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Fourth Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria

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Objectives: To collect recent data on the susceptibility of anaerobes to antimicrobial agents with known activity against anaerobes, and to compare them with results from previous Belgian multicentre studies.

Methods: Four hundred and three strict anaerobic clinical isolates were prospectively collected from February 2011 to April 2012 in eight Belgian university hospitals. MICs were determined by one central laboratory for 11 antimicrobial agents using Etest methodology.

Results: According to EUCAST breakpoints, >90% of isolates were susceptible to amoxicillin/clavulanate (94%), piperacillin/tazobactam (91%), meropenem (96%), metronidazole (92%) and chloramphenicol (98%), but only 70% and 40% to clindamycin and penicillin, respectively. At CLSI recommended breakpoints, only 71% were susceptible to moxifloxacin and 79% to cefoxitin. MIC_{50}/MIC_{90} values for linezolid and for tigecycline were 1/4 and 0.5/4 mg/L, respectively. When compared with survey data from 2004, no major differences in susceptibility profiles were noticed. However, the susceptibility of *Prevotella* spp. and other Gram-negative bacilli to clindamycin decreased from 91% in 1993–94 and 82% in 2004 to 69% in this survey. Furthermore, the susceptibility of clostridia to moxifloxacin decreased from 88% in 2004 to 66% in 2011–12 and that of fusobacteria from 90% to 71%.

Conclusions: Compared with previous surveys, little evolution was seen in susceptibility, except a decline in activity of clindamycin against *Prevotella* spp. and other Gram-negative bacteria, and of moxifloxacin against clostridia. Since resistance was detected to all antibiotics, susceptibility testing of anaerobic isolates is indicated in severe infections to confirm appropriateness of antimicrobial therapy.

Keywords: anaerobes, Etest, surveillance

Introduction

Anaerobes are important constituents of the bacterial flora of normal human skin and mucous membranes. They are a common cause of endogenous infection and can be responsible for a variety of clinical infections, including brain abscesses, head and neck, intra-abdominal, gynaecological, skin and soft tissue infections, deep abscesses and bacteraemia. These infections can be severe to life-threatening and are often caused by several aerobic and anaerobic pathogens. Because of their fastidious nature, the isolation by culture of anaerobic bacteria from clinical specimens may be difficult and requires appropriate collection, transportation and culture methods. Since anaerobic cultures are long and cumbersome and infections are often mixed, *in vitro* susceptibility testing is generally not performed routinely. Therefore, the treatment of these infections is mostly empirical and

includes an antimicrobial agent with known efficacy against anaerobes.

The spectrum of antibiotic resistance among anaerobes has increased during the last three decades and nowadays it includes even those antibiotics that were once considered to be universally active, such as carbapenems and nitroimidazoles, but whose activity may vary depending on region.[1] The CLSI[2] recommends periodic monitoring of regional and institutional resistance trends of clinically relevant isolates to guide empirical antimicrobial therapy of infections involving anaerobes.

Three multicentre surveys were previously conducted in Belgium: in 1987,[3] 1993–94[4] and 2004.[5] The objective of this study was to update the *in vitro* susceptibility and resistance levels of anaerobes and to compare them with results from previous studies. The impact of using either CLSI or EUCAST clinical breakpoints for anaerobes on the resistance rates was also evaluated.

Materials and methods

Bacteria

Strains were collected from 31 January 2011 to 7 April 2012 in eight Belgian university hospitals: Universitair Ziekenhuis Antwerpen (Antwerp); Cliniques Universitaires Saint-Luc (Brussels); Hôpital Universitaire Erasme (Brussels); Universitair Ziekenhuis Brussel (Brussels); Universitair Ziekenhuis Gent (Ghent); Universitair Ziekenhuis Leuven (Leuven); Centre Hospitalier Universitaire de Liège (Liège); and Centre Hospitalier Universitaire UCL Mont-Godinne-Dinant (Yvoir). Six of these centres participated in the previous surveys. Each centre was asked to prospectively collect up to 50 consecutive, 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 Universitair Ziekenhuis Brussel.

