Journal of Antimicrobial Chemotherapy (2007) **59**, 132–139
doi:10.1093/jac/dkl458
Advance Access publication 9 November 2006

JAC

Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria

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Received 4 July 2006; returned 7 August 2006; revised 11 September 2006; accepted 14 October 2006

***Objectives*: To collect recent data on the susceptibility of anaerobes and to compare them with results from previous studies.**

***Methods*: Four hundred and forty-three anaerobic clinical isolates from various body sites were prospectively collected from October 2003 to February 2005 in nine Belgian hospitals. MICs were determined for nine anti-anaerobic and three recently developed antibiotics.**

***Results*: Most Gram-negative bacilli except *Fusobacterium* spp. were resistant to penicillin. Piperacillin/tazobactam, metronidazole, chloramphenicol, meropenem and amoxicillin/clavulanic acid were very active against all groups, but only 86% of *Bacteroides fragilis* group strains were susceptible to the latter. Cefoxitin, cefotetan and clindamycin were less active. In particular, only 62%, 52% and 48% of *B. fragilis* group strains were susceptible, respectively. Clindamycin shows a continuing decrease in activity, as 83% were still susceptible in 1987 and 66% in 1993–94. Anti-anaerobic activity of the new antibiotics is interesting, with MIC_{50} and MIC_{90} of 1 and >32 mg/L for moxifloxacin, 2 and 4 mg/L for linezolid and 0.5 and 8 mg/L for tigecycline.**

***Conclusions*: The susceptibility of anaerobic bacteria remains stable in Belgium, except for clindamycin, which shows a continuous decrease in activity. However, for each of the tested antibiotics, at least a few resistant organisms were detected. Consequently, for severe infections involving anaerobic bacteria, it could be advisable to perform microbiological testing instead of relying on known susceptibility profiles. Periodically monitoring background susceptibility remains necessary to guide empirical therapy.**

Keywords: anaerobes, Etest, surveillance, empirical therapy

Introduction

Anaerobic bacteria are commonly found in polymicrobial infections. Antimicrobial therapy for the management of infections with a high probability of anaerobic aetiology includes an antimicrobial agent with known efficacy against anaerobes. Antibiotic resistance among anaerobic bacteria has increased in recent years and it has been reported against antibiotics that were previously thought to be universally active, such as carbapenems and imidazoles.[1] Since anaerobic cultures are cumbersome and susceptibility testing of anaerobic isolates is generally not performed routinely, it is important to have good knowledge of

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background susceptibility to avoid the use of inappropriate empirical antibiotics. Therefore, it is recommended to periodically monitor local and regional resistance patterns.[2] Two multicentre surveys have already been performed in Belgium, the first one in 1987[3] and the second one in 1993–94.[4] Since that time no published data are available about antimicrobial susceptibility of anaerobic bacteria. The objective of this study was to collect recent data on the susceptibility of anaerobes in our country. The results were analysed in reference to the previous surveys.

Materials and methods

Bacteria

Strains were collected from October 2003 to February 2005 in 8 Belgian university hospitals and one general hospital: Academisch Ziekenhuis Vrije Universiteit Brussel (Brussels), Hôpital Universitaire Erasme (Brussels), Cliniques Universitaires Saint-Luc (Brussels), Universitair Ziekenhuis Antwerpen (Antwerp), Universitair Ziekenhuis Leuven (Leuven), Centre Hospitalier Universitaire du Sart-Tilman (Liège), Cliniques Universitaires de Mont-Godinne (Yvoir), Algemeen Ziekenhuis Sint-Jan (Brugge) and Universitair Ziekenhuis Gent (Ghent). Six of these centres participated in the previous surveys. Each centre collected prospectively up to 50 unselected, non-duplicated clinically significant strict anaerobic isolates. Specimen source was recorded for each isolate. The isolates were sent for susceptibility testing to the microbiology laboratory of the Academisch Ziekenhuis Vrije Universiteit Brussel.

Identification

Species identification was performed by standard methods in the collecting laboratories. Identification was checked at the Academisch Ziekenhuis Vrije Universiteit Brussel by analysis of cellular fatty acid composition using the Microbial Identification System (MIS), followed by appropriate biochemical or enzymatic tests[5] if the MIS results did not support the identification of the collecting laboratory.

