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Antibiotic resistance among anaerobic Gram-negative bacilli: lessons from a French multicentric survey

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Abstract

Temporal changes of antibiotic susceptibilities among anaerobes in France are followed in our laboratory since 1992. For *Bacteroides* strains, resistance increased from 1992 to 1998 for amoxicillin-clavulanic acid, cefotetan and clindamycin. The present study evaluates the situation in 2000 for 434 Gram-negative anaerobic clinical isolates (obtained from 9 large university hospitals) by testing amoxicillin and ticarcillin alone or combined with clavulanic acid, cefoxitin, cefotetan, imipenem, clindamycin and metronidazole (using the NCCLS-approved method for MIC determination. The main genera tested included *Bacteroides* (359 strains of the *fragilis* group), *Prevotella* (40 strains), *Fusobacterium* (23 strains) and miscellaneous species (8 strains).

Resistance rates within the *B. fragilis* group were: amoxicillin-clavulanic acid 5.6%, ticarcillin 33%, ticarcillin-clavulanic acid 2%, cefoxitin 13%, cefotetan 44%, clindamycin 33%, imipenem 1% and metronidazole <1%, respectively. Only one strain of *B. fragilis* was resistant to metronidazole (MIC = 64 mg/L); due to the presence of the *nimA* gene on the chromosome. Resistance to imipenem or metronidazole was only found among the *B. fragilis* species. These two former drugs excepted, *B. fragilis* was less resistant to antibiotics than the other species. β-lactamase production was detected for 357/359 strains of the fragilis group, 26/40 stains of *Prevotella* and 3/23 strains of *Fusobacterium*.

Dynamic changes of antibacterial resistance are occurring within the *B. fragilis* group: decreased resistance to amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, imipenem while resistance for cefoxitin, cefotetan, clindamycin continues to increase. Regular antibiotic surveys are needed as a source of information to guide the empirical therapy of anaerobic infections.

Keywords: Anaerobic bacteria; Antibiotic resistance; Antibiotic survey; Bacteroides fragilis

1. Introduction

Anaerobes are implicated in serious human infections. Since most clinical microbiology laboratories perform limited anaerobic bacteriology and often no susceptibility tests, it is important to provide updated survey to guide physicians in the most effective choices for antianaerobe therapy. Since 1992, our laboratory follows changes in antibiotic susceptibilities of anaerobes in France, using the same methodology. The sole modification was the replacement of Wilkins Chalgren medium by Brucella-blood agar in 1999. Changes in the susceptibility patterns of anaerobic isolates emerge mainly among Gram-negative bacilli: although β-lactamase production and concomitant resistance to some β-lactams is the rule in the *Bacteroides fragilis* group, both phenomena are increasingly encountered mainly in the *Prevotella* and *Fusobacterium* species. Additionally

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clindamycin resistance is not unusual amongst anaerobes.

Within the *B. fragilis* group, antibiotic resistance increased from 1992 to 1998 for amoxicillin-clavulanic acid, cefotetan, and clindamycin [1–6]. Decreased susceptibility [7] to metronidazole (MIC = 8 or 16 mg/L) was observed in France since many years (2–4% of the *B. fragilis* group strains), but true resistance to metronidazole (MIC ⩾ 32 mg/L) has not been described in France during this period [1–6]. This study surveyed the antibiotic susceptibilities of 434 Gram-negative anaerobic isolates collected in 2000 from 9 large university hospitals against amoxicillin and ticarcillin alone or combined with clavulanic acid, cefoxitin, cefotetan, imipenem, clindamycin and metronidazole.

2. Material and methods

2.1. Strains

Each laboratory included in the study collected 50 consecutive, non-duplicate clinical isolates belonging to the *B. fragilis* group or other Gram-negative species and sent them to the laboratory of Lille. The 434 anaerobic strains were from human clinical samples (blood culture, peritonitis, chronic sinusitis and otitis, lung abscess…). They were identified according to classical methods [8], then subcultured in a Rosenow medium (Biorad®, France). When they were not immediately used for determination of MICs, the Rosenow broth was kept frozen at −20°C. *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741 and *Eggerthella lenta* ATCC 43055 were used as control strains.