Identification

Species identification was performed by standard methods in the collecting laboratories. Identification was verified at the Universitair Ziekenhuis Brussel by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT mass spectrometer with MALDI Biotyper 3.0 software and Reference Library 3.2.1.0 (Bruker Daltonik GmbH, Bremen, Germany)[6] or when necessary by analysis of cellular fatty acid composition using the Microbial Identification System (Microbial Identification Inc., Newark, DE, USA) followed by appropriate biochemical or enzymatic tests[7] and/or 16S rRNA gene sequencing.[6]

Susceptibility testing

Antimicrobial susceptibility testing was performed using Etest methodology (bioMérieux Benelux, Brussels, Belgium) as described previously.[8] The following antimicrobial agents were tested: penicillin, cefoxitin, amoxicillin/clavulanate, piperacillin/tazobactam, meropenem, clindamycin, metronidazole, chloramphenicol, moxifloxacin, tigecycline and linezolid. The medium used was Brucella agar (Becton-Dickinson, Erembodegem, Belgium) supplemented with 5% v/v laked sheep blood, haemin (5 mg/L) and vitamin K1 (1 mg/L), as recommended for the CLSI reference agar dilution procedure. The pre-reduced agar plates were inoculated with a suspension with a turbidity equivalent to that of a 1 McFarland standard (corresponding to an inoculum of 10^8 cfu/mL) and incubated anaerobically at 35°C for 48 h. *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741 and *Eggerthella lenta* ATCC 43055 were included as control strains in each test run. The isolates were categorized by EUCAST breakpoints[9] for penicillin, amoxicillin/clavulanate, piperacillin/tazobactam, meropenem, clindamycin, metronidazole and chloramphenicol. CLSI breakpoints were used for cefoxitin and moxifloxacin since EUCAST has not defined any breakpoints for these two agents. The CLSI and EUCAST breakpoints are shown in Table 1. Until now, neither of these organizations has recommended susceptibility and resistance breakpoints for linezolid and tigecycline against anaerobes. The US FDA susceptibility breakpoint to tigecycline for anaerobes is set at 4 mg/L.[10] For linezolid there are no FDA breakpoints available for anaerobic bacteria. Raw data from the previous surveys were used to reinterpret results using these new breakpoints.

In addition, a β-lactamase test was performed on each isolate by using the nitrocefin test.

Detection of the cfiA gene

PCR analysis was performed to detect the presence of the *cfiA* gene in *B. fragilis* isolates included in the present study as well as in the 2004 survey.[5] The annealing temperature of the *cfiA* gene detection method described by Sóki *et al*.[11] was increased to 62°C to avoid non-specific reactions. The presence of a PCR product of 728 bp was regarded as positive.[12]

Detection and typing of nim genes

Bacteroides and *Parabacteroides* spp. isolates of the present study and of the 2004 survey[5] were screened for *nim* genes with primers NIM-3 and NIM-5 and amplification conditions as described previously.[13] Presence of a PCR product of 458 bp was regarded as a presumptive positive. Confirmation of the presence and typing of the *nim* genes was done by nucleotide sequencing of the PCR product.[14]

Statistical analysis

MedCalc software (version 11.4.4.0; MedCalc Software bvba, Mariakerke, Belgium) was used to carry out Fisher's exact test.

Ethics approval

The protocol of this study was approved by the Ethics Committee of the Universitair Ziekenhuis Brussel (B.U.N. 143201111957).

Results and discussion

Four hundred and three isolates were collected from various sources: 154 from abdominal sites (38%), 66 from wounds and pus (16%), 59 from abscesses (15%), 42 from blood (10%), 14 from gynaecological and obstetrical sites (4%), 8 from the respiratory tract (2%), 8 from the CNS (2%), 5 from ear and sinus (1%) and 47 from miscellaneous other sites (12%). *Bacteroides* and *Parabacteroides* spp. were the most prevalent, accounting for 45% of the isolates. *Fusobacterium* spp. accounted for 5%, *Prevotella* spp. and other Gram-negative bacilli for 13%, *Clostridium* spp. for 9%, non-spore-forming Gram-positive bacilli for 10% and anaerobic cocci for 18%.

