moxifloxacin (6). The goal of this study was to assess the in vitro activities of cefoxitin, clindamycin, doripenem, ertapenem, meropenem, imipenem, metronidazole, moxifloxacin, piperacillin-tazobactam, and tigecycline against *B. fragilis* group organisms isolated from patients in Canadian hospitals in 2010-2011.

MATERIALS & METHODS

B. fragilis group isolates were collected by nine Canadian hospital laboratories from January, 2010 to August, 2011 and shipped to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada). Each laboratory was asked to collect 50 consecutive *B. fragilis* group isolates. Each isolate was deemed clinically significant by individual clinical microbiology laboratory algorithms; isolates were limited to one per patient. Isolate inclusion was independent of patient age. The coordinating laboratory confirmed the identities of all isolates using Vitek ANC identification cards and a Vitek 2 instrument (bioMerieux, Durham, NC) and ancillary tests as required.

In vitro antimicrobial susceptibilities to 10 antimicrobial agents were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI; 7). In-house prepared broth microdilution panels included amoxicillin-clavulanate, cefoxitin, clindamycin, doripenem, ertapenem, imipenem, piperacillin-tazobactam, metronidazole, moxifloxacin, and tigecycline. MICs were interpreted using breakpoints published in the M100-S21 (2011; 8) document for all agents except doripenem (susceptible, ≤1 μg/ml) and tigecycline (susceptible, 4 μg/ml; intermediate, 8 μg/ml; resistant, 16 μg/ml) for which U.S. Food and Drug Administration (US FDA) recommended MIC breakpoints were used.

CLSI M100-S21 MIC interpretative breakpoints are: clindamycin and moxifloxacin (susceptible, 2 μg/ml; intermediate, 4 μg/ml; resistant, 8 μg/ml); amoxicillin-clavulanate, ertapenem, and imipenem (susceptible, 4 μg/ml; intermediate, 8 μg/ml; resistant, 16 μg/ml); metronidazole (susceptible, 8 μg/ml; intermediate, 16 μg/ml; resistant, 32 μg/ml); cefoxitin (susceptible, 16 μg/ml; intermediate, 32 μg/ml; resistant, 64 μg/ml), and piperacillin-tazobactam (susceptible, 32 μg/ml; intermediate, 64 μg/ml; resistant, 128 μg/ml) (8).

RESULTS

TABLE 1. In vitro activities of antimicrobial agents against clinical isolates of *Bacteroides* species

