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Susceptibility trends of *Bacteroides fragilis* group isolates from Buenos Aires, Argentina

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ABSTRACT

The aim of this study was to analyze the susceptibility trends to seven antibiotics of *Bacteroides fragilis* group isolates based on three survey studies performed by the Committee of Anaerobic Bacteria between 1989 and 2002. Fifty three, 82 and 65 *B. fragilis* group isolates were collected during each period. The antimicrobial agents included were: ampicillin, ampicillin-sulbactam (2:1), cefoxitin, piperacillin, imipenem, clindamycin, and metronidazole. Minimal inhibitory concentrations (MICs) were determined according to the reference agar dilution method described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). The most active antibiotics for *B. fragilis* and non-*B. fragilis* species throughout the three periods were: imipenem with 99.1 and 100% of activity, respectively, and metronidazole with 100% of activity. The susceptibility to ampicillin-sulbactam showed a decrease, from 100% to 90.3% and to 82.4 % in the last period, for both *B. fragilis* and non-*B. fragilis* species, respectively. The overall susceptibility rates for cefoxitin, piperacillin, and clindamycin were significantly different between *B. fragilis* and non-*B. fragilis* species (84.2% vs. 56.5%; 85.9% vs. 66.7% and 88.8% vs. 64.7%, respectively, $p < 0.05$). Cefoxitin was the antibiotic that showed more variations as regards periods and species. The susceptibility rates for clindamycin were low, about 60%, for non-*B. fragilis* species during the last two periods. The variations observed in the susceptibility patterns of the *B. fragilis* group isolates emphasize the need to continue monitoring the emergence of resistance in order to guide the election of the most appropriate antibiotic therapy scheme for anaerobic infections.

Key words: *Bacteroides fragilis* group, susceptibility, antianaerobic drugs

RESUMEN

Tendencias en el perfil de sensibilidad de aislamientos del grupo *Bacteroides fragilis* obtenidos en Buenos Aires, Argentina. El objetivo de este estudio fue evaluar las variaciones en el perfil de sensibilidad frente a siete antimicrobianos de aislamientos del grupo *Bacteroides fragilis*, mediante el análisis de tres relevamientos realizados por la Subcomisión de Bacterias Anaerobias de la Asociación Argentina de Microbiología (años 1989-1991, 1996-1998 y 1999-2002). En los citados períodos se recolectaron 53, 82 y 65 aislamientos del grupo *B. fragilis*. Se evaluó la actividad de: ampicilina, ampicilina-sulbactama (2:1), cefoxitina, piperacilina, imipenem, clindamicina y metronidazol. La concentración inhibitoria mínima (CIM) se determinó utilizando el método de dilución en agar, según las normas del Clinical and Laboratory Standards Institute (CLSI, anteriormente NCCLS). En los tres períodos considerados, los antibióticos más activos frente a aislamientos de la especie *B. fragilis* como así también frente a aislamientos pertenecientes a otras especies del grupo *B. fragilis* fueron imipenem, con 99,1 y 100% de actividad, respectivamente, y metronidazol, con 100% de actividad. Con ampicilina-sulbactama se observó a lo largo del tiempo una disminución de la sensibilidad, desde el 100% en el primer período hasta un 90,3 y 82,4% en el último, para *B. fragilis* y para especies del grupo distintas de *B. fragilis,* respectivamente. Cuando se consideraron los tres períodos juntos, se observaron diferencias significativas entre la especie *B. fragilis* y los restantes aislamientos del grupo para cefoxitina, piperacilina y clindamicina (84,2% vs. 56,5%; 85,9% vs. 66,7% and 88,8% vs. 64,7%, respectivamente, $p < 0.05$). Cefoxitina fue el antibiótico que mostró mayores variaciones a través del tiempo y entre especies. Las tasas de sensibilidad a clindamicina fueron bajas (alrededor del 60%) entre los aislamientos no pertenecientes a la especie *B. fragilis* durante los últimos dos períodos. Las variaciones observadas en los perfiles de sensibilidad del grupo *B. fragilis* muestran la necesidad de vigilar periódicamente la emergencia de resistencia a los antimicrobianos, a fin de orientar el tratamiento de las infecciones por bacterias anaerobias.

Palabras clave: grupo *Bacteroides fragilis,* sensibilidad, antianaeróbicos

Bacteroides fragilis group constitutes the dominant anaerobic bacteria in the normal intestinal microflora, most frequently isolated in clinical infections and having the highest resistance rates to antimicrobial agents. However, *B. fragilis* is more susceptible to most antimicrobial agents than the other species within the group (5).

Most anaerobic infections are treated empirically based on susceptibility patterns reported in the literature. Studies carried out for the last ten years have demonstrated an association between antibiotic resistant *B. fragilis* and adverse outcome (11, 14). Thus, the increasing antimicrobial resistance among *B. fragilis* group should

be considered in the selection of empirical antimicrobial therapy.