Susceptibility testing

The antibiotic susceptibility was determined by Etest® methodology (AB Biodisk, Solna, Sweden), which was previously shown to produce accurate results for susceptibility testing of anaerobic bacteria.[6] Brucella agar supplemented with laked sheep blood, haemin and vitamin K1 was used as recommended for the CLSI (formerly NCCLS) reference agar dilution procedure.[2] The following antimicrobial agents were tested: penicillin, amoxicillin/clavulanic acid, clindamycin, metronidazole, meropenem, chloramphenicol, cefoxitin, cefotetan, moxifloxacin, linezolid and piperacillin/tazobactam. The agar plates were inoculated with a McFarland 1 suspension and incubated in anaerobiosis. The results were read after 48 h. For slow growers, reading was performed after 72 h. Interpretation was carried out according to the manufacturer's recommendations. Because of the unavailability of tigecycline Etest® strips when the study was performed the MIC of this antimicrobial agent was determined by the CLSI agar dilution procedure.[2] *Bacteroides fragilis* ATTC 25285, *Bacteroides thetaiotaomicron* ATCC 29741 and *Eubacterium lentum* ATCC 43055 were included as control strains in each test run. The isolates were categorized by using the following breakpoints for susceptible and resistant strains, respectively: penicillin[2] $\leq$0.5 and $\geq$2 mg/L, amoxicillin/clavulanic acid[2] $\leq$4/2 and $\geq$16/8, clindamycin[2] $\leq$2 and $\geq$8, metronidazole[2] $\leq$8 and $\geq$32, meropenem[2] $\leq$4 and $\geq$16, chloramphenicol[2] $\leq$8 and $\geq$32, cefoxitin[2] $\leq$16 and $\geq$64, cefotetan[2] $\leq$16 and $\geq$64, moxifloxacin[7] $\leq$1 and $\geq$4, linezolid[8] $\leq$4 and >4, piperacillin/tazobactam[2] $\leq$32/4 and $\geq$128/4 and tigecycline $\leq$2 and $\geq$8 (the latter as recommended by the manufacturer). In addition, a β-lactamase test was performed on each isolate by using the nitrocefin test. Since some breakpoints differ slightly from those used in the report of the 1987 survey and in addition an intermediate category has now been established, all 1987 data were computed again using the individual MIC results for comparison between the surveys.

Results

Four hundred and forty-three (443) anaerobic isolates were collected from various sources: 151 from abdominal sites, 98 from blood, 70 from wounds and pus, 46 from abscesses, 14 from the respiratory tract, 11 from gynaecological and obstetrical sites, 6 from the central nervous system, 6 from ear and sinus, and 41 from miscellaneous other sites.

Table 1 summarizes the susceptibility results for the different groups of anaerobes. Table 2 compares the percentages of susceptible strains in this present survey with those found in the 1993–94 and 1987 surveys. The distribution of individual species is presented in the footnotes of Table 1 for the strains of this study and can be found in original reports for the previous surveys.[3,4]

Overall, β-lactamases were detected in 62% of the 443 isolates. Most β-lactamase-producing strains belong to the *B. fragilis* group (98% β-lactamase-positive) and to the group of *Prevotella* spp. and other Gram-negative bacilli (70% β-lactamase-positive). As compared with the previous surveys of 1987 and 1993–94, the percentage of β-lactamase-producing strains increased in this last group from 31% to 57% and 70%, respectively. Among *Clostridium* spp. 5 β-lactamase-producing clostridia (3 *Clostridium clostridioforme*, 1 *Clostridium tertium* and 1 *Clostridium* spp.) out of 57 isolates (9%) were detected in this study. In 1993–94 only one β-lactamase-positive *Clostridium* isolate was found. In 1987 all clostridia were β-lactamase-negative. All other organisms including all *Fusobacterium* were β-lactamase-negative in the three surveys.

Penicillin, a compound very susceptible to β-lactamases, was active against only 1% of *B. fragilis* group strains, a result similar to that of the previous studies. The susceptibility of *Prevotella* spp. and other Gram-negative bacilli decreased markedly from 64% in 1987 to 48% in 1993–94 and to only 26% in this survey. Penicillin activity was much better against other anaerobic isolates. However, a decrease in the prevalence of penicillin-susceptible isolates was seen in *Clostridium* spp., non-spore-forming Gram-positive bacilli and anaerobic cocci from 91%, 93% and 92% in 1987 to 83%, 81% and 84% in this survey, respectively. In contrast, all *Fusobacterium* isolates in this survey were susceptible to penicillin compared with 70% and 88% in 1987 and 1993–94, respectively.

The activity of cefoxitin was less affected by the β-lactamases: 62% of *B. fragilis* group strains and 98% of *Prevotella* spp. and other Gram-negative bacilli were susceptible. Eighty-six percent (86%) of *B. fragilis* strains were susceptible as opposed to 30% of strains belonging to other species of the *B. fragilis* group. The susceptibility rates of all other groups of anaerobes were high (>90%).