In the 2011–12 survey, β-lactamases were detected in 52% of the 403 isolates. β-Lactamases were present in 96% of *Bacteroides* and *Parabacteroides* spp. and in 73% of *Prevotella* spp. strains. Among *Fusobacterium* spp. and *Clostridium* spp., three β-lactamase-producing fusobacteria (14%) and two β-lactamase-producing clostridia (5%) were detected. All other isolates were β-lactamase negative (Table S1, available as Supplementary data at *JAC* Online).

The numbers of isolates, MIC ranges, MIC_{50} and MIC_{90} values and the proportions of susceptible isolates are represented in Table 2. In order to allow valid comparison of results with earlier data, susceptibility rates found in previous Belgian surveys are

Table 1. EUCAST and CLSI breakpoints for tested antimicrobial agents

	EUCAST		CLSI		
Antimicrobial agent	susceptible	resistant	susceptible	intermediate	resistant
Penicillin	≤0.25	>0.5	≤0.5	1	≥2
Cefoxitin	NA	NA	≤16	32	≥64
Amoxicillin/clavulanate	≤4/2	>8/2	≤4/2	8/4	≥16/8
Piperacillin/tazobactam	≤8/4	>16/4	≤32/4	64/4	≥128/4
Meropenem	≤2	>8	≤4	8	≥16
Clindamycin	≤4	>4	≤2	4	≥8
Metronidazole	≤4	>4	≤8	16	≥32
Chloramphenicol	≤8	>8	≤8	16	≥32
Moxifloxacin	NA	NA	≤2	4	≥8
Tigecycline	NA	NA	NA	NA	NA
Linezolid	NA	NA	NA	NA	NA

NA, not available.
In EUCAST tables, the intermediate category is not listed. It is implied as the values between the susceptible breakpoint and the resistant breakpoint.[9]

also shown. These were recalculated from the original individual data when breakpoints were modified after the first publication.[3–5] The distribution of individual species is presented in the footnotes of Table 2 for the strains of this study and can be found in the original reports for the previous surveys. More detailed information with comparison of percentages of susceptible isolates according to CLSI and EUCAST breakpoints and individual results for the most prevalent species is available in Table S1, available as Supplementary data at *JAC* Online.

After reinterpretation of the raw data from our previous surveys, very few changes in susceptibility rates were observed. *Bacteroides* and *Parabacteroides* spp., well known as being more virulent and more resistant to antimicrobial agents than most other anaerobes, were still the most prevalent organisms. Metronidazole, chloramphenicol and meropenem remained very active against these organisms, with susceptibility rates of 100%, 97% and 92%, respectively. As in the previous survey,[15] a small number of *Bacteroides* and *Parabacteroides* spp. isolates harboured *nim* genes (2.8% versus 2.5% in 2004) (Table S2, available as Supplementary data at *JAC* Online). The *nimA* gene was detected in three isolates (one *B. fragilis*, one *B. thetaiotaomicron* and one *B. vulgatus/dorei*) and the *nimD* gene in two isolates (one *B. thetaiotaomicron* and one *B. vulgatus/dorei*). None of these genes, which can confer resistance to metronidazole, was expressed in the present survey.[16,17]

Thirteen percent of *B. fragilis* isolates and 4.5% of other *Bacteroides* and *Parabacteroides* spp. isolates were intermediate or resistant to meropenem according to EUCAST breakpoints in this survey. While 9 of the 10 meropenem non-susceptible *B. fragilis* isolates belonged to division II (*cfiA*-positive) strains in 2004,[12,18] in 2011–12 only 2 resistant *B. fragilis* isolates harboured this gene, suggesting a combination of overexpressed CepA chromosomal cephalosporinase and porin impermeability in the remaining 7 non-susceptible isolates (Table S3, available as Supplementary data at *JAC* Online).

The most salient difference between EUCAST and CLSI is the lower EUCAST breakpoint for piperacillin/tazobactam. While as many as 98% of all isolates were considered susceptible to piperacillin/tazobactam when using CLSI breakpoints, only 91% were susceptible when using EUCAST breakpoints, to be compared with a susceptibility rate of 94% to amoxicillin/clavulanate, equal for CLSI and EUCAST. Susceptibility to piperacillin/tazobactam, like that to amoxicillin/clavulanate, varied among *Bacteroides* and *Parabacteroides* species, ranging from 100% to 60%; exact figures by species are represented in Table S1, available as Supplementary data at *JAC* Online.

