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2 Title: Epidemiology and microbiological features of anaerobic bacteremia in two French

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5 Running Title: Anaerobic bacteremia

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20 Abstract:

21 Bacteremia implicating anaerobic bacteria (BIAB) represents 2 to 6% of all episodes of
22 bacteremia and is associated with high mortality. In this retrospective study from June 2015
23 to December 2016, we compared BIAB frequency in two hospital centers in Montpellier
24 (France): Montpellier university hospital (MUH) and a center specialized in cancer (ICM).
25 Among the 2465 microbiologically relevant episodes of bacteremia, we identified 144 (5.8%)
26 in which anaerobic bacteria were implicated. BIAB frequency was higher at ICM than MUH
27 (10.4%, vs. 4.9%, $p < 0.01$). Poly-microbial bacteremia was more frequent among the BIAB
28 episodes (31.9% vs. 11.0% for aerobic-only bacteremia, $p < 0.01$). *Bacteroides* and *Clostridium*
29 were the most frequently identified genera of anaerobic bacteria (64 and 18 episodes,
30 respectively), with the *B. fragilis* group (BFG) involved in 68/144 episodes. We could perform
31 antibiotic susceptibility typing in 106 of the 144 anaerobic isolates, including 67 BFG isolates.
32 All isolates but one were susceptible to metronidazole. In the BFG, sporadic resistant or
33 intermediate results were found for amoxicillin-clavulanate (5/67), piperacillin-tazobactam
34 (2/67) and imipenem (1/67). BFG isolates were susceptible also to cefoxitin (90.8%),
35 rifampicin (97.0%) and tigecyclin (91.0%). Multidrug resistance in this group (7 isolates) was
36 mostly due to acquired resistance to moxifloxacin, clindamycin and tigecyclin. This study
37 shows that BIAB frequency can vary among hospitals and services. They should especially be
38 taken into account in centers specialized in cancer treatment. However, the implicated
39 bacteria remain frequently susceptible to the most used antibiotics used against anaerobic
40 bacteria, although resistance does exist.

41

42

43 Introduction

44 Anaerobic bacteria are implicated in 2 to 6% of bacteremia episodes [1–4]. Conflicting data
45 have been published on their incidence (increase, decrease or stability) during the last
46 decades [2,5–7]. Mortality caused by bacteremia implicating anaerobic bacteria (BIAB) is
47 high, from 13 to 25% [1,2,8–10], and even higher in patients who are inappropriately treated
48 [3]. They should especially be taken into account in centers specialized in cancer treatment
49 [1–4,9]. Antimicrobial resistance in BIAB remains rare: most of anaerobic bacteria are
50 susceptible to metronidazole, to the association of amoxicillin and clavulanic acid, and to
51 penems [1,4,10–12]. Resistance to clindamycin and fluoroquinolones has been more
52 frequently reported, although about half of the isolates are still susceptible [1,4,11,12].
53 Importantly, a clone of multi-resistant *Bacteroides fragilis* has been recently described in
54 Europe [13]. The most frequent sources of BIAB are intra-abdominal and female genital tract
55 infections [14]. Multiple conditions have been associated with higher risk of BIAB, such as
56 solid and hematological malignancies and surgery of the gastro-intestinal tract or the
57 urogenital system [14].

58 BIAB incidence in different centers has not been compared in recent studies. This could be a
59 limit in epidemiology studies because the combination and frequency of local risk factors
60 could influence their incidence and spread. Therefore, the aim of our study was to compare
61 BIAB incidence, their microbial epidemiology, and the resistance to antibiotics of the
62 involved anaerobic bacteria in two different French hospitals.

63

64 Material and methods

65 Blood cultures were received from two different hospital centers: Montpellier University
66 Hospital (MUH), a training hospital of 2000 beds, and the Institute of Cancer in Montpellier
67 (ICM), a center dedicated to cancer treatment with 210 beds.

68 For every positive blood culture identified in our bacteriology laboratory between 1 June
69 2015 and 31 December 2016, the following data were retrieved: patients' demographic data
70 (anonymized at the first step of data analysis except birth date), hospital service where the
71 patient was hospitalized at sampling time, and results of the identification and antibacterial
72 susceptibility testing (AST) assays of the isolated bacteria. AST data for anaerobic isolates
73 (categorization in term of susceptibility and minimum inhibitory concentrations (MIC)) were
74 retrieved from the SIRWEB software database.