Cefotetan was less active than cefoxitin against anaerobic Gram-negative bacilli: 52% of *B. fragilis* group strains and 86%

Table 1. Antimicrobial activities of 12 antibiotics against different groups of anaerobes

Organism	Antimicrobial agent	Range (mg/L)	MIC_{50}	MIC_{90}	%S	%I	%R
Bacteroides fragilis group[a]	penicillin	0.25–>32	>32	>32	1	0	99
(238 strains) (98%	cefotetan	2–>256	16	>256	52	5	43
β-lactamase-positive)	cefoxitin	0.5–>256	16	128	62	18	20
	amoxicillin/clavulanate	0.25–32	0.5	8	86	9	5
	piperacillin/tazobactam	<0.016–>256	1	32	95	3	2
	meropenem	0.016–32	0.125	1	97	2	1
	clindamycin	<0.016–256	4	>256	48	13	39
	metronidazole	<0.016–32	0.5	1	99	0	1
	chloramphenicol	0.5–16	4	8	99	1	0
	moxifloxacin	0.125–>32	1	>32	52	16	32
	linezolid	0.5–16	2	4	99	0	1
	tigecycline	0.125–32	1	8	73	8	19
B. fragilis (135 strains)	penicillin	0.25–>32	>32	>32	1	0	99
(98% β-lactamase-positive)	cefotetan	2–>256	8	64	83	3	14
	cefoxitin	2–256	8	32	86	7	7
	amoxicillin/clavulanate	0.25–16	0.5	4	92	7	1
	piperacillin/tazobactam	<0.016–8	0.5	2	100	0	0
	meropenem	0.064–32	0.125	0.5	96	2	2
	clindamycin	0.032–>256	2	>256	60	10	30
	metronidazole	0.064–32	0.5	1	99	0	1
	chloramphenicol	1–8	4	8	100	0	0
	moxifloxacin	0.125–>32	1	>32	68	5	27
	linezolid	0.5–8	2	4	99	0	1
	tigecycline	0.25–32	1	8	80	2	18
B. fragilis group without	penicillin	0.25–>32	>32	>32	1	0	99
B. fragilis (103 strains)	cefotetan	2–>256	>256	>256	11	8	81
(99% β-lactamase-positive)	cefoxitin	0.5–>256	32	256	30	33	37
	amoxicillin/clavulanate	0.25–32	1	16	78	11	11
	piperacillin/tazobactam	0.06–>256	16	64	89	7	4
	meropenem	0.016–8	0.25	1	98	2	0
	clindamycin	<0.016–>256	4	>256	33	17	50
	metronidazole	<0.016–32	0.5	1	99	0	1
	chloramphenicol	0.5–16	4	8	98	2	0
	moxifloxacin	0.125–>32	2	>32	30	30	40
	linezolid	0.5–16	2	4	98	0	2
	tigecycline	0.125–32	1	8	64	15	21
Fusobacterium	penicillin	<0.002–0.25	0.016	0.125	100	0	0
spp.[b] (30 strains)	cefotetan	0.016–8	0.062	1	100	0	0
(0% β-lactamase-positive)	cefoxitin	<0.016–8	0.064	1	100	0	0
	amoxicillin/clavulanate	<0.016–4	0.032	0.5	100	0	0
	piperacillin/tazobactam	<0.016–8	0.016	1	100	0	0
	meropenem	<0.002–0.5	0.006	0.064	100	0	0
	clindamycin	<0.016–8	0.032	0.125	90	3	7
	metronidazole	<0.016–0.5	0.016	0.125	100	0	0
	chloramphenicol	0.25–2	0.25	0.5	100	0	0
	moxifloxacin	0.016–>32	0.5	2	80	10	10
	linezolid	0.03–1	0.25	0.25	100	0	0
	tigecycline	0.03–0.25	0.125	0.25	100	0	0

Table 1. (*continued*)