2.2. Antibacterial agents

Drug substances supplied were as follows metronidazole (Aventis, Paris), clindamycin (Pharmacia-Upjohn, Paris, France), amoxicillin, ticarcillin and claculanic acid (Smith Kline Beecham, Nanterre, France), cefotetan (Astra-Zeneca, Rueil-Malmaison, France), cefoxitin and imipenem (Merck Sharp Dohme, Paris, France). Antibacterials were reconstituted according to each manufacturer's instructions. Serial two-fold dilutions of antibacterial agents were prepared on the day of the test and added to the media in various concentrations.

2.3. Determination of minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations of nine antibacterial agents were determined by the agar dilution technique as recommended by the NCCLS, using 10^5 CFU of inoculum per spot and Brucella base-sheep-blood agar [9]. The plates were incubated in anaerobic chamber (Ruskin, Jouan, France) for 48 h at 35°C. MICs were defined as the lowest concentration of antibacterial agent resulting in a marked change in the appearance of growth as compared to the control plate.

Amoxicillin and ticarcillin were diluted with clavulanate tested at a constant concentration of 2 mg/L [10], as is usual in most European countries. To conform to the interpretative categories of the NCCLS, we added two plates containing 8/4 and 16/8 mg/L amoxicillin and clavulanic acid combinations respectively.

Resistance rates for amoxicillin, co-amoxiclav (amoxicillin + clavulanic acid), ticarcillin alone or combined with clavulanic acid, cefoxitin, cefotetan, imipenem, clindamycin and metronidazole were calculated at the NCCLS breakpoints. A reduced susceptibility breakpoint of ⩾ 8 mg/L was used for metronidazole [10].

2.4. Beta-lactamase production

<p> β-lactamase production was detected using a nitrocephin test (cefinase®, Biomérieux).

2.5. Statistical analysis

The statistical significance of difference in resistance rates for all pairwise comparisons was performed by using Pearson's chi-square test or the Fischer exact test.

3. Results and discussion

3.1. Species distribution

As expected (Table 1), the main part of the 359 clinical isolates of the *B. fragilis* group were *B. fragilis* (53%) followed by *B. thetaiotaomicron* (17%) and *B. vulgatus* (8%). *B. ovatus*, *B. distasonis*, *B. uniformis* and *B. caccae* were present at lower rates (5.6%, 5.3%, 5.3% and 4.4% respectively) and *B. eggerthii*, *B. merdae* and *B. stercoris* were rarely isolated (<1%). Isolation rates of the *B. fragilis* species are similar to those recently reported by Aldridge et al. [11].

3.2. MICS determination

The number of isolates, the range of MICs, MIC_{50} and MIC_{90} for each species are shown in Table 2 (*B. fragilis* group) and Table 5 (Gram-negative other than the *B. fragilis* group). The MICs for the three control strains were always within recommended limits.

3.2.1. Bacteroides fragilis group (359 isolates)

Low resistance rates (<5%) were noted for amoxicillin or ticarcillin combined with clavulanic acid, imipenem and metronidazole (Table 3). In contrast, high resistance rates were observed for clindamycin

Table 1
Distribution of Gram-negative anaerobic isolates

Organism	Number		
Bacteroides fragilis group	359	Including	
B. fragilis			189
B. thetaiotaomicron			60
B. ovatus			20
B. distasonis			19
B. caccae			16
B. merdae			2
B. vulgatus			30
B. uniformis			19
B. eggerthii			2
B. stercoris			2
Other *Bacteroides*	7	Including	
B. splanchnicus			3
B. capillosus			2
B. ureolyticus			2
Porphyromonas spp	4		
Prevotella spp	40	Including	
Pr. bivia			14
Pr. buccae			7
Black-pigmented *Prevotella*			14
Other non-pigmented *Prevotella*			5
Fusobacterium spp	23	Including	
F. nucleatum			13
F. mortiferum			2
Other *Fusobacterium*			8
Anaerorhabdus furcosus	1		
Total	434		

(32%) and cefotetan (44%). The majority of the investigated *B. fragilis* group strains were susceptible to metronidazole. Low-level resistance or reduced susceptibility (MIC 8–16 mg/L) was observed in 16 isolates (10 strains of *B. fragilis*, 3 *B. thetaiotaomicron*, 2 *B. vulgatus* and 1 *B. distasonis*). The susceptibility to ticarcillin/clavulanic acid was very similar to that of imipenem, with some minor exceptions. In total, 8 strains had MICs $\geqslant$128 mg/L for ticarcillin/clavulanic acid and of these three were also resistant to imipenem and one had reduced susceptibility (MIC = 8 mg/L). By comparison with the ticarcillin/clavulanic acid combination, resistance to co-amoxiclav was slightly more frequent as we isolated 20 resistant strains but 17 of them remained susceptible to imipenem.