75 From these data, all putative bacteremia episodes were determined for each patient and
76 each bacterial species, using a bioinformatics pipeline. Briefly, a 5-day interval between two
77 positive blood cultures was chosen to define two separate bacteremia episodes. For each
78 episode, only the species with i) at least one positive blood culture for aerobic and anaerobic
79 species that are known to be systematic pathogens in blood cultures (*e. g.*, *Staphylococcus*
80 *aureus*, *Enterobacterales*, *Bacteroides* spp., etc.), ii) at least two positives blood cultures for
81 an aerobic species known as possible contaminant and with comparable AST results (*i.e.*,
82 coagulase-negative *Staphylococcus* or *Corynebacterium* spp.), or iii) the presence of more
83 than one positive blood culture for an anaerobic species known as probable contaminant
84 (*e.g.*, *Cutibacterium acnes*) were retained (see supplementary data to see which species are
85 associated to which part of the algorithm). Fungi were excluded. Each bacteremia episode
86 was associated with the service where the earliest positive blood culture was sampled.
87 Episodes occurring in patients younger than 16 years old were automatically excluded.

88 During this period, blood cultures were always concomitantly performed in BacT/Alert FA
89 (aerobic) and FN Plus (anaerobic) bottles, containing antimicrobial-absorbing beads. Bottles
90 were incubated and screened using the BacT/ALERT 3D system. Aerobic bottles were
91 incubated for 5 days (standard conditions), or for 10 and 21 days when fungemia and
92 endocarditis, respectively, were suspected. Anaerobic bottles were incubated in standard
93 conditions for 7 days, or for 21 days when endocarditis was suspected. For each positive
94 blood culture, the bacteria retrieved after subculture were identified Matrix Assisted Laser
95 Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) using a Bruker
96 Microflex system (Bruker Daltonics, Bremen, Germany) and the Bruker database. The
97 European Network for the Rapid Identification of Anaerobes (ENRIA) database was also
98 interrogated when needed [15]. AST was performed using the disk diffusion method
99 according to the recommendations by the “Comité de l’Antibiogramme de la Société
100 Française de Microbiologie” (Antibiogram Committee of the French Microbiology Society;
101 CA-SFM) for aerobic bacteria (version 2015) and for anaerobic bacteria (version 2013). For
102 anaerobes, AST was carried out only for species with known possible resistance (mostly
103 *Bacteroides*) and in specific contexts. The MIC values were determined using strips for
104 amoxicillin-clavulanic acid, cefoxitin, metronidazole and tigecyclin. Resistance for other
105 antibiotics was tested using discs: piperacillin 75 µg, piperacillin 75 µg + tazobactam 10 µg,
106 imipenem 10 µg, moxifloxacin 5 µg, rifampicin 30 µg and clindamycin 2 UI. For each genus
107 and species, and for each antibiotic, the number of AST, the percentage of susceptible
108 isolates, and when possible their MIC₅₀, MIC₉₀ and MIC range were calculated. Multiple
109 resistances (i.e., association of acquired resistance against at least three categories of
110 antibiotics, adapted from the definitions for *Enterobacterales* (former *Enterobacteriaceae*)
111 and *Pseudomonas aeruginosa* [16]) in the same isolate were verified manually. Categories of

112 molecules used were penicillins + β -lactams inhibitors (amoxicillin-clavulanic acid,
113 piperacillin-tazobactam), cephamycins (cefoxitin), and carbapenems (imipenem),
114 fluoroquinolones (moxifloxacin), ansamycins (rifampicin), lincosamides (clindamycin),
115 nitroimidazole derivatives (metronidazole), and cyclins (tigecyclin). Statistical analyses were
116 performed with the scipy package v1.3.0, in python 3.6.

117 The scripts used are published on GitHub (DOI 10.5281/zenodo.3601471). The different
118 dataset can be requested to the corresponding, upon motivated request.

119

120 Results

121 Among the 82 463 records of blood cultures retrieved, 2 465 episodes of bacteremia were
122 identified, including 144 BIAB episodes (5.8%). Bacteremia was poly-microbial in 301
123 episodes (12.2%), including 46 episodes involving at least one anaerobe (31.5% of all BIAB),
124 and 13 episodes involving only anaerobes (9.0% of all BIAB episodes). Poly-microbial
125 bacteremia was significantly associated with the presence of anaerobes ($p < 0.001$, χ^2 test).
126 Overall, the mean number of bacteria species isolated in each episode was 2.29, and
127 increased to 2.61 for BIAB episodes (range: 1 to 6 and 1 to 5, respectively).