Organism	Antimicrobial agent	Range (mg/L)	MIC_{50}	MIC_{90}	%S	%I	%R
Prevotella species and other Gram-negative bacilli[c] (50 strains) (70% β-lactamase-positive)	penicillin	0.004–>32	32	>32	26	2	72
	cefotetan	0.03–128	4	32	86	6	8
	cefoxitin	<0.016–64	1	16	98	0	2
	amoxicillin/clavulanate	<0.016–4	0.125	2	100	0	0
	piperacillin/tazobactam	<0.016–>256	0.125	1	98	0	2
	meropenem	0.03–0.5	0.064	0.125	100	0	0
	clindamycin	<0.016–>256	0.032	>256	82	0	18
	metronidazole	<0.016–8	0.125	1	100	0	0
	chloramphenicol	0.125–4	1	4	100	0	0
	moxifloxacin	0.03–>32	0.5	16	70	6	24
	linezolid	0.25–8	1	2	98	0	2
	tigecycline	0.062–2	0.25	0.5	100	0	0
Clostridium spp.[d] (57 strains) (9% β-lactamase-positive)	penicillin	0.032–>32	0.125	2	83	5	12
	cefotetan	0.064–>256	0.125	4	95	2	3
	cefoxitin	0.25–>256	1	16	91	4	5
	amoxicillin/clavulanate	<0.016–8	0.064	1	97	3	0
	piperacillin/tazobactam	0.032–>256	0.125	8	97	0	3
	meropenem	0.003–8	0.016	1	98	2	0
	clindamycin	0.016–>256	1	256	63	14	23
	metronidazole	<0.016–16	0.5	2	98	2	0
	chloramphenicol	1–32	4	8	95	3	2
	moxifloxacin	0.125–>32	0.5	32	86	2	12
	linezolid	0.5–16	2	4	98	0	2
	tigecycline	0.064–8	0.5	4	84	14	2
Non-spore-forming Gram-positive bacilli[e] (31 strains) (0% β-lactamase-positive)	penicillin	<0.002–4	0.032	2	81	3	16
	cefotetan	0.125–>256	0.5	>256	81	0	19
	cefoxitin	0.032–16	0.25	8	100	0	0
	amoxicillin/clavulanate	<0.016–2	0.064	0.5	100	0	0
	piperacillin/tazobactam	0.016–32	0.125	32	100	0	0
	meropenem	0.008–0.5	0.064	0.5	100	0	0
	clindamycin	<0.016–>256	0.064	1	90	0	10
	metronidazole	0.016–>256	>256	>256	35	0	65
	chloramphenicol	0.064–32	0.25	4	97	0	3
	moxifloxacin	0.032–64	0.125	1	90	7	3
	linezolid	0.064–8	0.25	1	97	0	3
	tigecycline	0.064–0.5	0.125	0.25	100	0	0
Anaerobic cocci[f] (37 strains) (0% β-lactamase-positive)	penicillin	0.002–>32	0.064	8	84	2	14
	cefotetan	0.064–2	0.5	2	100	0	0
	cefoxitin	<0.016–4	0.25	2	100	0	0
	amoxicillin/clavulanate	<0.016–4	0.064	1	100	0	0
	piperacillin/tazobactam	<0.016–64	0.064	32	92	8	0
	meropenem	<0.002–0.064	0.016	0.064	100	0	0
	clindamycin	<0.016–>256	0.25	4	89	6	5
	metronidazole	0.016–2	0.25	1	100	0	0
	chloramphenicol	0.25–256	0.5	1	97	0	3
	moxifloxacin	0.032–64>32	0.25	>32	68	10	22
	linezolid	0.25–2	0.5	1	100	0	0
	tigecycline	0.032–1	0.125	0.5	100	0	0

Table 1. (*continued*)

Organism	Antimicrobial agent	Range (mg/L)	MIC_{50}	MIC_{90}	%S	%I	%R
All anaerobes (443 strains) (62% β-lactamase-positive)	penicillin	<0.002–>32	>32	>32	34	1	65
	cefotetan	0.016–>256	8	>256	70	4	26
	cefoxitin	<0.016–>256	8	64	78	10	12
	amoxicillin/clavulanate	<0.016–32	0.5	4	92	5	3
	piperacillin/tazobactam	<0.016–>256	0.5	32	96	2	2
	meropenem	<0.002–32	0.125	0.5	98	1	1
	clindamycin	<0.016–>256	1	>256	63	10	27
	metronidazole	<0.016–>256	0.5	2	95	0	5
	chloramphenicol	0.064–256	4	8	98	1	1
	moxifloxacin	0.016–>32	1	>32	64	12	24
	linezolid	0.03–16	2	4	99	0	1
	tigecycline	0.03–32	0.5	8	84	6	10