Currently available antibiotics with activity against these organisms include clindamycin, metronidazole, b-lactams and b-lactam/b-lactamase inhibitor combinations. However, increased resistance to these agents has frequently been reported in recent years, e.g. the emerging resistance to metronidazole in several countries, the high prevalence of clindamycin resistance, and the isolation of *B. fragilis* group strains resistant to carbapenems and to b-lactam/b-lactamase inhibitor combinations (5).

Therefore, there is an ongoing need for documentation of the changing susceptibility patterns of these microorganisms. In the present study, we analyze the susceptibility trends of *B. fragilis* group strains isolated between 1989 and 2002 to seven antibiotics usually used in Argentina.

The Committee of Anaerobic Bacteria of SADEBAC - Asociación Argentina de Microbiología, coordinated three survey studies on antimicrobial resistance in anaerobic bacteria during the following periods: 1st period, 1989 to 1991 (1); 2nd period, 1996 to 1998 (7); and 3rd period, 1999 to 2002 (6). Fifty three, 82 and 65 non-duplicated clinical isolates of *B. fragilis* group were collected during each period. The isolates were obtained from seven medical centers in Ciudad Autónoma de Buenos Aires: Centro de Educación Médica e Investigaciones Clínicas Dr. Norberto Quirno (CEMIC), Hospital de Agudos Enrique Tornú, Hospital de Enfermedades Infecciosas Francisco J. Muñiz, Hospital Italiano, Hospital Nacional de Pediatría Juan P. Garrahan, Instituto de Investigaciones Médicas Alfredo Lanari, and Sanatorio Mitre. The species distribution in each period is shown in Table 1. Isolates were identified according to conventional biochemical methods.

The following seven antimicrobial agents were included in this study: ampicillin, ampicillin-sulbactam (2:1), cefoxitin, piperacillin, imipenem, clindamycin, and metronidazole. Minimal inhibitory concentrations (MICs) were determined according to the reference agar dilution method described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (8-10). Briefly, agar dilution test plates were inoculated with 10^5 CFU/spot using a Steers multipoint replicator, and incubated at 37 °C during 48 h in jars under anaerobic atmosphere generated by commercially envelopes (GenBox, bioMérieux, Marcy l´Étoile, France).

MIC was defined as the lowest concentration of antibacterial agent that inhibited visible growth. The following CLSI recommended susceptibility breakpoint values (µg/ml) were used (11): ampicillin £1, ampicillin/sulbactam £8/4, piperacillin £32, imipenem £4, cefoxitin £16, metronidazole £8, and clindamycin £2.

Susceptibility rates were compared using the chi-square test, Fisher exact test or the maximum probability method.

Table 2 shows the susceptibility pattern analysis of *B. fragilis* group against 7 antibiotics through the three mentioned periods. Trends were analyzed comparing *B. fragilis* and non-*B. fragilis* species susceptibility rates for each period.

All isolates (n=200) were susceptible to metronidazole, which turned out to be the most active antimicrobial agent tested. Sporadic reports of metronidazole resistant *B. fragilis* group have been published since 1978. However, in recent years some cases of clinical failures were reported. Fortunately, these resistances are still rare (13).

Although imipenem resistance due to metallo-b-lactamases has first been reported in 1986 by Cuchural *et al.* (4), it does not seem to be increasing in the world (3, 15). In this study, only in the first period we found one strain with imipenem MIC = 8 µg/ml; while during the followings, MICs values were 2 µg/ml or below. Interestingly, the strain which showed imipenem MIC = 8 µg/ml was considered susceptible because at that time, the

Table 1. Distribution of isolates for susceptibility studies during the three periods.

Microorganism	Nº of isolates		
	1989-1991	1996-1998	1999-2002
Bacteroides fragilis group	53	82	65
Bacteroides fragilis	40	45	31
Non-*Bacteroides fragilis*	13	37	34
Bacteroides distasonis	4	2	1
Bacteroides caccae	0	3	5
Bacteroides merdae	0	2	2
Bacteroides ovatus/thetaiotaomicron	4	22	12
Bacteroides uniformis	1	4	7
Bacteroides vulgatus	4	0	1
Others(1)	0	4	6

(1)Others: *Bacteroides fragilis* group non *B. fragilis*, non-identified species.

imipenem susceptible breakpoint was £8 µg/ml (8). Nowadays, this susceptibility value would be considered intermediate. Carbapenems resistance is encoded by the *cfiA* gene, also called *ccrA* gene, expressing a class B metallo-b-lactamase which confers resistance to all b-lactam antibiotics. A small percentage of *B. fragilis* strains carrying the *cfiA* gene, expresses the protein at a high enough level to classify the strain as resistant (12). Strains with intermediate level resistance to carbapenems, including some susceptible strains, could reach high level expression of this enzyme as a consequence of *in vitro* selection with imipenem. Thus, patients infected with these strains, would need antibiotic treatment other than b-lactams. Imipenem is frequently used in Argentina for severely ill patients. Although we have not found resistant strains to imipenem during the last two periods, we suggest testing imipenem activity on isolates from these patients.

