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. MIC50/MIC90 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 bacteremia. 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. The CLSI 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, 1993–94 and 2004. 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); Université Libre de Bruxelles (Brussels); Université catholique de Louvain (Ghent); Université Libre de Bruxelles (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. Species name 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) 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 and/or 16S rRNA gene sequencing.

#### Susceptibility testing

Antimicrobial susceptibility testing was performed using Etest methodology (bioMérieux Benelux, Brussels, Belgium) as described previously. The following antimicrobial agents were tested: penicillin, cefoxitin, amoxicillin/clavulanate, piperacillin/tazobactam, meropenem, clindamycin, metronidazole, chloramphenicol, maxifloxacin, 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 10⁶ cfu/mL) and incubated anaerobically at 35 °C for 48 h. Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741 and Eggertella lenta ATCC 43055 were included as control strains in each test run. The isolates were categorized by EUCAST breakpoints 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. 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. The annealing temperature of the cfiA gene detection method described by Söki et al. was increased to 62 °C to avoid non-specific reactions. The presence of a PCR product of 728 bp was regarded as positive.

#### Detection and typing of nim genes

Bacteroides and Parabacteroides spp. isolates of the present study and of the 2004 survey were screened for nim genes with primers NIM-3 and NIM-5 and amplification conditions as described previously. 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.

#### 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-sporing-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. B. 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, MIC₉₀ and MIC₅₀ 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...
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,\(^3\)\(^-\)\(^5\) 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.\(^6\)\(^,\)\(^17\)

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 (cpfA-positive) strains in 2004,\(^12\)\(^,\)\(^18\) in 2011–12 only 2 resistant B. fragilis isolates harboured this gene, suggesting a combination of overexpressed CpfA 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 1. EUCAST and CLSI breakpoints for tested antimicrobial agents

| Antimicrobial agent | EUCAST susceptible | EUCAST resistant | CLSI susceptible | CLSI intermediate | CLSI resistant |
|---------------------|---------------------|------------------|-----------------|------------------|---------------|
| Penicillin          | \(<0.25\)            | \(>0.5\)         | \(<0.5\)        | 1                | \(\geq2\)    |
| Cefotaxin           | NA                  | NA               | \(\leq16\)      | 32               | \(\geq64\)   |
| 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               | NA              | NA               | NA           |
| 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.\(^9\)
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

| Organism/antimicrobial agent | MIC range (mg/L) | MIC50/MIC90 (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                  | <0.016 to >256   | 2/64              | 79    | 78   | 83      | 97   |
| 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               | 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. (n) | 180 |       |       | 238  | 163     | 119  |
| penicillin                 | 0.032 to >32     | >32/>32           | 3     | 1    | 1       | 2    |
| cefoxitin                  | 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               | 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                  | 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               | 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                  | 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               | 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) | MIC50/MIC90 (mg/L) S (%) |
|-----------------------------|----------------------------------|-----------------|------------------------|
| Fusobacterium spp. (n)      |                                  |                 |                        |
| penicillin                  | 0.004 to >32 0.016/32 81         | <0.016 – 4      | 100 100 NT              |
| cefoxitin<sup>a</sup>       | <0.016 – 4     | 0.125/4 100     | 100 100 100             |
| 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 100 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<sup>b</sup>    | 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 (n) | 52 | 0.002 to >32 4/>32 35 | 26 48 59 |
| penicillin                  | <0.016 – 8     | 0.5/2 100       | 98 96 NT                |
| cefoxitin<sup>a</sup>       | <0.016 – 8     | 0.125/1 100     | 100 100 95              |
| amoxicillin/clavulanate     | <0.016 – 2     | 0.125/6 100     | 100 100 100             |
| 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 100 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<sup>b</sup>    | 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. (n)        | 38               | 0.032 to >32 0.125/2 71 | 77 81 84 |
| penicillin                  | 0.25 to >256   | 1/32 90        | 91 90 NT                |
| cefoxitin<sup>a</sup>       | <0.016 – 4     | 0.064/0.5 100   | 97 100 100              |
| amoxicillin/clavulanate     | 0.016 to >256  | 0.125/8 95     | 91 98 NT                |
| piperacillin/tazobactam     | 0.004 – 2      | 0.032/1 100     | 98 NT 100 NT            |
| meropenem                   | <0.016 to >256 | 0.5/32 82       | 77 83 87                |
| clindamycin                 | <0.016 to >256 | 0.25/2 100      | 98 100 100              |
| metronidazole               | <0.016 – 4     | 0.25/2 100      | 98 100 100              |
| chloramphenicol             | 0.25 – 8       | 4/8 100        | 95 100 96               |
| moxifloxacin<sup>b</sup>    | 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 (n) | 40 | 0.008 – 8 0.032/2 80 | 81 73 93 |
| penicillin                  | 0.016 – 64     | 0.25/16 95     | 100 86 NT               |
| cefoxitin<sup>a</sup>       | 0.016 – 64     | 0.064/0.5 100   | 100 100 100             |
| amoxicillin/clavulanate     | 0.016 – 2      | 0.125/0.25 90  | 84 91 NT                |
| piperacillin/tazobactam     | <0.016 – 64    | 0.25/4 100     | 100 NT 100 NT           |
| meropenem                   | 0.016 – 0.5    | 0.125/0.25 100 | 100 NT 100 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<sup>b</sup>    | 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 | MIC range (mg/L) | MIC50/MIC90 (mg/L) | S (%) | 2004 | 1993–94 | 1987 |
|-----------------------------|------------------|--------------------|-------|------|---------|------|
| Anaerobic cocci\(^9\) (n)  |                  |                    |       |      |         |      |
| penicillin                  | <0.002 to >32    | 0.064/0.5          | 88    | 37   | 57      | 47   |
| 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  | 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.