128 The number of bacteremia episodes was 2040 at MUH and 425 at ICM among which 4.9%
129 ($n=100$) and 10.4% ($n=44$) were BIAB, respectively ($p < 0.001$; χ^2 test). In hospital services
130 with more than 10 episodes of bacteremia, BIAB frequency ranged from 0 to 27.3% (mean:
131 4.1%) at MUH and from 4.2 to 19.2% (mean: 10.8%) at ICM. At MUH, the highest BIAB
132 frequency was detected in services specialized in abdominal surgery (including an intensive

133 care unit) and at the clinical hematology department. At ICM, the highest frequency was
134 observed in the intensive care unit and in the two surgical departments.

135 Among the blood cultures, 26 different genera and 56 different species of anaerobic bacteria
136 were identified (table 1). The most frequently detected genera were *Bacteroides* (n=64),
137 *Clostridium* (n=18), *Lactobacillus* (n=13), and *Eggerthella* (n=13). The most frequently
138 observed anaerobic species were *Bacteroides fragilis* (37 episodes), *Bacteroides*
139 *thetaiotaomicron* (n=15), and *Eggerthella lenta* (n=13). The *B. fragilis* group (BFG) was
140 detected in 68 episodes (46.6% of all BIAB episodes). All the identified *Bacteroides* species
141 belonged to the BFG.

142 AST was performed in 106 isolates. Metronidazole was the most frequently tested antibiotic,
143 and the only tested antibiotic in 20 isolates (18.9%). Only one isolate (*B. fragilis*) was
144 resistant to metronidazole. The AST results for metronidazole are summarized in table 2.

145 Four isolates showed intermediate susceptibility to amoxicillin-clavulanic acid (n=1 *B. fragilis*,
146 n=1 *Dysgonomonas capnocytophagoides*, and n=2 *B. thetaiotaomicron*), and two
147 *Parabacteroides distasonis* isolates were resistant. One *B. thetaiotaomicron* was resistant to
148 piperacillin-tazobactam and two isolates (n=1 *Bacteroides ovatus* and n=1 *Veillonella*
149 *parvula*) showed intermediate susceptibility. Susceptibility to imipenem was intermediate in
150 three isolates (n=1 *B. thetaiotaomicron* and n=2 *Lactobacillus rhamnosus*).

151 Sixty-seven isolates with AST belonged to the BFG (AST was not performed on 3 isolates).

152 The results of these isolates are summarized in table 3. AST was not realized in 3 cases.

153 Globally, isolates from the BFG were highly susceptible to piperacillin-tazobactam,
154 imipenem, rifampicin and metronidazole (susceptibility rates of 97.0%, 98.5%, 97.0%, and
155 98.5%, respectively). Results for amoxicillin-clavulanic acid, cefoxitin and tigecyclin were

156 slightly lower (92.5%, 90.8%, and 91.0%, respectively). Five of the six isolates non-susceptible
157 to cefoxitin were identified as *B. thetaiotaomicron*. Isolates were inconstantly susceptible to
158 piperacillin, moxifloxacin and clindamycin (50.7%, 58.2% and 50.8%, respectively).

159 Multi-drug resistance was detected in seven *Bacteroides* isolates (6.6% of all anaerobic
160 bacteria isolates with AST, and 10.4% of all *Bacteroides* with AST; table 4). Multi-drug
161 resistance was mostly due to acquired resistance to moxifloxacin (n=7/7), clindamycin
162 (n=6/7), and tigecyclin (n=4/7). Some isolates had paradoxical results: susceptible to
163 amoxicillin-clavulanic acid and piperacillin-tazobactam and intermediate to imipenem, or
164 susceptible to amoxicillin-clavulanic acid and resistant to piperacillin-tazobactam.

165

166 Discussion

167 This study found an important difference in BIAB frequency in two French hospital centers,
168 and in the different services of each hospital (from 0% to 27.3%). Anaerobe presence in
169 bacterial cultures related to bacteremia episodes was significantly associated with poly-
170 microbial bacteremia. AST data analysis showed that resistance to the most frequently used
171 anti-anaerobe antibiotics was rare, although a small fraction of multi-drug resistant isolates
172 was detected.

173 The difference between hospital centers could easily be explained by the different patient
174 populations treated. MUH is a multi-disciplinary teaching hospital in which some wards are
175 specialized in cancer treatment, but others (such as rheumatology, neurology, and
176 cardiology) display very few risk factors for BIAB. Conversely, ICM is a center specialized in
177 cancer treatment. Therefore, the higher BIAB frequency at ICM could be explained by the

178 fact that malignancies are one of the prominent risk factors for BIAB [8,14]. Comparison of
179 service-specific data shows higher frequency heterogeneity at MUH than ICM. This may be
180 again explained by the difference in the incidence of risk factors among MUH services. This is
181 also supported by the high BIAB frequency in some MUH services, sometimes higher than
182 those observed at ICM.