Clindamycin showed a clear trend towards decreasing activity against *Prevotella* spp. and other Gram-negative bacilli. In comparison with the first two surveys (>90% susceptibility) and the third survey (82%), there was a further decrease in susceptibility to clindamycin (69%) (Fisher's exact test, second survey versus present survey; $P=0.044$), which was also noted in the study of Glupczynski *et al.*[19] The activity of clindamycin against *Bacteroides* and *Parabacteroides* spp., with a susceptibility rate of 58%, is insufficient for treatment of infections where these organisms are prevalent, such as abdominal infections.

Although EUCAST mentions that newer fluoroquinolone agents have enhanced intrinsic activity against anaerobes, there is insufficient evidence that anaerobes are a good target for therapy with moxifloxacin and no breakpoints have been made available by this committee. When using CLSI breakpoints, susceptibility of anaerobes to moxifloxacin slightly decreased from 75% in 2004 to 71% in 2011–12. However, a significant decrease in susceptibility to moxifloxacin was observed for clostridia (from 88% in 2004 to 66% in 2011–12) (Fisher's exact test; $P=0.019$) and a trend of decreasing activity against fusobacteria (from 90% to 71%) (Fisher's exact test; $P=0.14$). The rates of resistance to moxifloxacin have been shown to vary considerably between countries.[1] A randomized clinical trial in the treatment of intra-abdominal infections suggested that moxifloxacin could be a valuable treatment option for a range of community-acquired intra-abdominal infections with mild to moderate severity.[20] However, MIC_{90} values of *B. fragilis* and *B. thetaiotaomicron* to moxifloxacin in this clinical trial were 4 mg/L compared with >32 mg/L recorded in the present *in vitro* survey. As only 71% of anaerobes were susceptible in our survey, we believe that this drug should not be used in our country for the empirical treatment of anaerobic infections.

Table 2. Antimicrobial activities of 11 antibiotics against different groups of anaerobes and comparison of the percentage of susceptible isolates with previous Belgian surveys; raw data of the previous surveys were used to reinterpret results using the current breakpoints

	2011–12			Isolates susceptible (%)		
Organism/antimicrobial agent	MIC range (mg/L)	MIC_{50}/MIC_{90} (mg/L)	S (%)	2004	1993–94	1987
All isolates (*n*)		403		443	323	274
penicillin	<0.002 to >32	8/>32	40	32	37	44
cefoxitin[a]	<0.016 to >256	2/64	79	78	83	NT
amoxicillin/clavulanate	<0.016 to >256	0.25/4	94	92	96	97
piperacillin/tazobactam	<0.016 to >256	0.25/8	91	83	91	NT
meropenem	0.002 to >32	0.125/1	96	96	NT	NT
clindamycin	<0.016 to >256	1/>256	70	72	81	90
metronidazole	<0.016 to >256	0.25/2	92	94	93	96
chloramphenicol	0.032 to >32	4/8	98	98	100	99
moxifloxacin[a]	0.016 to >32	0.5/>32	71	75	NT	NT
linezolid	0.016 to >256	1/4	NB	NB	NT	NT
tigecycline	<0.016 to >32	0.5/4	NB	NB	NT	NT
Bacteroides and *Parabacteroides* spp.[b] (*n*)		180		238	163	119
penicillin	0.032 to >32	>32/>32	3	1	1	2
cefoxitin[a]	0.125 to >256	16/128	56	62	72	NT
amoxicillin/clavulanate	0.032–32	1/8	87	86	93	96
piperacillin/tazobactam	<0.016 to >256	2/32	85	77	98	NT
meropenem	0.032 to >32	0.25/2	92	93	NT	NT
clindamycin	<0.016 to >256	4/>256	58	61	77	88
metronidazole	0.064–4	0.25/0.5	100	99	98	100
chloramphenicol	0.064–16	8/8	97	99	100	99
moxifloxacin[a]	0.064 to >32	2/>32	62	68	NT	NT
linezolid	0.5–16	2/4	NB	NB	NT	NT
tigecycline	0.064–64	2/8	NB	NB	NT	NT
B. fragilis (*n*)		69		135	98	68
penicillin	16 to >32	>32/>32	0	1	1	0
cefoxitin[a]	4 to >256	8/32	84	86	86	NT
amoxicillin/clavulanate	0.25–8	0.5/4	96	92	95	97
piperacillin/tazobactam	0.032 to >256	0.25/2	96	100	98	NT
meropenem	0.064 to >32	0.125/4	87	93	NT	NT
clindamycin	0.032 to >256	1/>256	77	70	88	91
metronidazole	0.125–2	0.25/0.5	100	99	100	100
chloramphenicol	2–16	8/8	99	100	100	99
moxifloxacin[a]	0.25 to >32	0.5/>32	70	73	NT	NT
linezolid	1–16	2/4	NB	NB	NT	NT
tigecycline	0.5 to >32	2/8	NB	NB	NT	NT
Bacteroides and *Parabacteroides* spp. without *B. fragilis* (*n*)		111		103	65	51
penicillin	0.032 to >32	>32/>32	5	1	0	4
cefoxitin[a]	0.125–256	32/128	39	30	51	NT
amoxicillin/clavulanate	0.032–32	1/8	81	78	89	94
piperacillin/tazobactam	<0.016 to >256	4/32	78	47	71	NT
meropenem	0.032–32	0.25/2	96	94	NT	NT
clindamycin	<0.016 to >256	8/>256	46	51	60	84
metronidazole	0.064–4	0.25/0.5	100	98	94	100
chloramphenicol	0.064–16	8/8	96	98	100	100
moxifloxacin[a]	0.064 to >32	2/>32	58	60	NT	NT
linezolid	0.5–16	2/4	NB	NB	NT	NT
tigecycline	0.064–32	1/8	NB	NB	NT	NT