[a]Includes *Bacteroides fragilis* (135 strains), *Bacteroides caccae* (6 strains), *Bacteroides distasonis* (6 strains), *Bacteroides eggerthii* (1 strain), *Bacteroides ovatus* (12 strains), *Bacteroides stercoris* (3 strains), *Bacteroides thetaiotaomicron* (46 strains), *Bacteroides uniformis* (7 strains) and *Bacteroides vulgatus* (22 strains).
[b]Includes *Fusobacterium mortiferum* (1 strain), *Fusobacterium necrophorum* (10 strains), *Fusobacterium nucleatum* (16 strains), *Fusobacterium varium* (3 strains).
[c]Includes *Bacteroides capillosus* (1 strain), *Bacteroides ureolyticus* (1 strain), *Bilophila wadsworthia* (2 strain), *Capnocytophaga ochracea* (1 strain), *Capnocytophaga sputigena* (1 strain), *Porphyromonas endodontalis* (1 strain), *Prevotella bivia* (8 strains), *Prevotella buccae* (5 strains), *Prevotella denticola* (1 strain), *Prevotella intermedia/nigrescens* (3 strains), *P. intermedia/nigrescens* (2 strains), *Prevotella melaninogenica* (4 strains), *P. melaninogenica* group (2 strains), *Prevotella oralis* (5 strains), *Prevotella oris* (3 strains), *Prevotella* species (9 strains), *Prevotella tannerae* (1 strain).
[d]Includes *Clostridium bifermentans* (1 strain), *Clostridium clostridioforme* (3 strains), *Clostridium glycolicum* (2 strains), *Clostridium innocuum* (1 strain), *Clostridium perfringens* (37 strains), *Clostridium ramosum* (3 strains), *Clostridium septicum* (2 strains), *Clostridium sordelli* (1 strain), *Clostridium* species (2 strains), *Clostridium sporogenes* (1 strain), *Clostridium subterminale* (1 strain) and *Clostridium tertium* (3 strains).
[e]Includes *Eggerthella lenta* (6 strains), *Eubacterium limosum* (1 strain), *Eubacterium* species (2 strains), *Lactobacillus* species (1 strain), *Propionibacterium acnes* (19 strains), *Solobacterium moorei* (2 strains).
[f]Includes *Anaerococcus hydrogenalis* (1 strain), *Anaerococcus vaginalis* (3 strains), *Finegoldia magna* (8 strains), *Micromonas micros* (9 strains), *Peptostreptococcus anaerobius* (1 strains), *Peptostreptococcus* species (2 strains), *Peptostreptococcus asaccharolyticus* (1 strain), *Peptoniphilus asaccharolyticus* (4 strains), *Peptoniphilus ivorii* (1 strain) and *Veillonella parvula* (7 strains).

of *Prevotella* spp. and other Gram-negative bacilli were susceptible. Susceptibility of *B. fragilis* strains to cefotetan was 83% in contrast to 11% of the other species in the *B. fragilis* group.

Ninety-eight per cent (98%) of all isolates were susceptible to meropenem. Six of 135 *B. fragilis* strains were intermediate (MIC = 8 mg/L) or resistant to meropenem (MIC $\geq$ 16 mg/L). In addition, one *Bacteroides caccae* isolate, one *Bacteroides stercoris* isolate and one *Clostridium glycolicum* isolate were intermediate to meropenem. In 1993–94 all isolates were susceptible to imipenem.

Two β-lactam/β-lactamase inhibitor combinations were tested in this study: amoxicillin/clavulanic acid and piperacillin/tazobactam. The activity of the β-lactam antibiotic was partially restored by the addition of a β-lactamase inhibitor. Overall activities of amoxicillin/clavulanic acid and piperacillin/tazobactam in this survey were 92% and 96%, respectively. In the previous surveys susceptibility to these combinations was >95% (piperacillin/tazobactam was not tested in 1987). Resistance to amoxicillin/clavulanic acid is limited to the *B. fragilis* group, with the exception of a few intermediate *Clostridium* spp. strains. In the *B. fragilis* group, 92% of *B. fragilis* and 78% of other *B. fragilis* group strains were susceptible to amoxicillin/clavulanic acid in this survey, as compared with 95% and 89% in 1993–94 and 97% and 94% in 1987, respectively. Four hundred and twenty-six (426) isolates were susceptible to piperacillin/tazobactam, 10 intermediate (5 *B. thetaiotaomicron*, 2 *Bacteroides vulgatus* and 3 *Veillonella parvula*) and 7 resistant (1 *B. stercoris*, 2 *B. thetaiotaomicron*, 1 *Bacteroides uniformis*, 1 *Bilophila wadsworthia* and 2 *C. clostridioforme*).