3.3. *Bacteroides fragilis* (189 isolates) (Table 2)

One strain of *B. fragilis* isolated from a peritoneal fluid in Nancy was resistant to metronidazole (MIC = 64 mg/L), meanwhile 10 strains had reduced susceptibility (MIC of 8 or 16 mg/L). High-level resistance to metronidazole is rare, here it could be afterwards linked to the presence of two copies of the *nimA* gene on the chromosome of this strain (H. Marchandin, personal communication). Three strains were resistant to imipenem and all β-lactams. There was no high-level cross-resistance to imipenem and metronidazole.

With exception of imipenem and metronidazole, *B. fragilis* isolates were more susceptible to most antibiotics than other *Bacteroides* species (Table 3); the striking differences are obvious for clindamycin and cefotetan (Table 4). Resistance to clindamycin (21%) varied between institutions (from 3% in Lyon Hospital to 36% in Bichat Hospital, Paris).

3.3.1. *Other bacteroides species (150 isolates)*

No resistance to neither imipenem nor metronidazole could be detected. Reduced susceptibility to metronidazole was noted only for 3 strains *of B. thetaiotaomicron*,

Table 2
Antimicrobial activities of the various antimicrobials against 359 isolates of *B. fragilis* group

Antimicrobial agents and species groups	MIC (mg/L)		
	Range	50%	90%
Bacteroides fragilis (189)			
Amoxicillin	0.5–>64	32	>64
Amoxicillin + clavulanic acid	⩽0.06–>64	0.25	2
Ticarcillin	1–>256	32	>256
Ticarcillin + clavulanic acid	⩽0.125–>256	0.5	4
Cefoxitin	1–>128	8	64
Cefotetan	1–>128	8	64
Imipenem	⩽0.03–128	0.125	0.5
Clindamycin	⩽0.06–>256	1	>256
Metronidazole	0.125-64	1	4
Bacteroides thetaiotaomicron (60)			
Amoxicillin	0.125–>64	32	>64
Amoxicillin + clavulanic acid	0.125–64	0.5	4
Ticarcillin	0.5–>256	64	>256
Ticarcillin + clavulanic acid	0.5–128	4	16
Cefoxitin	8–128	32	64
Cefotetan	32–>128	128	>128
Imipenem	0.06–2	0.5	1
Clindamycin	⩽0.06–>256	4	>256
Metronidazole	0.125–8	1	2
Bacteroides ovatus (20)			
Amoxicillin	32–>64	32	>64
Amoxicillin + clavulanic acid	0.25–64	0.5	16
Ticarcillin	16–>256	64	>256
Ticarcillin + clavulanic acid	0.25–256	4	16
Cefoxitin	8–64	128	>128
Cefotetan	32–>128	8	64
Imipenem	0.125–1	0.25	0.5
Clindamycin	⩽0.06–>256	8	>256
Metronidazole	0.5–4	1	2
B. distasonis (19)			
Amoxicillin	⩽0.06–>64	16	>64
Amoxicillin + clavulanic acid	⩽0.06–>64	1	16
Ticarcillin	4–>256	32	>256
Ticarcillin + clavulanic acid	⩽0.125–>256	8	64
Cefoxitin	4–64	16	32

Table 2 (*continued*)