Organism (no.tested) /antimicrobial agent	MIC (μg/mL) 50%	90%	Range	% Susceptible	% Intermediate	% Resistant
All *Bacteroides* spp.[a] (377)						
Amoxicillin-Clavulanate	1	8	≤0.12-32	87	6.6	6.4
Piperacillin-Tazobactam	0.25	8	≤0.015->256	99.5	0	0.5
Cefoxitin	16	64	≤1->128	66.1	18.3	15.7
Ertapenem	0.5	2	0.12->32	97.6	1.6	0.8
Imipenem	0.25	1	0.03->32	99.2	0.3	0.5
Doripenem[b]	≤0.25	1	≤0.25->2	91.8	NA[c]	NA[c]
Clindamycin	2	>16	≤0.25->16	52.5	14.1	33.4
Metronidazole	1	4	≤0.12-32	99.7	0	0.3
Moxifloxacin	2	>16	0.12->16	55.7	10.3	34
Tigecycline[b]	1	8	≤0.06-32	80.9	11.1	8
Bacteroides fragilis (183)						
Amoxicillin-Clavulanate	0.5	8	0.25-32	88.5	6	5.5
Piperacillin-Tazobactam	0.12	1	≤0.015->256	98.9	0	1.1
Cefoxitin	8	32	2->128	84.2	8.2	7.7
Ertapenem	0.25	4	0.12->32	96.2	2.7	1.1
Imipenem	0.12	1	0.06->32	98.9	0	1.1
Doripenem	≤0.25	2	≤0.25->2	88.5	NA	NA
Clindamycin	1	>16	≤0.25->16	66.1	7.7	26.2
Metronidazole	1	2	0.25-4	100	0	0
Moxifloxacin	1	16	0.25->16	64.5	8.2	27.3
Tigecycline	1	8	0.25-16	84.7	9.8	5.5
Bacteroides fragilis group (67)						
Amoxicillin-Clavulanate	1	8	0.25-32	86.7	4.5	9
Piperacillin-Tazobactam	0.5	8	≤0.015-32	100	0	0
Cefoxitin	16	128	≤1->128	59.7	14.9	25.4
Ertapenem	1	4	0.12-16	97	1.5	1.5
Imipenem	0.25	2	0.06-8	98.5	1.5	0
Doripenem	≤0.25	1	≤0.25->2	91	NA	NA
Clindamycin	2	>16	≤0.25->16	50.8	9	40.3
Metronidazole	2	4	≤0.12-8	100	0	0
Moxifloxacin	2	>16	0.12->16	59.7	10.5	29.9
Tigecycline	1	8	0.12-32	80.6	13.4	6
B. ovatus (58)						
Amoxicillin-Clavulanate	1	16	0.25-32	77.6	10.3	12.1
Piperacillin-Tazobactam	2	16	≤0.015-32	100	0	0
Cefoxitin	4	>128	4->128	39.7	29.3	31
Ertapenem	1	4	0.12-4	100	0	0
Imipenem	0.25	0.5	0.12-2	100	0	0
Doripenem	0.5	1	≤0.25-2	96.6	NA	NA
Clindamycin	4	>16	1->16	27.6	29.3	43.1
Metronidazole	1	4	≤0.12-8	100	0	0
Moxifloxacin	4	>16	0.5->16	29.3	20.7	50
Tigecycline	1	16	≤0.06-32	72.4	10.3	17.2
B. thetaiotamicron (32)						
Amoxicillin-Clavulanate	1	8	0.25-16	87.5	9.4	3.1
Piperacillin-Tazobactam	8	8	≤0.015-16	100	0	0
Cefoxitin	32	32	8-64	31.3	65.6	3.1
Ertapenem	1	2	0.12-2	100	0	0
Imipenem	0.25	0.5	0.06-0.5	100	0	0
Doripenem	0.5	0.5	≤0.25-2	96.9	NA	NA
Clindamycin	4	>16	≤0.25->16	25	28.1	46.9
Metronidazole	1	2	≤0.12-32	96.9	0	3.1
Moxifloxacin	4	>16	0.5->16	34.4	15.6	50
Tigecycline	2	16	0.5-16	71.9	15.6	12.5
B. stercoris (22)						
Amoxicillin-Clavulanate	0.5	2	0.25-4	100	0	0
Piperacillin-Tazobactam	0.25	8	≤0.015-16	100	0	0
Cefoxitin	8	64	≤1-128	68.2	13.6	18.2
Ertapenem	0.5	1	0.12-2	100	0	0
Imipenem	0.12	0.5	0.06-1	100	0	0
Doripenem	≤0.25	0.5	≤0.25-2	95.5	NA	NA
Clindamycin	2	>16	≤0.25->16	63.6	18.2	18.2
Metronidazole	2	4	0.5-4	100	0	0
Moxifloxacin	1	8	0.25->16	63.6	0	36.4
Tigecycline	1	8	0.5-16	77.3	13.6	9.1

[a] The 377 isolates included 183 *B. fragilis*, 67 *B. fragilis* group, 58 *B. ovatus*, 32 *B. thetaiotamicron*, 22 *B. stercoris*, nine *Parabacteroides distasonis/merdae*, four *B. vulgatus*, one *B. buccae*, and one *B. caccae*. Individual species data is only presented where isolates numbers were ≥10.
[b] Clinical and Laboratory Standards Institute (M100-S21, 2011) MIC interpretative breakpoints not available. FDA MIC interpretative breakpoints used.
[c] NA – not available.

TABLE 2. Distribution of MICs for antimicrobial agents tested against 377 isolates of *Bacteroides* species

Antimicrobial agent	Number of isolates for which the antimicrobial agent MIC (μg/ml) was: (Cumulative % inhibition)[a] ≤0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Piperacillin-Tazobactam	34 (9.0)	13 (12.5)	13 (15.9)	76 (36.1)	64 (53.1)	24 (59.4)	29 (67.1)	27 (74.3)	31 (82.5)	43 (93.9)	19 (98.9)	**2 (99.5)**	2[b] (100)
Imipenem		1 (0.3)	76 (20.4)	101 (47.2)	85 (69.8)	65 (87.0)	31 (95.2)	13 (98.7)	**2 (99.2)**	1 (99.5)	1 (99.7)	1 (100)	
Ertapenem				57 (15.1)	103 (42.4)	52 (56.2)	92 (80.6)	38 (90.7)	**26 (97.6)**	6 (99.2)	1 (99.5)	2 (100)	
Doripenem					238 (63.1)	79 (84.1)	**29 (91.8)**	31[c] (100)					
Metronidazole				3 (0.8)	9 (3.2)	44 (14.9)	161 (57.6)	121 (89.7)	34 (98.7)	**4 (99.7)**	0 (99.7)	1 (100)	
Tigecycline			1 (0.3)	5 (1.6)	18 (6.4)	85 (28.9)	130 (63.4)	35 (72.7)	**31 (80.9)**	42 (92.0)	28 (99.5)	2 (100)	
Amoxicillin-Clavulanate				1 (0.3)	35 (9.5)	132 (44.6)	61 (60.7)	65 (78.0)	**34 (87.0)**	25 (93.6)	19 (98.7)	5 (100)	
Moxifloxacin				1 (0.3)	15 (4.2)	89 (27.9)	49 (40.8)	**56 (55.7)**	39 (66.0)	47 (78.5)	81[d] (100)		
Clindamycin					39 (10.3)	28 (17.8)	78 (38.5)	**53 (52.5)**	53 (66.6)	10 (69.2)	116[d] (100)		
Cefoxitin							3 (0.8)	1 (1.1)	41 (11.9)	132 (46.9)	**72 (66.0)**	69 (84.4)	59[b] (100)