Chloramphenicol was very active against all anaerobic isolates (98% overall) with susceptibility exceeding 95% in all groups. Susceptibility to metronidazole remains stable. Overall susceptibility was 95% and was high in all groups except non-spore-forming Gram-positive bacilli: all *Propionibacterium acnes* (19 isolates) and one *Lactobacillus* sp. were resistant. Overall activity of clindamycin decreased from 83% in 1987 to 72% in 1993–94 and to 63% in this survey. Only 33% of non-*B. fragilis* strains in the *B. fragilis* group were susceptible to clindamycin, as compared with 60% of *B. fragilis* strains.

For linezolid, moxifloxacin and tigecycline no CLSI breakpoints for anaerobes are available. Overall, MIC_{50} and MIC_{90} were 2 and 4 mg/L for linezolid and 99% of isolates were susceptible to $\leq$4 mg/L linezolid. Resistance to linezolid (MIC > 4 mg/L) was found in only six isolates, belonging to the following species: *B. fragilis* (MIC = 8 mg/L), *Bacteroides ovatus* (MIC = 8 mg/L), *B. uniformis* (MIC = 16 mg/L), *B. wadsworthia* (MIC = 8 mg/L), *Eubacterium* species (MIC = 8 mg/L) and *Clostridium subtermina* (MIC = 16 mg/L). These isolates were susceptible to amoxicillin/clavulanic acid, meropenem and metronidazole.

For moxifloxacin, MIC_{50} and MIC_{90} were 1 and >32 mg/L. Overall susceptibility to 1 mg/L moxifloxacin was 64%. In the

Table 2. Percentage of susceptible isolates for each antimicrobial agent tested during the three surveys: comparison of results from this study with previous surveys[3,4]

	Penicillin			Amoxicillin/ clavulanate			Clindamycin			Metronidazole			Chloramphenicol		
Organisms	1987	1993–94	2004	1987	1993–94	2004	1987	1993–94	2004	1987	1993–94	2004	1987	1993–94	2004
Bacteroides fragilis group	2	2	1	96	93	86	83	66	48	100	98	99	99	100	99
B. fragilis	0	2	1	97	95	92	90	85	60	100	100	99	99	100	100
B. fragilis group without *B. fragilis*	4	3	1	94	89	78	75	37	33	100	95	99	100	100	98
Fusobacterium spp.	70	88	100	100	100	100	90	69	90	100	100	100	100	100	100
Prevotella species and other Gram-negative bacilli	64	48	26	95	100	100	90	91	82	100	96	100	100	100	100
Clostridium spp.	91	90	83	100	100	97	82	74	63	100	100	98	96	100	95
Non-spore-forming Gram-positive bacilli	93	77	81	100	100	100	93	82	90	36	36	35	100	100	97
Anaerobic cocci	92	81	84	98	96	100	94	89	89	94	95	100	98	100	97
Total	46	38	34	97	96	92	83	72	63	95	94	95	97	99	98

B. fragilis group, 68% of *B. fragilis* and 30% of non-*B. fragilis* strains were susceptible to moxifloxacin at this breakpoint. In other groups susceptibility was 80% for *Fusobacterium* spp., 70% for *Prevotella* spp. and other Gram-negative bacilli, 86% for *Clostridium* spp., 90% for non-spore-forming Gram-positive bacilli and 68% for anaerobic cocci.

MIC_{50} and MIC_{90} of tigecycline were 0.5 and 8 mg/L, respectively. At the susceptibility breakpoint of $\leq$2 mg/L, 84% of isolates were susceptible to tigecycline; 73% in the *B. fragilis* group and 84% in *Clostridium* spp. All other isolates were susceptible at this breakpoint.

Discussion

Mixed polymicrobial infections involving anaerobic bacteria are most commonly treated empirically, without any laboratory documentation by cultures and susceptibility testing. Over the past 20 years, however, significant antibiotic resistance has been identified among several species of anaerobic bacteria. Many Gram-negative anaerobes presently display unpredictable susceptibilities to antimicrobial agents, which may result in an inappropriate choice of empirical antimicrobial therapy. Until recently, there was no consensus about the influence of antimicrobial resistance on the clinical outcome of infections involving anaerobes.[9,10] The often polymicrobial nature of the infection and the contribution of surgical drainage may indeed obscure the importance of resistant organisms. Two studies underscore the importance of appropriate choice of therapy. A Finnish retrospective study[9] including 57 patients with clinically significant anaerobic bacteraemia evaluated the effect of the choice of antimicrobial therapy on the outcome for patients. Twenty-eight patients received appropriate antimicrobial treatment; for 18 patients (32%) an initially inappropriate therapy was changed on the basis of the bacteriological results and for 11 patients the treatment remained unchanged and was not adjusted to the laboratory results. In these three groups, the mortality rate was 18%, 17% and 55%, respectively. Failure to adjust therapy according to the bacteriological results thus had a serious impact on outcome. A recent multicentre prospective observational trial[10] on bacteraemia with *Bacteroides* spp. showed that ineffective therapy results in adverse clinical outcomes such as higher mortality, more clinical failure and bacteriological persistence as compared with effective therapy. However, since anaerobic susceptibility results often are not obtained within a useful time frame, periodic surveys are still needed for conducting empirical therapy.