Antimicrobial agents and species groups	MIC (mg/L)		
	Range	50%	90%
Cefotetan	4–>128	64	>128
Imipenem	0.06–2	0.5	1
Clindamycin	0.5–>256	>256	>256
Metronidazole	0.25–8	1	2
B. caccae (16)			
Amoxicillin	8–>64	32	64
Amoxicillin + clavulanic acid	0.125–1	0.25	0.5
Ticarcillin	16–>256	32	64
Ticarcillin + clavulanic acid	⩽0.125–4	1	2
Cefoxitin	4–32	32	32
Cefotetan	4–>128	64	>128
Imipenem	0.06–1	0.25	0.5
Clindamycin	⩽0.06–>256	2	>256
Metronidazole	0.25–1	1	1
B. vulgatus (30)			
Amoxicillin	4–>64	>64	>64
Amoxicillin + clavulanic acid	0.125–>64	0.5	8
Ticarcillin	4–>256	128	>256
Ticarcillin + clavulanic acid	⩽0.125–16	0.5	2
Cefoxitin	2–128	8	64
Cefotetan	4–>128	8	128
Imipenem	0.125–2	0.5	1
Clindamycin	⩽0.06–>256	1	>256
Metronidazole	0.5–16	1	2
B. uniformis (19)			
Amoxicillin	16–>64	32	>64
Amoxicillin + clavulanic acid	0.125–64	0.25	4
Ticarcillin	16–>256	64	>256
Ticarcillin + clavulanic acid	⩽0.125–128	1	16
Cefoxitin	2–64	16	64
Cefotetan	4–>128	64	>128
Imipenem	0.125–1	0.25	0.5
Clindamycin	⩽0.06–>256	2	>256
Metronidazole	0.5–2	1	2
Bacteroides fragilis group (359)			
Amoxicillin	0.06–>64	32	>64
Amoxicillin + clavulanic acid	⩽0.06–>64	0.25	4
Ticarcillin	0.5–>256	32	>256
Ticarcillin + clavulanic acid	⩽0.125–>256	1	16
Cefoxitin	1–>128	16	64
Cefotetan	1–>128	32	>128
Imipenem	⩽0.03–128	0.25	1
Clindamycin	⩽0.06–>256	2	>256
Metronidazole	0.125–64	1	2

2 *B. vulgatus* and 1 *B. distasonis*. Considering the other antibiotics, resistance rates were generally higher (Table 3). Resistance rates for clindamycin were in the 35–55% range. Cefotetan resistance among strains of *B. thetaiotaomicron* and *B. ovatus* was very frequent (98%) (Table 4). Higher resistance rates among non-*B. fragilis* species were already stated in USA [12].

3.3.2. Importance of the species level when considering antibiotic resistance

Considering cefotetan and clindamycin, significant differences in susceptibility were noted among the various species (Table 4). *B. fragilis* is more susceptible than *B. thetaiotaomicron* ($p<0,0001$), *B. ovatus* ($p<0,0001$), *B. vulgatus* ($p=0.021$) and other species ($p<0,0001$). *B. thetaiotaomicron* is more resistant than *B. distasonis* ($p=0,0002$), *B. vulgatus* ($p<0,0001$) and other species ($p<0,0001$), but did not differ from *B. ovatus* ($p=0.92$). We did not find significant differences in resistance to clindamycin and cefotetan for *B. distasonis* and *B. vulgatus* ($p=0.135$) or other species ($p=0.864$). Thus results for the *B. fragilis* group could

Table 4
Resistance of the *B. fragilis* group species to cefotetan and clindamycin

Species group	No.	Cefotetan-R		Clindamycin-R	
		No. of strains	%	No. of strains	%
1. B. fragilis					
B. fragilis	189	36	19	40	21
2. *B. thetaiotaomicron* and *B. ovatus*					
B. thetaiotaomicron	60	56	98	28	47
B. ovatus	20	19	98	11	55
3. Other species					
B. distasonis					
B. caccae, *B. merdae*	37	23	62	17	46
B. vulgatus	30	10	33	13	43
Other Bacteroides	23	14	61	8	35
Overall isolates	359	158	44	117	33

Table 3
Antimicrobial resistance in the *B. fragilis* group

Species group (No.)	Resistance rates % (antimicrobial breakpoint in mg/L)							
	AMC (⩾16/8)	TIC (⩾128)	TIM (⩾128/2)	FOX (⩾64)	CTT (⩾64)	IMP (⩾16)	MTZ (⩾32)	CLI (⩾8)
B. fragilis (189)	4.8	28.6	1.6	11	19	1.6	0.5	21
Non-*B. fragilis* (170)	6.5	39.4	2.9	15.2	72	0	0	45.3
B. fragilis group (359)	5.6	33.7	2.2	13	44	0.8	0.3	32.6

AMX = amoxicillin, AMC = amoxicillin + clavulanic acid, TIC = ticarcillin, TIM = ticarcillin + clavulanic acid, FOX = cefoxitin, CTT = cefotetan, IMP = imipenem, MTZ = metronidazole, CLI = clindamycin.