[a] Susceptible MIC breakpoint indicated in bold type for each antimicrobial agent..
[b] 2/2 (piperacillin-tazobactam) and 21/59 (cefoxitin) isolate MICs were >64 μg/ml.
[c] 19/31 (doripenem) isolate MICs were >2 μg/ml.
[d] 48/81 (moxifloxacin) and 112/116 (clindamycin) isolate MICs were >16 μg/ml.

TABLE 3. Distribution of resistance and MDR[a] phenotpyes for 377 *B. fragilis* group isolates

Number of agents to which isolate was resistant	n	% of all isolates	% of MDR isolates	Most Frequent Resistance Phenotypes (n, %)
0	98	26.0	0	None
1	100	26.5	0	Moxifloxacin (37, 37.0%) Clindamycin (30, 30.0%) Cefoxitin (20, 20.0%) Tigecycline (9, 9.0%)
2	88	23.3	0	Amoxicillin-Clavulanate, Cefoxitin (27, 30.7%) Amoxicillin-Clavulanate, Clindamycin (20, 22.7%) Clindamycin, Moxifloxacin (19, 21.6%)
3	53	14.1	58.2%	Cefoxitin, Clindamycin, Moxifloxacin (19, 35.8%) Clindamycin, Moxifloxacin, Tigecycline (12, 22.6%) Amoxicillin-Clavulanate, Cefoxitin, Clindamycin (7, 13.2%) Amoxicillin-Clavulanate, Clindamycin, Moxifloxacin (6, 11.3%) Cefoxitin, Moxifloxacin, Tigecycline (4, 7.5%)
4	29	7.7	31.9%	Cefoxitin, Clindamycin, Moxifloxacin, Tigecycline (16, 55.2%) Amoxicillin-Clavulanate, Cefoxitin, Clindamycin, Moxifloxacin (7, 24.1%) Amoxicillin-Clavulanate, Clindamycin, Moxifloxacin, Tigecycline (4, 13.8%) Amoxicillin-Clavulanate, Clindamycin, Metronidazole, Moxifloxacin (1, 3.6%)
5	7	1.9	7.7%	Amoxicillin-Clavulanate, Cefoxitin, Clindamycin, Moxifloxacin, Tigecycline (7, 100%)
6	1	0.3	1.1%	Amoxicillin-Clavulanate, Cefoxitin, Clindamycin, Imipenem, Piperacillin-Tazobactam, Tigecycline (1, 100%)
7	1	0.3	1.1%	Amoxicillin-Clavulanate, Cefoxitin, Clindamycin, Imipenem, Moxifloxacin, Piperacillin-Tazobactam, Tigecycline (1, 100%)

[a] Multidrug-resistance defined as resistance to ≥3 antimicrobial classes (amoxicillin-clavulanate, cefoxitin, clindamycin, imipenem, metronidazole, moxifloxacin, piperacillin-tazobactam, tigecycline).

CONCLUSIONS

- Metronidazole, piperacillin-tazobactam, imipenem, and ertapenem were the agents with the greatest susceptibilities (>97%) against *B. fragilis* group isolates collected from across Canada in 2010-2011 (Table 1).
- The susceptibility rates for metronidazole, piperacillin-tazobactam, and imipenem and their MIC distributions (Table 2) remain unchanged compared with Canadian data published in 1992 (4).
- In contrast to metronidazole, piperacillin-tazobactam, and imipenem, rates of resistance for *B. fragilis* group have increased for amoxicillin-clavulanate, from 0.8% (1992) to 6.4% (2010-2011), and for clindamycin, from 9% (1992) to 33.4% (2010-2011).
- 26.0, 26.5, 23.3, and 24.1% of recent *B. fragilis* group isolates, respectively, were resistant to 0, 1, 2, and ≥3 antimicrobial agent classes (Table 3).
- Metronidazole, piperacillin-tazobactam, imipenem and ertapenem are highly active in vitro against current clinical isolates of *Bacteroides* species regardless of resistance to other frequently tested antimicrobial agents.

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