183 In this study, BIAB occurrence was reported as the percentage of bacteremia episodes with
184 anaerobic bacteria among all bacteremia episodes. Other approaches have been used in
185 other studies, such as the number of BIAB cases per year, per patient-days, per inhabitant or
186 per admission; or the number of positive anaerobic cultures per sampled blood cultures; or
187 the percentage of anaerobic bacteria isolated from blood cultures per bacteria [2–6,8–10].

188 Our approach should be independent of the total number of patients, the length of hospital
189 stay, and the frequency or indications of blood cultures because the number of BIAB
190 episodes was normalized to the total number of bacteremia cases. The overall BIAB
191 frequency at MUH was comparable to that reported by De Keukeleire et al. [2] in a Belgian
192 university hospital. On the other hand, the overall frequency at ICM was much higher than
193 that at those in other recent studies that were not performed in cancer-specific centers.

194 However, the main bias of our approach is the algorithm used to differentiate true
195 bacteremia episodes from contaminations, and one episode from another. The number of
196 bottles with the same bacteria and isolation of the same bacteria with the same antibiotic
197 susceptibility pattern in multiple bottles are frequently used to detect false positives in
198 blood cultures [17], but misinterpretation is always possible. In our study, antibiotic
199 susceptibility patterns were used for aerobic bacteria because in our laboratory, AST of
200 coagulase-negative staphylococci is systematically performed on two different isolates and
201 the results are compared to conclude whether this is a true bacteremia or a contamination.

202 However, episodes with different antibiotic susceptibility patterns are sometimes considered
203 as real episodes by the medical team in charge, since no other explanation for the sepsis can
204 be found. Contamination of multiple blood culture samples by the same strain is also
205 possible, and may be falsely interpreted as a real bacteremia. Hopefully, these two error
206 types are rare, and may cancel each other out on a large scale. An alternative way to
207 determine whether a positive blood culture is due to a true bacteremia or a contamination
208 would have been to use the time-to-positivity of the blood culture, which relates to the
209 biomass present in the blood culture. However, the time-to-positivity depends on several
210 factors *e.g.* time of transportation, volume of blood in each blood culture, antimicrobial
211 therapy administered before sampling. These factors are not standardly recorded in the
212 participating hospitals and are therefore unknown. Another bias in the algorithm is the
213 interval between two positive blood cultures in order to consider it as two separate
214 bacteremia episodes. The 5-day interval was chosen because all bacteremia episodes must
215 be evaluated and treated rapidly. When treatment is not appropriate, other blood cultures
216 may be performed within this interval. A longer period, on the other hand, could falsely
217 create multi-bacterial episodes in the case of episodes occurring close together.

218 The frequency of poly-microbial bacteremia in the BIAB group observed in our study (31.9%)
219 is comparable to that of other studies (from 12.9% to 38%) [1–3,9,10].

220 In our study, BIAB microbial epidemiology was similar to that of previous works. *Bacteroides*
221 spp. was the most frequently implicated genus, followed by *Clostridium* spp. [1–4,9]. The
222 distribution of the different species included in the BFG was identical to what published
223 previously: *B. fragilis* was the most frequent, followed by *B. thetaiotaomicron*, *P. distasonis*,
224 and then *B. ovatus* and *B. vulgatus* [1–3,18]. This distribution is similar to that of the BFG

225 species in infections described by Nagy *et al.*, except for *P. distasonis*, but this was attributed
226 by the authors to a taxonomic change [19]. *Cutibacterium* frequency was as low as in the
227 study by Vena *et al.*, where this genus was only considered when more than two samples
228 were positive, because it is considered to be a frequent blood culture contaminant [9]. On
229 the other hand, *Lactobacillus* spp. was more frequently isolated compared to other studies.
230 The ENRIA MALDI-TOF database allowed us to identify other genera unfrequently found in
231 BIAB, such as *Tissierella praeacuta*. It should be noticed that the recovery of anaerobic
232 bacteria differs between the different blood culture systems. With each system, some
233 species are more easily retrieved, resulting in distribution differences [20,21]. This is
234 particularly true for *Fingoldia magna*, rarely described in the literature, and *C. acnes* [21]. *E.*
235 *lenta* detection has been reported by Bact/ALERT users [3,10,22] as in our study, but not by
236 users of the BACTEC system.