Continued

Table 2. *Continued*

Organism/antimicrobial agent	2011–12			Isolates susceptible (%)		
	MIC range (mg/L)	MIC_{50}/MIC_{90} (mg/L)	S (%)	2004	1993–94	1987
Fusobacterium spp.[c] (*n*)		21		30	16	10
penicillin	0.004 to >32	0.016/>32	81	100	81	50
cefoxitin[a]	<0.016–4	0.125/4	100	100	100	NT
amoxicillin/clavulanate	<0.016–4	0.064/1	100	100	100	100
piperacillin/tazobactam	<0.016–8	0.032/4	100	100	100	NT
meropenem	0.002–1	0.016/0.125	100	100	NT	NT
clindamycin	<0.016 to >256	0.064/16	81	93	75	90
metronidazole	<0.016–0.25	0.064/0.125	100	100	100	100
chloramphenicol	0.125–8	0.5/4	100	100	100	100
moxifloxacin[a]	0.125 to >32	0.5/>32	71	90	NT	NT
linezolid	0.064–8	0.25/1	NB	NB	NT	NT
tigecycline	<0.016–1	0.125/0.25	NB	NB	NT	NT
Prevotella spp. and other Gram-negative bacilli[d] (*n*)		52		50	23	39
penicillin	<0.002 to >32	4/>32	35	26	48	59
cefoxitin[a]	<0.016–8	0.5/2	100	98	96	NT
amoxicillin/clavulanate	<0.016–2	0.125/1	100	100	100	95
piperacillin/tazobactam	<0.016–32	0.125/0.5	98	98	100	NT
meropenem	0.002–0.25	0.064/0.125	100	100	NT	NT
clindamycin	<0.016 to >256	0.032/>256	69	82	91	92
metronidazole	<0.016 to >256	0.25/2	96	98	91	100
chloramphenicol	0.032–8	2/4	100	100	100	100
moxifloxacin[a]	0.064 to >32	0.5/4	77	76	NT	NT
linezolid	0.016 to >256	1/2	NB	NB	NT	NT
tigecycline	0.016–4	0.25/1	NB	NB	NT	NT
Clostridium spp.[e] (*n*)		38		57	42	45
penicillin	0.032 to >32	0.125/2	71	77	81	84
cefoxitin[a]	0.25 to >256	1/32	90	91	90	NT
amoxicillin/clavulanate	<0.016–4	0.064/0.5	100	97	100	100
piperacillin/tazobactam	0.016 to >256	0.125/8	95	91	98	NT
meropenem	0.004–2	0.032/1	100	98	NT	NT
clindamycin	<0.016 to >256	0.5/32	82	77	83	87
metronidazole	<0.016–4	0.25/2	100	98	100	100
chloramphenicol	0.25–8	4/8	100	95	100	96
moxifloxacin[a]	0.032 to >32	0.5/>32	66	88	NT	NT
linezolid	0.25–16	2/4	NB	NB	NT	NT
tigecycline	0.016–16	0.25/4	NB	NB	NT	NT
Non-spore-forming Gram-positive bacilli[f] (*n*)		40		31	22	14
penicillin	0.008–8	0.032/2	80	81	73	93
cefoxitin[a]	0.016–64	0.25/16	95	100	86	NT
amoxicillin/clavulanate	0.016–2	0.064/0.5	100	100	100	100
piperacillin/tazobactam	<0.016–64	0.25/4	90	84	91	NT
meropenem	0.016–0.5	0.125/0.25	100	100	NT	NT
clindamycin	<0.016 to >256	0.064/>256	85	90	86	93
metronidazole	<0.016 to >256	>256/>256	25	35	36	36
chloramphenicol	0.064–8	0.5/4	100	97	100	100
moxifloxacin[a]	0.064 to >32	0.125/2	93	97	NT	NT
linezolid	0.064–16	0.125/1	NB	NB	NT	NT
tigecycline	0.032–0.5	0.125/0.5	NB	NB	NT	NT