Ampicillin-sulbactam was one of the most active b-lactams studied. There has been a worrying increase in the number of strains displaying decreased susceptibility

Table 2. Susceptibility patterns of *Bacteroides fragilis* group isolates through the three periods.

Antibiotic Microorganism (n)	MIC (mg/ml)			Susceptibility (%)	Overall susceptibility of the 3 periods (%)	p(1)
	Range	50%	90%			
Ampicillin						
B. fragilis						
1st period (40)	£ 0.5 - > 64	16	> 64	7.5		
2nd period (45)	2 - > 64	32	> 64	0	2.6	
3rd period (31)	1 - > 64	32	> 64	0		> 0.05
Non- *B. fragilis*						
1st period (13)	£ 0.25 - > 64	16	> 64	7.7		
2nd period (37)	0.125 - > 64	16	> 64	5.4	4.8	
3rd period (34)	0.5 - > 64	32	> 64	2.9		
Ampicillin/sulbactam						
B. fragilis						
1st period (40)	£ 0.25 - 4	0.5	2	100		
2nd period (45)	1 - 16	2	8	91.1	94.0	
3rd period (31)	0.5 - 32	2	8	90.3		
Non- *B. fragilis*						> 0.05
1st period (13)	£ 0.25 - 8	1	8	100		
2nd period (37)	0.5 - 16	2	8	91.9	89.4	
3rd period (34)	0.5 - 32	2	16	82.4		
Cefoxitin						
B. fragilis						
1st period (40)	£ 0.5 - >128	8	32	**95.0**(2)		
2nd period (45)	4 - 64	16	32	**75.6**(2, a)	84.2	
3rd period (31)	1 - 64	8	64	**82.8**(b)		< 0.001
Non- *B. fragilis*						
1st period (13)	£ 0.5 - 64	16	16	**92.9**(3, 4)		
2nd period (37)	2 - 64	16	64	**40.5**(3, a)	56.5	
3rd period (34)	£ 2 - 64	32	64	**58.8**(4, b)		
Piperacillin						
B. fragilis						
1st period (40)	2 - > 128	4	16	**95.0**(5)		
2nd period (45)	NP	NP	NP	NP	85.9	
3rd period (31)	£ 1 - >128	8	128	**74.2**(5)		
Non- *B. fragilis*						< 0.05
1st period (13)	4 - > 128	16	> 128	78.6		
2nd period (37)	NP	NP	NP	NP	66.7	
3rd period (34)	2 - >128	16	> 128	61.8		

Table 2. continuation

Antibiotic Microorganism (n)	MIC (mg/ml) Range	50%	90%	Susceptibility (%)	Overall susceptibility of the 3 periods (%)	p(1)
Imipenem						
Bacteroides fragilis						
1st period (40)	£ 0.5 - 8	£ 0.5	0.5	97.5		
2nd period (45)	0.06 - 2	0.25	1	100	99.1	
3rd period (31)	£ 0.015 - 2	0.06	0.25	100		
Non-*B. fragilis*						> 0.05
1st period (13)	£ 0.5 - 2	£ 0.5	£ 0.5	100		
2nd period (37)	£ 0.03 - 2	0.25	1	100	100	
3rd period (34)	£ 0.015 - 1	0.125	0.5	100		
Clindamycin						
Bacteroides fragilis						
1st period (40)	0.5 - 32	£ 0.5	4	97.5		
2nd period (45)	< 0.125 - > 256	1	> 256	**84.4(c)**	88.8	
3rd period (31)	£ 0.25 - > 8	1	> 8	**83.9(d)**		
Non- *B. fragilis*						< 0.001
1st period (13)	£ 0.5 - >32	0.5	> 32	85.7		
2nd period (37)	£ 0.03 - >256	2	8	**64.9(c)**	64.7	
3rd period (34)	£ 0.25 - > 8	2	> 8	**55.9(d)**		
Metronidazole						
B. fragilis						
1st period (40)	£ 0.5 - 4	£ 0.5	1	100		
2nd period (45)	0.25 - 8	1	2	100	100	
3rd period (31)	0.5 - 2	1	2	100		
Non- *B. fragilis*						> 0.05
1st period (13)	£ 0.5 - 2	£ 0.5	1	100		
2nd period (37)	0.25 - 4	1	2	100	100	
3rd period (34)	0.25 - 4	1	2	100		