237 AST was mostly performed on isolates identified as belonging to the BFG (63% of AST
238 assays), due to its frequency of isolation in BIAB (46.6%). As this group is known for its high
239 antibiotic resistance rates [23], this could have led to a distortion of the results toward
240 higher resistance rates. Yet, these rates remained low for the most prevalent anti-anaerobic
241 bacteria antibiotics (piperacillin-tazobactam, metronidazole, imipenem), and are comparable
242 with those of other studies, even though they were performed in Asia [1,4,11,12]. The
243 breakpoints used in our study are those of the CA-SFM version 2013 that have not been
244 updated since the adoption of the European Committee on Antimicrobial Susceptibility
245 Testing (EUCAST) methods. EUCAST (v8.1) only proposes breakpoints for anaerobic bacteria
246 (the same as those used by CA-SFM 2013), but has none for ceftiofur, tigecyclin,
247 moxifloxacin and rifampicin. Therefore, our AST results should be interpreted with caution
248 while waiting for new guidelines for anaerobic bacteria AST.

249 In the BFG, the frequency of susceptibility to clindamycin (50.8%) was equivalent to that of
250 previous studies on anaerobic bacteria [1,4,11,12], but lower than that published for
251 European collections in other contexts [19,24,25]. This could be due to the increasing
252 prevalence of clindamycin resistance in anaerobic bacteria in Europe [25]. The BFG
253 susceptibility to moxifloxacin was low (58.2%), as described in the literature [26]. A small
254 percentage of isolates were non-susceptible to tigecyclin, as previously reported [25].

255 Seven multi-drug resistant BFG isolates were found in this study. None had a resistance
256 profile compatible with the clone described by SÓki *et al.* (most were susceptible to
257 piperacillin-tazobactam or imipenem, and all to metronidazole), thus *cfiA* and *nim* genes may
258 not be present, or not expressed [13]. In our study, multi-drug resistance was mostly due to
259 resistance to moxifloxacin and clindamycin, associated with resistance to at least another
260 antibiotic. Major anti-anaerobic bacteria antibiotics were not affected in these isolates. The
261 definition of multi-drug resistance for anaerobic bacteria used in our study was interpolated
262 from that for aerobic bacteria proposed by Magiorakos *et al.*: “acquired non-susceptibility to
263 at least one agent in three or more antimicrobial categories” [16]. This definition has a major
264 pitfall, as resistance to moxifloxacin and clindamycin in the BFG is frequent (58.2% and
265 50.8%, respectively), the cumulative probability of having at least one resistance within any
266 of the six other categories of antibiotics is high (28.0% in our dataset, independently of the
267 results for moxifloxacin and clindamycin), inflating the percentage of multi-drug resistant
268 isolates, although these isolates remains susceptible to numerous antibiotics. Thus, criteria
269 for multi-drug resistance in *Bacteroides* should be clearly established with differences
270 between major (for example metronidazole, imipenem) and minor (moxifloxacin,
271 clindamycin) molecules. Moreover, a new definition for extensively drug-resistant
272 *Bacteroides* should be created.

273 To conclude, BIAB frequency was nearly two-fold higher at ICM, although some MUH
274 services had higher frequencies. This underlines the need for clinicians to take into account
275 the factors leading to an higher risk of BIAB, which can be a deadly condition [1,2,8–10]. *In*
276 *vitro* susceptibility of the implicated anaerobic bacteria remains high, therefore empirical
277 antimicrobial therapy against anaerobic bacteria should be effective. The development of
278 multi-drug resistance is a concern because these molecules are mainly broad-spectrum
279 antibiotics. Therefore, AST must be performed as frequently as possible, particularly for
280 isolates belonging to the BFG, and new strategies should be developed to reduce the interval
281 between blood culture positivity and AST results.