Continued

Table 2. *Continued*

Organism/antimicrobial agent	2011–12			Isolates susceptible (%)		
	MIC range (mg/L)	MIC_{50}/MIC_{90} (mg/L)	S (%)	2004	1993–94	1987
Anaerobic cocci[g] (*n*)		72		37	57	47
penicillin	<0.002 to >32	0.064/0.5	88	81	77	85
cefoxitin[a]	<0.016–32	0.5/2	99	100	100	NT
amoxicillin/clavulanate	<0.016 to >256	0.125/0.5	97	100	96	98
piperacillin/tazobactam	<0.016–128	0.064/1	96	86	88	NT
meropenem	0.002–16	0.032/0.25	99	100	NT	NT
clindamycin	<0.016 to >256	0.25/>256	83	95	89	94
metronidazole	<0.016 to >256	0.25/1	99	100	95	94
chloramphenicol	0.5 to >32	2/4	99	97	100	98
moxifloxacin[a]	0.016 to >32	0.125/16	81	78	NT	NT
linezolid	0.125–2	0.5/1	NB	NB	NT	NT
tigecycline	0.032–2	0.125/0.5	NB	NB	NT	NT

NT, not tested; NB, no EUCAST or CLSI breakpoints available; S, susceptible.
[a]No EUCAST breakpoints available; CLSI breakpoints were used.
[b]Includes *Bacteroides caccae* (3 isolates), *B. cellulosilyticus* (1 isolate), *B. coprocola* (1 isolate), *B. fragilis* (69 isolates), *B. massiliensis* (1 isolate), *B. nordii* (1 isolate), *B. ovatus/xylanisolvens* (19 isolates), *B. pyogenes* (5 isolates), *B. salyersiae* (3 isolates), *B. salyersiae/nordii* (1 isolate), *Bacteroides* sp. (1 isolate), *B. tectus* (1 isolate), *B. thetaiotaomicron* (40 isolates), *B. uniformis* (8 isolates), *B. vulgatus/dorei* (17 isolates), *Parabacteroides distasonis* (8 isolates) and *P. merdae* (1 isolate).
[c]Includes *Fusobacterium gonidiaformans* (2 isolates), *F. mortiferum* (2 isolates), *F. necrophorum* (5 isolates), *F. nucleatum* (7 isolates) and *F. varium* (5 isolates).
[d]Includes *Campylobacter rectus* (1 isolate), *Dialister micraerophilus* (2 isolates), *Porphyromonas asaccharolytica* (1 isolate), *P. somerae* (2 isolates), *Prevotella baroniae* (1 isolate), *P. bergensis* (1 isolate), *P. bivia* (10 isolates), *P. buccae* (5 isolates), *P. buccalis* (1 isolate), *P. denticola* (1 isolate), *P. disiens* (8 isolates), *P. histicola* (1 isolate), *P. loescheii* (1 isolate), *P. melaninogenica* (2 isolates), *P. nanceiensis* (2 isolates), *P. nigrescens* (4 isolates), *P. oris* (1 isolate), *P. salivae* (1 isolate), *Prevotella* sp. (4 isolates), *P. timonensis* (1 isolate), *P. veroralis* (1 isolate) and *Sutterella wadsworthensis* (1 isolate).
[e]Includes *Clostridium bifermentans* (1 isolate), *C. bolteae/clostridioforme* (3 isolates), *C. cadaveris* (1 isolate), *C. citroniae* (1 isolate), *C. clostridioforme* (1 isolate), *C. hathewayi* (3 isolates), *C. innocuum* (1 isolate), *C. limosum* (1 isolate), *C. perfringens* (17 isolates), *C. ramosum* (4 isolates), *C. septicum* (1 isolate), *Clostridium* sp. (1 isolate), *C. sporogenes* (2 isolates) and *C. tertium* (1 isolate).