Table 5
Antimicrobial activities of the various antimicrobials against Gram-negative anaerobes other than the *B. fragilis* group (75 isolates)

Antimicrobial agents and species groups	MIC (mg/L)		
	Range	50%	90%
Bacteroides spp (8)			
Amoxicillin	⩽0.06–32		
Amoxicillin + clavulanic acid	⩽0.06–0.5		
Ticarcillin	0.125–64		
Ticarcillin + clavulanic acid	⩽0.125–1		
Cefoxitin	⩽0.25–16		
Cefotetan	⩽0.25–64		
Imipenem	⩽0.03–0.125		
Clindamycin	⩽0.06–1		
Metronidazole	0.125–4		
Prevotella spp (40)			
Amoxicillin	⩽0.06– > 64	4	64
Amoxicillin + clavulanic acid	⩽0.06–1	⩽0.06	0.25
Ticarcillin	⩽0.125–128	4	32
Ticarcillin + clavulanic acid	⩽0.125–1	⩽0.125	0.5
Cefoxitin	<0.25–16	1	8
Cefotetan	⩽0.25–32	2	8
Imipenem	⩽0.03–0.5	0.06	0.25
Clindamycin	⩽0.06– > 256	0.125	0.5
Metronidazole	⩽0.06–8	1	4
Porphyromonas spp (4)			
Amoxicillin	⩽0.06–0.125		
Amoxicillin + clavulanic acid	⩽0.06		
Ticarcillin	⩽0.125–0.5		
Ticarcillin + clavulanic acid	⩽0.125		
Cefoxitin	⩽0.25–0.5		
Cefotetan	⩽0.25–1		
Imipenem	⩽0.03–0.06		
Clindamycin	⩽0.06–1		
Metronidazole	0.125–8		
Fusobacterium spp (23)			
Amoxicillin	⩽0.06–32	⩽0.06	32
Amoxicillin + clavulanic acid	⩽0.06–2	⩽0.06	0.5
Ticarcillin	⩽0.125–32	32	256
Ticarcillin + clavulanic acid	⩽0.125–16	⩽0.125	1
Cefoxitin	⩽0.25–16	<0.25	8
Cefotetan	⩽0.25–16	<0.25	8
Imipenem	⩽0.03–4	0.06	0.5
Clindamycin	⩽0.06–4	<0.06	1
Metronidazole	⩽0.06–4	0.125	2

be separated in three groups: (i) *B. fragilis*, (ii) *B. thetaiotaomicron* and *B. ovatus* (iii) other species.

Using the Z Fisher test for each group of species, resistance to clindamycin and cefotetan were linked together ($p<0.01$), although there was no relationship in their resistance mechanisms.

3.3.3. Other Gram-negative anaerobes

As previously stated [13], β-lactamase production was detected among 26/40 *Prevotella* species (65%) and 3/23 strains of *Fusobacterium* (13%); thus, these strains were reported as resistant to amoxicillin (Table 5).

Resistant strains to co-amoxiclav, ticarcillin + clavulanic acid, cefoxitin imipenem and metronidazole could not be found among *Prevotella* and *Fusobacterium*; only one strain of *Prevotella melaninogenica* and one strain of *P. buccae* were resistant to clindamycin. The strain of *Anaerorhabdus furcosus* and all strains of *Porphyromonas* were susceptible to all β-lactams tested as well as to clindamycin and metronidazole.

3.4. Temporal evolution of susceptibilities within the B. fragilis group

Ninety-eight percent of the strains were resistant to amoxicillin (breakpoint 2 mg/L). If a higher breakpoint (⩾64 mg/L) was used to detect highly resistant isolates, 30% of the strains reached that level, which is a major increase in resistance since the last French survey from 1999 [6], when 25% of the investigated strains had a MIC⩾64 mg/L. Similarly, ticarcillin resistance is slowly increasing (Table 6 and Fig. 1). In 1999, resistance to imipenem was high comparatively to the previous years. Production of a carbapenemase leads to cross-resistance to all β-lactams that may hide any evolution of resistance among combinations of clavulanic acid with penicillins. Considering the imipenem-susceptible strains, resistance to co-amoxiclav is increasing from 2.3% in 1994 to 4.8% in 2000 (Fig. 2). Resistance to clindamycin (32%) and cefotetan (44%) is steadily increasing (Table 6, Fig. 3). In the survey from 1999 [6], only five *B. fragilis* strains (2.2%) with reduced susceptibility to metronidazole were reported (MIC = 8 mg/L) compared to the present findings (4.5%), where 16 isolates had MICs ⩾8 mg/L. Decreased susceptibility to metronidazole, clindamycin, and cefotetan is illustrated in Fig. 3. Several reports from different parts of the world indicate an increase of resistance, although variations are considerable [14–17].