\(^9\)Includes Bacteroides caccae (3 isolates), B. cellulosityicus (1 isolate), B. caprocola (1 isolate), B. fragilis (69 isolates), B. massiliensis (1 isolate), B. nordii (1 isolate), B. ovatus/lyxanisolvens (19 isolates), B. pyogenes (5 isolates), B. slyersiae (3 isolates), B. slyersiae/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).

\(^\)Includes Fusobacterium goniodiformans (2 isolates), F. mortiferum (2 isolates), F. necrophorum (5 isolates), F. nucleatum (7 isolates) and F. varium (5 isolates).

\(^\)Includes Campylobacter rectus (1 isolate), Dialister microaerophilus (2 isolates), Porphyromonas asaccharolytica (1 isolate), P. somerae (2 isolates), Prevotella baronae (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. loeschei (1 isolate), P. melanolinogenica (2 isolates), P. nanceiensis (2 isolates), P. nigrescens (4 isolates), P. oris (1 isolate), P. salivae (1 isolate), Prevotella sp. (4 isolates), P. timonenensis (1 isolate), P. veroralis (1 isolate) and Sutterella wadsworthensis (1 isolate).

\(^\)Includes Clostridium bifermantans (1 isolate), C. bolteae/clastidiiformae (3 isolates), C. cadaveris (1 isolate), C. citroniae (1 isolate), C. clostidiformae (1 isolate), C. hathewayi (3 isolates), C. innocuum (1 isolate), C. lmxurum (1 isolate), C. perforingis (17 isolates), C. ramosum (4 isolates), C. septicum (1 isolate), Clostridium sp. (1 isolate), C. sporogenes (2 isolates) and C. tertium (1 isolate).

\(^\)Includes Actinobaculum massiliense (1 isolate), A. schoali (1 isolate), Actinomyces odontolyticus (1 isolate), A. radiae (1 isolate), Actinomyces sp. (2 isolates), Anaerotrunctus sp. (1 isolate), Bifidobacterium dentium (1 isolate), Colinella 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 Sackia exigua (1 isolate).

\(^\)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), Peptastreptococcus anaerobius (12 isolates), P. anaerobius/stomaties (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.\(^\)\(^2\)\(^1\)

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 ≤ 4 mg/L, and results are largely comparable to those of other surveys.\(^2\)\(^2\)\(^,\)\(^2\)\(^3\)\(^,\)\(^2\)\(^4\) However, clinical data on linezolid in the treatment of anaerobic infections are limited to only a few case reports\(^2\)\(^4\)\(^,\)\(^2\)\(^5\) 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 clindamycin. 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 JACOnline (http://jac.oxfordjournals.org/).

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