The comparison of the results of the present survey with those of the previous studies (1987 and 1993–94) shows some important evolutions in the antibiotic susceptibility of anaerobic bacteria in Belgium. The most striking evolution is the regular increase in clindamycin resistance: non-susceptible strains increased from 17% in 1987, to 28% in 1993–94 and to 37% in 2003–2004. This can be explained only partially by a different species distribution (more Gram-negative bacilli in 2003–2004). The decrease in activity against *B. fragilis* group strains and clostridia to 48% and to 63%, respectively, makes clindamycin useless for empirical treatment of severe anaerobic infections.

Overall, β-lactamase-producing strains increased from 41% in 1987 and 48% in 1993–94 to 62% in this survey. This rise can be partly attributed to a change in the species distribution. In 1987 and 1993–94, 56% and 58% of Gram-negative bacilli, respectively, were included in contrast to 71% in this survey. As expected, a high rate of β-lactamase-producing strains was recorded in the *B. fragilis* group (98%), corroborating the CLSI recommendation to report all members of the *B. fragilis* group as resistant to penicillin.[2] Remarkable was the continuous increase in β-lactamase positivity, up to 70% of the *Prevotella* spp. and other Gram-negative bacilli group, and the appearance of β-lactamase-positive *Clostridium* spp. strains (9%).

Overall 66% of strains were found to be not susceptible to penicillin. This antibiotic is no longer useful in empirical treatment of anaerobic infections. Previously it was considered the drug of choice for anaerobic infections above the diaphragm. However, taking into account the increasing resistance to penicillin of *Prevotella* spp., often present in oropharyngeal

flora, this policy must be reconsidered as well. In patients with gas gangrene, penicillin G in high dosages is still considered to be the drug of choice. However, animal studies have demonstrated that protein synthesis inhibitors were better inhibitors of toxin synthesis than were cell-wall-active agents. For this reason, it is now recommended to combine clindamycin with penicillin in serious clostridial infections if clindamycin is still active.[11]

The activity of cefoxitin in this survey, once considered as the most active cephalosporin against anaerobes, was comparable with the results of the previous study (1993–94) except for the *B. fragilis* group. Eighty-six per cent of *B. fragilis* were susceptible in both surveys. Susceptibility of other species of the *B. fragilis* group, still 51% in 1993–94, was further reduced to 30%. In all other groups more than 90% of the isolates were found to be susceptible to cefoxitin. Cefotetan was even less active than cefoxitin against the *B. fragilis* group. Only 11% of non-*B. fragilis* species were susceptible. Because of the high rate of resistance of the *B. fragilis* group to cefoxitin and cefotetan, these agents are not recommended for empirical treatment of serious *Bacteroides* infections. Their role in prophylaxis of surgical site infection in abdominal and pelvic surgery should also be reconsidered seriously.

In the two β-lactam/β-lactamase inhibitor combinations tested in this study, amoxicillin/clavulanic acid and piperacillin/tazobactam, the activity of the β-lactam antibiotic was restored by the addition of a β-lactamase inhibitor in most organisms. In the previous study (1993–94) these agents showed an overall activity in excess of 95%. Overall activity is still 96% for piperacillin/tazobactam; in contrast it is reduced to 92% for amoxicillin/clavulanic acid. Decrease in susceptibility is especially pronounced in non-*B. fragilis* species of the *B. fragilis* group for both combinations: 78% for amoxicillin/clavulanic acid and 89% for piperacillin/tazobactam. Within this group, the resistance appeared evenly distributed, except for *Bacteroides distasonis*: three of the six isolates were resistant and one intermediate.

The majority of isolates were susceptible to carbapenems. All strains were susceptible to imipenem in 1993–94. In this survey only a few isolates of the *B. fragilis* group and of clostridia were not susceptible to meropenem.

Chloramphenicol preserves an excellent activity against all anaerobes. This antibiotic with good tissue penetration can be of use in the treatment of cases where its benefit exceeds the risks of toxicity. Metronidazole resistance remains exceptional.