4. Conclusions

This study illustrates the dynamic changes that are occurring among antibacterial resistance of anaerobic pathogens when compared to previously published surveys. The antibacterial resistance among the *B. fragilis* group in France like in most countries of Europe is increasing. Resistance to either metronidazole or imipenem could only be detected for *B. fragilis*. Considering the other antibiotics, the non-*B. fragilis* species are more resistant to most antibiotics than *B. fragilis*. The former group could be separated in two sub-groups (i) *B. thetaiotaomicron–B. ovatus*, (ii) the other species. The activity of cefoxitin, an antibiotic used mainly in prophylaxis of intra-abdominal surgery, remains good. In contrast as resistance to cefotetan and

Table 6
Evolution of the antimicrobial resistance among strains of the *B. fragilis* group strains in the 1992–2000 period in France

Year (reference)	No. of strains	Resistance rates (antimicrobial breakpoint in mg/L)							
		AMC[a] (⩾16/8)	AMC^R IMP^S (⩾16/8)	TIC (⩾128)	TIM (⩾128/2)	CTT (⩾64)	IMP (⩾8)	CLI (⩾4)	MTZ (⩾4)
1992 [1]	61	3	0	27	3	24	3	12	0
1994 [2]	222	2.7	2.3	24	0.9	28	0.4	14	2
1995 [3]	129	4.6	3.1	23	1,5	ND	1.5	19	0
1996 [4]	199	4	1.5	22	1.5	27.1	1.5	20.6	0.4
1998 [5]	209	3.8	3.8	22.2	0	28.6	0	29.2	1.9
1999 [6]	228	10.1	5.7	32.8	6.6	35.1	4.4	28.1	2.2
2000 (this study)	359	5.6	4.8	33.7	2.2	44	0.8	32.6	4.7

AMC^R IMP^S Resitance rate to co-amoxiclav among imipenem-susceptible strains.
[a] legends as in Table 3.

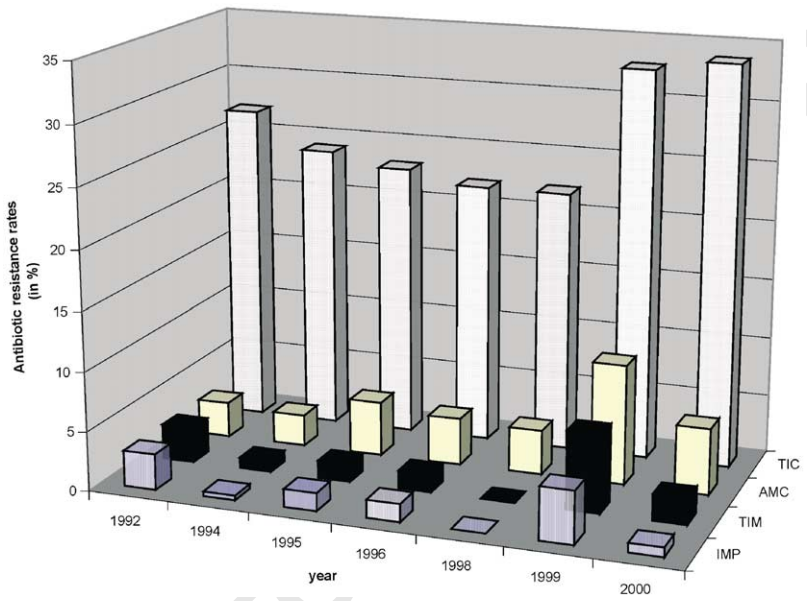

Fig. 1. *B. fragilis* group: evolution of the antimicrobial resistance from 1992 to 2000 (ticarcillin (TIC), amoxicillin + clavulanic acid (AMC), ticarcillin + claulanic acid (TIM) and imipenem (IMP)).