(1) p: statistical significance between susceptibility rates of *B. fragilis* and non-*B. fragilis* species considering all three periods; NP: not performed; numbers **2** to **5**: statistical significance between periods of the same species or group of species, i.e. *B. fragilis* and non-*B.fragilis* isolates; letters **a** to **d**: statistical significance between *B. fragilis* and non-*B. fragilis* species for the same period; boldface: statistical significance, p< 0.05.

to ampicillin-sulbactam, from 100% to 90.3% (p=0.08) and to 82.4% (p > 0.05) in the last period, for both *B. fragilis* and non-*B. fragilis* species, respectively. This trend had previously been observed by Bianchini H. (2). These rates are somewhat higher than those shown by Snydman *et al.* (15), who reported an overall resistance of 2.4%, and observed a decrease from 3.6% to 1.7%, between 1997 and 2000. However, ampicillin-sulbactam and other b-lactams with b-lactamase inhibitors as piperacillin-tazobactam continue to be the most active b-lactams following the carbapenems (3, 5, 15). In the last years, a 100% activity of piperacillin-tazobactam against *B. fragilis* group was reported, as it had been observed in our last survey study (6).

The overall susceptibility rates for cefoxitin, piperacillin, and clindamycin were significantly different between *B. fragilis* and non-*B. fragilis* species (84.2% vs. 56.5%; 85.9% vs. 66.7% and 88.8% vs. 64.7%, respectively, p< 0.05).

Cefoxitin was the most variable antibiotic with respect to periods and species. There was a significant susceptibility decrease between 1989-1991, and 1996-1998 periods for both groups, *B. fragilis* (95% vs. 75.6%, p < 0.05) and non-*B. fragilis* species (92.9% vs. 40.5%, p < 0.05). Although susceptibility rates showed to be lower in the third period than in the first one, they were higher with respect to the second period, even though they did not demonstrate significant differences in the *B. fragilis* isolates (Table 2). Furthermore, when comparing *B. fragilis* with non-*B. fragilis* species, the susceptibility rates were significantly higher for *B. fragilis* in the second and third periods (p < 0.05) (Figure 1A).

The overall rate of susceptibility to clindamycin (88.8%) observed for *B. fragilis* was higher than that observed in

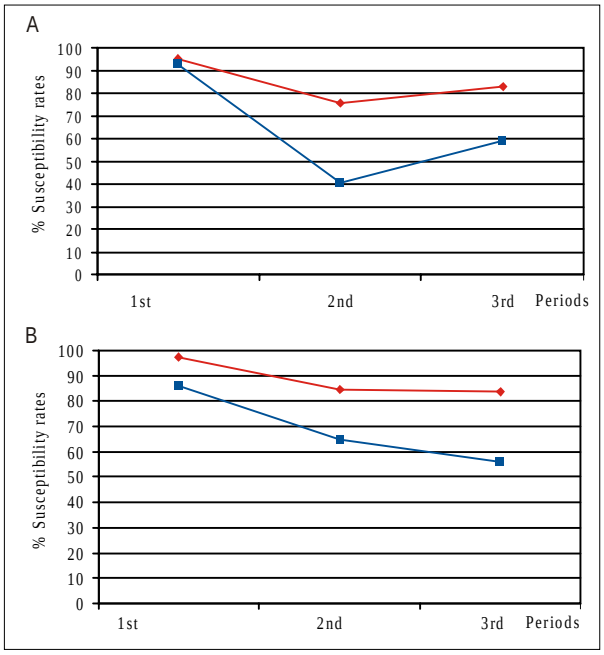

Figure 1. *B. fragilis*; non-*B. fragilis* species. Variations in susceptibility percentage at specified breakpoints for *B. fragilis* and non *B. fragilis* species over three periods for A- cefoxitin and B- clindamycin.

other studies (~ 67%) (3, 16) but similar to others (84%) (15). Our data of susceptibilities were low, about 60%, for non-*B. fragilis* species during the last two periods. The reports in the literature are quite variable for the different species of non-*B. fragilis* with values ranging from 25% to 80% (3, 15, 16). Similar to cefoxitin, non-*B. fragilis* species showed susceptibility rates significantly lower than *B. fragilis* isolates comparing the second and third periods (Figure 1B).

There was a trend to higher MIC values among the antimicrobial agents tested as shown in Table 2. The exceptions were imipenem and metronidazole. Nevertheless, the difference on the susceptibility rates among periods was not always significant. If this resistance increase is due to overuse of antibiotics in our country, it is difficult to prove.

The variations in susceptibility patterns observed during these 14 years, emphasize the need to continue monitoring the emergence of resistance against the most frequently antianaerobic drugs used. This would provide information to guide empirical treatment, although the results in one region or medical center, might not be applicable to another. Also, because susceptibility rates may vary among members of the *B. fragilis* group, it would be necessary to perform, routine identification to species level in clinical laboratories.

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