[f]Includes *Actinobaculum massiliense* (1 isolate), *A. schaalii* (1 isolate), *Actinomyces odontolyticus* (1 isolate), *A. radingae* (1 isolate), *Actinomyces* sp. (2 isolates), *Anaerotruncus* sp. (1 isolate), *Bifidobacterium dentium* (1 isolate), *Collinsella aerofaciens* (1 isolate), *Eggerthella lenta* (4 isolates), *Flavonifractor plautii* (1 isolate), *Mobiluncus* sp. (1 isolate), *Propionibacterium acnes* (22 isolates), *P. avidum* (1 isolate), *P. granulosum* (1 isolate) and *Slackia exigua* (1 isolate).
[g]Includes *Acidaminococcus fermentans* (1 isolate), *Anaerococcus* sp. (2 isolates), *A. vaginalis* (1 isolate), *Finegoldia magna* (21 isolates), *Parvimonas micra* (16 isolates), *Peptoniphilus harei* (7 isolates), *Peptoniphilus* sp. (1 isolate), *Peptostreptococcus anaerobius* (12 isolates), *P. anaerobius/stomatis* (2 isolates), *Staphylococcus saccharolyticus* (1 isolate), *Veillonella* sp. (6 isolates), *V. parvula* (1 isolate) and *V. parvula/dispar* (1 isolate).

In vitro results indicate that tigecycline may be useful in the treatment of infections involving anaerobic bacteria. Until now no breakpoints for susceptibility testing of anaerobic bacteria have been proposed by EUCAST or CLSI, because no correlation could be found between MIC values, pharmacokinetic/pharmacodynamic data and clinical outcome.[9] Only FDA-approved breakpoints are available, which correspond to the MIC distribution of anaerobic organisms in clinical trials. Although the proposed breakpoint cut-off of 4 mg/L is not fully supported by pharmacodynamic values based on serum concentrations, high tissue concentrations reached at infection sites or in abscesses could support it.[21]

In this survey as well as in the 2004 study, linezolid showed good *in vitro* activity against all anaerobic bacteria, with overall 97% of isolates having an MIC $\leq$4 mg/L, and results are largely comparable to those of other surveys.[22,23] However, clinical data on linezolid in the treatment of anaerobic infections are limited to only a few case reports[24,25] and no official breakpoints are available.

In conclusion, the overall susceptibility of anaerobes showed little evolution in comparison with our previous surveys, except a decline in the activity of clindamycin against *Prevotella* spp. and other Gram-negative isolates and of moxifloxacin against clostridia. However, the use of EUCAST breakpoints reduced the percentage of strains susceptible to piperacillin/tazobactam to 91%. Meropenem and metronidazole remain the two most potent agents for the treatment of anaerobic infections, although organisms resistant to each of them were detected. *In vitro* susceptibility testing of anaerobic isolates is indicated in severe infections to confirm the appropriateness of antimicrobial therapy.

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Supplementary data

Tables S1, S2 and S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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