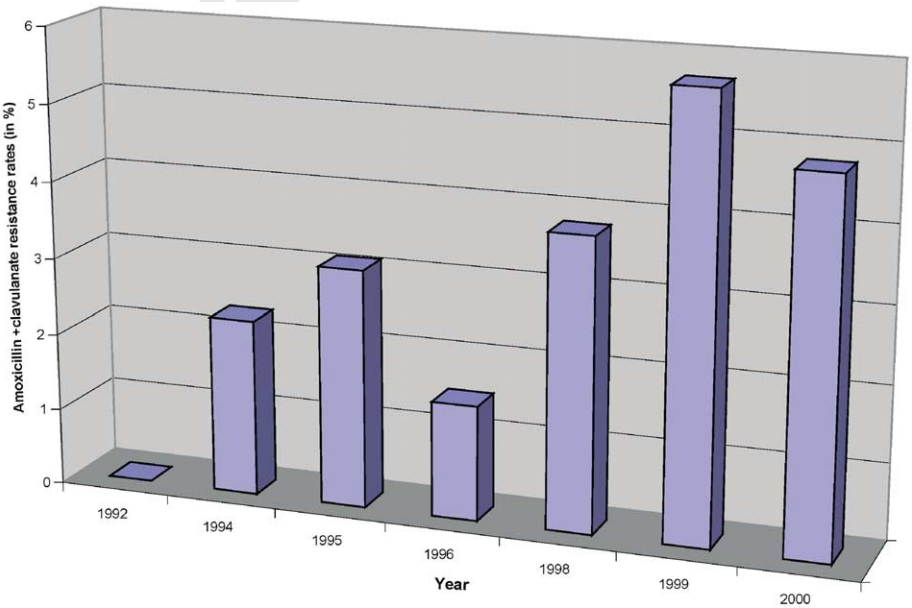

Fig. 2. Evolution of amoxicillin + clavulanate resistance rates among impienem-susceptible *B. fragilis* strains from 1992 to 2000.

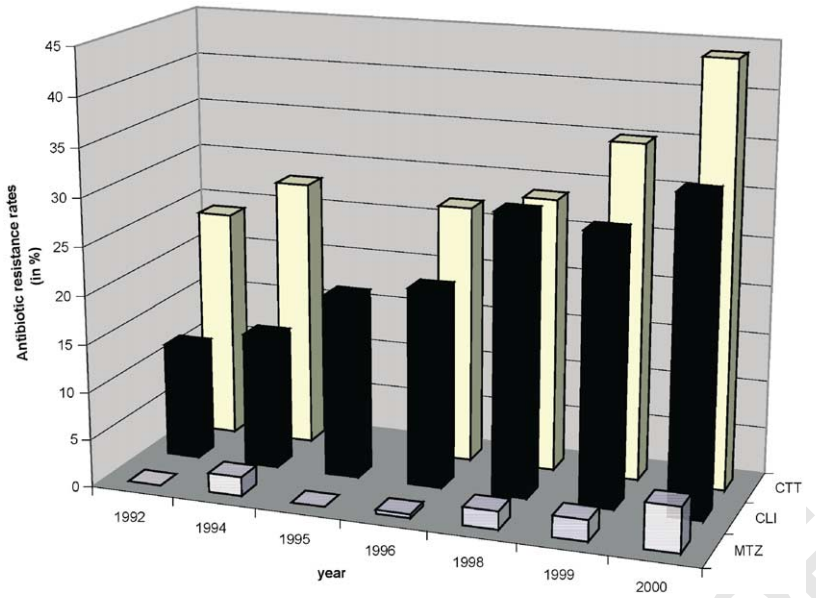

Fig. 3. *B. fragilis* group: evolution of the antimicrobial resistance from 1992 to 2000 (cefotetan (CTT), clindamycin (CLI) and metronidazole (MTZ)).

clindamycin is increasing their anti-anaerobe activity may not be warranted during empirical treatments.

Combination of penicillins with clavulanic acid, imipenem and metronidazole are the more potent antibacterial agents against Gram-negative anaerobes. Reduced susceptibility to metronidazole seems to increase nowadays. Continuous surveillance of antibacterial susceptibility is necessary in Gram-negative anaerobic bacteria, especially the *B. fragilis* group.

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