282

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Table 1: Anaerobic bacteria found in bacteriemia episodes involving anaerobic bacteria (n=144)

Genus and species of anaerobic bacteria isolated in blood cultures ^a	occurrences ^b (% of all BIAB)	Genus and species of anaerobic bacteria isolated in blood cultures ^a	occurrences ^b (% of all BIAB)
GRAM NEGATIVE		GRAM POSITIVE	
Bacilli		Spore forming bacilli	
<i>Bacteroides</i>	64 (44.4)	<i>Clostridium</i> and related genera	18 (12.5)
- <i>fragilis</i>	37 (25.7)	- <i>ramosum</i>	4 (2.8)
- <i>thetaiotaomicron</i>	15 (10.4)	- <i>tertium</i>	3 (2.1)
- <i>ovatus</i>	6 (4.2)	- <i>butyricum</i>	2 (1.4)
- <i>vulgatus</i>	5 (3.5)	- <i>cadaveris</i>	2 (1.4)
- <i>uniformis</i>	3 (2.1)	- <i>clostridioforme</i>	2 (1.4)
- <i>finnegoldii</i>	1 (0.7)	- <i>perfringens</i>	2 (1.4)
- <i>nordii</i>	1 (0.7)	- <i>glycolicum</i>	1 (0.7)
<i>Parabacteroides distasonis</i>	6 (4.2)	- <i>Hungatella hathewayi</i>	1 (0.7)
<i>Fusobacterium</i>	7 (4.9)	- <i>innocuum</i>	1 (0.7)
- <i>nucleatum</i>	5 (3.5)	- <i>septicum</i>	1 (0.7)
- <i>necrophorum</i>	2 (1.4)	<i>Flavonifractor plautii</i>	1 (0.7)
<i>Prevotella</i>	7 (4.9)	Non-spore forming bacilli	
- <i>buccae</i>	3 (2.1)	<i>Lactobacillus</i>	13 (9.0)
- <i>melaninogenica</i>	1 (0.7)	- <i>plantarum</i>	4 (2.8)
- <i>nanceiensis</i>	1 (0.7)	- <i>ramnosus</i>	4 (2.8)
- <i>nigrescens</i>	1 (0.7)	- <i>paracasei</i>	2 (1.4)
- <i>sp</i>	1 (0.7)	- <i>salivarius</i>	1 (0.7)
<i>Leptotrichia</i>	3 (2.1)	- <i>sp</i>	2 (1.4)
- <i>trevisanii</i>	2 (1.4)	<i>Eggerthella lenta</i>	13 (9.0)
- <i>sp</i>	1 (0.7)	<i>Actinotignum schaalii</i>	5 (3.5)
<i>Dialister pneumosintes</i>	3 (2.1)	<i>Bifidobacterium</i>	4 (2.8)
<i>Porphyromonas asaccharolytica</i>	2 (1.4)	- <i>breve</i>	2 (1.4)
<i>Capnocytophaga canimorsus</i>	1 (0.7)	- <i>dentium</i>	1 (0.7)
<i>Desulfovibrio sp</i>	1 (0.7)	- <i>longum</i>	1 (0.7)
<i>Dysgonomonas capnocytophagoides</i>	1 (0.7)	<i>Cutibacterium acnes</i>	3 (2.1)
<i>Sutterella wadsworthensis</i>	1 (0.7)	<i>Actinomyces</i>	2 (1.4)
<i>Tissierella praeacuta</i>	1 (0.7)	- <i>oris</i>	1 (0.7)
Cocci		- <i>turicensis</i>	1 (0.7)
<i>Veillonella</i>	4 (2.8)	<i>Slackia exigua</i>	2 (1.4)
- <i>atypica</i>	2 (1.4)	<i>Alloscardovia omnicoles</i>	1 (0.7)
- <i>parvula</i>	2 (1.4)	<i>Trueperella bernardiae</i>	1 (0.7)
- <i>sp</i>	1 (0.7)	Cocci	
		<i>Parvimonas micra</i>	3 (2.1)
		<i>Ruminococcus gnavus</i>	1 (0.7)

AB anaerobic bacteria, BIAB bacteriemia involving anaerobic bacteria.

^a Genera are in bold. When only one species was isolated in a genus, lines were merged. Genus and species in a genus are ordered from the most to the least frequent. "sp" species were either not specified in the ENRIA database, or results were not sufficient to discriminate among species. ^b As multiple species of the genus could be found in a BIAB episode, the sum of the occurrences of all species from a genus can be higher than the occurrence of that genus.