Linezolid, the first of a new class of antimicrobial agents, the oxazolidinones, showed promising activity against the tested isolates. Four hundred and thirty-seven of 443 isolates were susceptible to linezolid if $\leq$4 mg/L was used as the breakpoint. Experience with linezolid in the treatment of anaerobic infections is, however, still limited and clinical trials would be useful.

Activity of moxifloxacin was less favourable especially against the *B. fragilis* group. At concentrations of 1, 2 and 4 mg/L moxifloxacin inhibited only 64%, 76% and 83% of isolates, respectively. Twelve per cent of the isolates had an MIC of moxifloxacin >32 mg/L. When MIC distributions for *B. fragilis* were examined, a bimodal distribution was observed with a first peak at 0.5 mg/L and a smaller peak at >32 mg/L, while the distribution presented on the website of EUCAST shows only one peak at 0.5 mg/L (data not shown). It is possible that the wide usage of quinolones in Belgium—ranking third in a recent European survey on use of quinolones in ambulatory medicine—already selected this resistant subpopulation.[12]

For tigecycline, a new glycylcycline derivate of minocycline, a susceptibility breakpoint of $\leq$2 mg/L was used in clinical trials. Subsequently FDA proposed $\leq$4 mg/L for anaerobes based on MICs for responsive organisms. If this breakpoint is used instead of 2 mg/L, susceptibility to tigecycline rises to 81% for the *B. fragilis* group and to 98% for *Clostridium* spp.

In conclusion, piperacillin/tazobactam, meropenem and metronidazole remain very useful antimicrobial agents for the treatment of anaerobic infections. However, resistant organisms were detected for each of these agents. Therefore susceptibility testing of anaerobic isolates is indicated in severe infections to confirm appropriateness of antimicrobial therapy. Periodically monitoring background susceptibility is necessary to guide empirical treatment. New antimicrobial agents with interesting anti-anaerobic activity are available and should be evaluated in clinical trials.

Acknowledgements

This study was performed under the auspices of the BVIKM-SBIMC (Belgische Vereniging voor Infectiologie en Klinische Microbiologie/Société Belge d'Infectiologie et de Microbiologie Clinique) and the BICS (Belgian Infection Control Society). It was supported by grants from Bayer HealthCare Pharmaceuticals, Brussels, Pfizer Global Pharmaceuticals, Brussels, Wyeth Pharmaceuticals, Louvain la Neuve, AstraZeneca, Brussels and GlaxoSmithKline, Genval.

Transparency declarations

The following declarations were made by the authors of this paper about the study period. I. W. received support for attendance to an international conference from Wyeth Pharmaceuticals Belgium. D. P. received support from Bristol-Myers Squibb Belgium, Pfizer Belgium and GlaxoSmithKline Belgium for attendance to international conferences and is member of the Advisory Board Tigecycline (Wyeth Pharmaceuticals Belgium). M. D. is a member of the Infectious Diseases Advisory Board (GlaxoSmithKline Belgium) and the Advisory Board for the Belgian/Luxembourg Version of the Sanford Guide to Antimicrobial Therapy (GlaxoSmithKline Belgium). B. G. received support from Pfizer Belgium, Wyeth Pharmaceuticals and GlaxoSmithKline Belgium for attendance to international conferences and is a member of the Advisory Board for the Belgian/Luxembourg Version of the Sanford Guide to Antimicrobial Therapy (GlaxoSmithKline Belgium). M. I. received support from Bristol-Myers Squibb Belgium, GlaxoSmithKline Belgium and from Wyeth Pharmaceuticals Belgium for attendance to international conferences. P. M. received support from Wyeth Pharmaceuticals Belgium for attendance to an international conference. M. S. received research grant support from Pfizer Belgium, Wyeth Pharmaceuticals Belgium, travel support from Pfizer Belgium and GlaxoSmithKline Belgium for attendance to international conferences and is member of the Advisory Board Tigecycline (Wyeth Pharmaceuticals Belgium), Infectious Diseases Advisory Board (GlaxoSmithKline Belgium) and the Advisory Board for the Belgian/Luxembourg Version of the Sanford Guide to Antimicrobial Therapy (GlaxoSmithKline Belgium). J. V. is member of the Advisory Board Tigecycline (Wyeth Pharmaceuticals Belgium) and received support for attendance to international conferences from Pfizer

Belgium and Bristol-Myers Squibb Belgium. S. L. received support for attendance to an international conference from Wyeth Pharmaceuticals Belgium and was a member of the Advisory Board for the Belgian/Luxembourg Version of the Sanford Guide to Antimicrobial Therapy (GlaxoSmithKline Belgium). I. V., M. R., K. V., G. C. and Y. G. have none to declare.

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