Table 2 : Results of antibiotic susceptibility testing of metronidazole

Genus and species of anaerobic bacteria isolated in blood cultures	Metronidazole AST results (breakpoint > 4 mg/L)			
	n susceptible/n tested (%)	MIC50	MIC90	MIC range or value
<i>Bacteroides</i>	61/62 (98.4)	.5	1.5	.064-6
- <i>fragilis</i>	34/35 (97.1)	.38	1	.094-6
- <i>thetaiotaomicron</i>	13/13 (100)	.75	1.25	.25-2
- <i>vulgatus</i>	5/5 (100)	.38	1.5	.064-2
- <i>ovatus</i>	4/4 (100)	NA	1.5	.5-2
- <i>uniformis</i>	3/3 (100)	NA	NA	.19-.5
- <i>finnegoldii</i>	1/1 (NA)	NA	NA	1.5
- <i>nordii</i>	1/1 (NA)	NA	NA	.19
<i>Clostridium</i>	11/11 (100)	.25	1.5	<.016-2
- <i>ramosum</i>	2/2 (100)	NA	NA	.125-.75
- <i>tertium</i>	2/2 (100)	NA	NA	.5-.75
- <i>cadaveris</i>	2/2 (100)	NA	NA	.047-.047
- <i>clostridioforme</i>	1/1 (NA)	NA	NA	<.016
- <i>perfringens</i>	2/2 (100)	NA	NA	1.5-2
- <i>glycolicum</i>	1/1 (NA)	NA	NA	.032
- <i>innocuum</i>	1/1 (NA)	NA	NA	1.5
<i>Fusobacterium nucleatum</i>	1/1 (NA)	NA	NA	.016
<i>Prevotella</i>	3/3 (100)	NA	NA	.019-.75
- <i>buccae</i>	2/2 (100)	NA	NA	.19-.75
- <i>melaninogenica</i>	2/2 (100)	NA	NA	.5-.5
<i>Parabacteroides distasonis</i>	5/5 (100)	1.5	3	.75-4
<i>Veillonella</i>	2/2 (100)	NA	NA	.75-1
- <i>parvula</i>	1/1 (NA)	NA	NA	.75
- <i>sp</i>	1/1 (NA)	NA	NA	1
<i>Dialister pneumosintes</i>	1/1 (NA)	NA	NA	2
<i>Porphyromonas asaccharolytica</i>	1/1 (NA)	NA	NA	.032
<i>Ruminococcus gnavus</i>	1/1 (NA)	NA	NA	.064
<i>Tissierella praeacuta</i>	1/1 (NA)	NA	NA	.064
TOTAL	87/88 (98.9)	.5	1.5	<.016-6

NA: not applicable: the percentage of susceptible results was not calculated for n=1, and MIC50 and MIC90 were not calculated for n<5.

AST antibiotic susceptibility typing.

Table 3 : Results of antibiotic susceptibility testing for *Bacteroides fragilis* group's isolates

Genus and species of anaerobic bacteria isolated in blood cultures	Results of AST per antibiotic													
	Number of susceptible results/total number of results (percentage of susceptible results) ± MIC50, MIC90 and range for MIC/value													
	AMC (MIC)				PIP		PIT		CXI (MIC)				IMI	
Breakpoints (CASFM 2013)	S ≤ 4 mg/L ; R > 8 mg/L				R > 18 mm		S ≥ 21 mm R < 19 mm		R > 32 mg/L				S ≥ 24 mm R < 17 mm	
<i>Bacteroides</i>	59/62 (95.2)	.75	4	.094-8	32/62 (51.6)	60/62 (96.8)	54/60 (90.0)	8	32	1.5->256	61/62 (98.4)			
- <i>fragilis</i>	34/35 (97.1)	.5	3	.094-6	22/35 (62.9)	35/35 (100)	33/34 (97.1)	6	24	1.5->256	35/35 (100)			
- <i>thetaiotaomicron</i>	11/13 (84.6)	2	4	.25-8	4/13 (30.8)	12/13 (92.3)	8/13 (61.5)	32	64	12-64	12/13 (92.3)			
- <i>vulgatus</i>	5/5 (100)	1	3	.094-4	2/5 (40.0)	5/5 (100)	5/5 (100)	4	16	2-16	5/5 (100)			
- <i>ovatus</i>	4/4 (100)	NA	NA	.19-2	3/4 (75.0)	3/4 (75.0)	4/4 (100)	NA	NA	12-24	4/4 (100)			
- <i>uniformis</i>	3/3 (100)	NA	NA	.25-3	1/3 (33.3)	3/3 (100)	3/3 (100)	NA	NA	2-3	3/3 (100)			
- <i>finnegoldii</i>	1/1 (NA)	NA	NA	2	0/1 (NA)	1/1 (NA)	1/1 (NA)	NA	NA	6	1/1 (NA)			
- <i>nordii</i>	1/1 (NA)	NA	NA	1.5	0/1 (NA)	1/1 (NA)	1/1 (NA)	NA	NA	32	1/1 (NA)			
<i>Parabacteroides distasonis</i>	3/5 (60.0)	2	16	1-24	2/5 (40.0)	5/5 (100)	5/5 (100)	24	24	16-24	5/5 (100)			
TOTAL	62/67 (92.5)	1	4	.094-24	34/67 (50.7)	65/67 (97.0)	59/65 (90.8)	8	32	1.5->256	66/67 (98.5)			

Table 3 : Results of antibiotic susceptibility testing for Bacteroides fragilis group's isolates (continued)

Genus and species of anaerobic bacteria isolated in blood cultures	Results of AST per antibiotic												
	Number of susceptible results/total number of results (percentage of susceptible results) ± MIC50, MIC90 and range for MIC/value												
	MOX	RIF		CLI		MET (MIC)			TIG (MIC)				
Breakpoints (CASFM 2013)	S ≥ 21 mm R < 18 mm	S ≥ 19 mm R < 14 mm	R < 15 mm		R > 4 mg/L			S ≤ 4 mg/L ; R > 8 mg/L					
Bacteroides	34/62 (54.8)	60/61 (98.4)	31/62 (50.0)	61/62 (98.4)	.5	1.5	.064-6	56/61 (91.8)	.25	4	.032-8		
- <i>fragilis</i>	20/35 (57.1)	35/35 (100)	21/35 (60.0)	34/35 (97.1)	.38	1	.094-6	31/34 (91.2)	.38	4	.047-8		
- <i>thetaiotaomicron</i>	8/13 (61.5)	12/13 (92.3)	4/13 (30.8)	13/13 (100)	.75	1	.25-2	11/13 (84.6)	.5	4	.032-6		
- <i>vulgatus</i>	2/5 (40.0)	5/5 (100)	2/5 (40.0)	5/5 (100)	.38	1.5	.064-2	5/5 (100)	.125	1	.032-2		
- <i>ovatus</i>	3/4 (75.0)	4/4 (100)	1/4 (25.0)	4/4 (100)	NA	NA	.5-2	4/4 (100)	NA	NA	.047-1.5		
- <i>uniformis</i>	1/3 (33.3)	3/3 (100)	2/3 (66.7)	3/3 (100)	NA	NA	.19-.5	3/3 (100)	NA	NA	.064-.094		
- <i>finnegoldii</i>	0/1 (NA)		0/1 (NA)	1/1 (NA)	NA	NA	1.5	1/1 (NA)	NA	NA	.047		
- <i>nordii</i>	1/1 (NA)	1/1 (NA)	1/1 (NA)	1/1 (NA)	NA	NA	.19	1/1 (NA)	NA	NA	1		
Parabacteroides distasonis	5/5 (100)	5/5 (100)	3/5 (60.0)	5/5 (100)	1.5	3	.75-4	5/5 (100)	.5	1	.25-2		
TOTAL	39/67 (58.2)	65/66 (97.0)	34/67 (50.8)	66/67 (98.5)	.5	1.5	.064-6	61/66 (91.0)	.25	4	.032-8		

NA: not applicable: the percentage of susceptible results was not calculated for n=1, and MIC50 and MIC90 were not calculated for n<5.

AMC, amoxicillin-clavulanic acid; AST, antibiotic susceptibility testing; CLI, clindamycin; CXI, ceftiofur; IMI imipenem; MIC, minimum inhibitory concentration; MOX, moxifloxacin; MET, metronidazole; PIP, piperacillin; PIT, piperacillin-tazobactam; RIF, rifampicin; TIG, tigecycline.

Table 4: Antibiotic susceptibility testing of multi-drug resistant *Bacteroides fragilis* group's isolates

Species	AMC	PIT	CXI	IMI	MOX	RIF	CLI	MET	TIG	N. of families with resistance
<i>B. thetaiotaomicron</i>	S	S	R	I	R	R	R	S	S	5
<i>B. fragilis</i>	I	S	S	S	R	S	R	S	I	4
<i>B. thetaiotaomicron</i>	I	S	R	S	R	S	S	S	I	4
<i>B. thetaiotaomicron</i>	I	S	R	S	R	S	R	S	S	4
<i>B. thetaiotaomicron</i>	S	R	S	S	I	S	R	S	S	3
<i>B. fragilis</i>	S	S	S	S	R	S	R	S	I	3
<i>B. thetaiotaomicron</i>	S	S	S	S	R	S	R	S	I	3

AMC, amoxicillin-clavulanic acid; PIT, piperacillin-tazobactam; CXI, ceftiofur; IMI, imipenem; MOX, moxifloxacin; RIF, rifampicin; CLI clindamycin; MET, metronidazole; TIG, tigecycline.