Original Article

Anaerobic Bacteraemia Revisited: Species and Susceptibilities

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Abstract

Introduction: This retrospective study was performed to evaluate the frequency of anaerobic bacteraemia over a 10-year period, and to provide updated antibiotic susceptibilities for the more clinically relevant anaerobes causing blood stream infection. Materials and Methods: Data were retrieved from the laboratory information system for the period 2003 to 2012. During this time, blood cultures were inoculated in Bactec™ Plus vials (BD, USA) and continuously monitored in the Bactec™ 9000 blood culture system (BD, USA). Anaerobic organisms were identified using commercial identification kits, predominantly API 20 A (bioMérieux, France) supplemented with Vitek ANC cards (bioMérieux, France) and AN-Ident discs (Oxoid, United Kingdom). A representative subset of isolates were retrieved from 2009 to 2011 and antimicrobial susceptibilities to penicillin, amoxicillin-clavulanate, clindamycin, imipenem, moxifloxacin, piperacillin-tazobactam and metronidazole were determined using the Etest method. Results: Anaerobes comprised 4.1% of all positive blood culture with 727 obligate anaerobes recovered over the 10-year period, representing a positivity rate of 0.35%. The only significant change in anaerobe positivity rates occurred between 2003 and 2004, with an increase of 0.2%. The *Bacteroides fragilis* group (45%) were the predominant anaerobic pathogens, followed by *Clostridium* species (12%), *Propioniobacterium* species (11%) and *Fusobacterium* species (6%). The most active in vitro antibiotics were imipenem, piperacillin-tazobactam, amoxicillin-clavulanate and metronidazole, with susceptibilities of 95.0%, 93.3%, 90.8% and 90.8% respectively. Resistance was high to penicillin, clindamycin and moxifloxacin. However, there were apparent differences for antibiotic susceptibilities between species. Conclusion: This study indicates that the anaerobes comprise a small but constant proportion of bloodstream isolates. Antibiotic resistance was high to some antibiotics, but metronidazole, the beta-lactam/beta-lactamase inhibitors and carbapenems retained good in vitro activity.

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Introduction

Over the last 2 decades, the importance of anaerobic bacteraemia has undergone various shifts in opinion. Early studies in the 1970s reported that anaerobes accounted for 2% to 20% of bacteraemia.[1,2] However, by the mid 1980s, multiple centres reported declining rates of anaerobic bacteraemia,[3-5] and several authors suggested that the routine inoculation of anaerobic blood cultures should be discontinued. The rationale for this argument was that the inoculation of 2 aerobic blood cultures would increase the yield of obligate aerobes,[6,7] and that antimicrobial susceptibilities of anaerobes were also predictable. The late 1990s saw several studies reporting a resurgence of anaerobic bacteraemia,[8,9] although this was also clearly dependent on local epidemiology.[10,11]

Antibiotic therapy for anaerobes is usually empiric. Anaerobic susceptibility testing is not performed routinely, as it is both costly and methodologically demanding, and time consuming. Nonetheless, the prevalence of antibiotic resistance in anaerobes is increasing, both to commonly used antibiotics (e.g. metronidazole and clindamycin)[12] and broad-spectrum antibiotics (e.g. the carbapenems).[13] Clinical data suggest that antibiotic resistance impacts both microbiological cure and patient mortality for patients with anaerobic bacteraemia.[14]

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There is little susceptibility data for anaerobic infections in Singapore. Our laboratory first published data on antibiotic susceptibilities for anaerobes isolated from diabetic foot infections in Singapore.[15] However, the sample size was small and data from soft tissue infections may not be representative of invasive bloodstream isolates. The Clinical Laboratory Standards Institute (CLSI) recommends that at least 50 to 100 strains should be tested to obtain accurate representation of the susceptibility pattern of local isolates. Reliable antibiotic data is required to guide antibiotic choices for both empiric and definitive treatment for anaerobic bacteraemia.

This study was performed in an 800-bed hospital that provides medical, surgical, orthopaedic and geriatric medical care to the eastern region of Singapore. The aims of the study were to determine the relative frequency of anaerobic bacteraemia over a 10-year period, to analyse the relative distribution of anaerobic species isolated from blood cultures and to determine the antibiotic susceptibilities of a representative panel of anaerobes.

Materials and Methods

Bacterial species identification and basic patient epidemiological data were extracted from the laboratory information system. Duplicate isolates from the same patient (defined as similar isolates within a 30-day period) were excluded from analysis. During the period from 2003 to 2012, standard clinical practice in the hospital included routine aerobic and anaerobic blood cultures which were performed using BACTEC™ Plus Aerobic/F and Plus Anaerobic/F vials (BD, USA). Blood cultures were incubated for 5 days in a continuous monitoring blood culture system (Bactec 9000 series, BD, USA). Anaerobic vials which flagged positive for growth were subcultured on to blood agar with 5% sheep blood (BD, USA), MacConkey agar (Oxoid, UK), chocolate agar (BD, USA) and CDC anaerobe 5% sheep blood agar (BD, USA). Blood agar and MacConkey agar were incubated at 35°C in ambient atmosphere, while chocolate agar and CDC anaerobe were incubated in 5% CO_2 and anaerobic conditions, respectively. Media were routinely incubated for 2 days, with additional incubation for up to 4 days if a slow growing anaerobe was suspected. Initial tests performed on suspected anaerobic isolates included confirmatory aerotolerance testing for strict anaerobic growth and Gram-stain. Further speciation by commercial identification kits was performed, supplemented by additional phenotypic tests, where required. The primary identification kit used was the API 20A (bioMérieux, France), with additional AN-Ident discs[16] (Oxoid, United Kingdom) used from 2003 to 2007. From 2008, isolates were predominantly identified using Vitek® ANC identification cards tested on the Vitek® Compact system supplemented with API 20A, where necessary.

Antibiotic susceptibility testing was performed on a retrospective collection of 119 anaerobic isolates from blood culture, collected from 2008 to 2010. Banked isolates were stored at -70°C in commercial cryovials, and retrieved from initial storage by subculture on CDC anaerobe 5% sheep blood agar (BD, USA). Isolates were passaged by subculture at least twice before further testing was performed. The isolates included *Bacteroides* spp. (n = 69), *Clostridium* spp. (n = 28), *Fusobacterium* spp. (n = 9) and other species.[13] These were chosen to represent the most common and/or most pathogenic anaerobic isolates from blood cultures. Antimicrobial susceptibility testing was performed by Etest, according to manufacturer's instructions.[17] Testing was performed on Brucella agar with 5% sheep blood, vitamin K and hemin (BD, USA), with inoculating suspensions prepared using Brucella broth (BD, USA). The antibiotics tested were amoxicillin-clavulanate, clindamycin, imipenem, metronidazole, moxifloxacin, penicillin and piperacillin-tazobactam. All testing media was pre-reduced anaerobically overnight to achieve optimal conditions. Plates were incubated at 35°C in an anaerobic workstation system to achieve rapid anaerobiosis. Etest results were read after 48 to 72 hours, with the duration of incubation depending on the time required to achieve satisfactory confluent growth. Antibiotic susceptibilities were interpreted according to current CLSI breakpoints.[18]

Results

During the 10-year period, 421,185 blood culture vials were processed in the laboratory (49.6% comprising of anaerobic blood cultures). A total of 727 obligate anaerobes were recovered from these cultures, representing a positivity rate of 0.35%. Anaerobes comprised 4.1% of 17,800 organisms recovered from blood cultures over the same period (Table 1). The only significant change in anaerobe positivity rates occurred between 2003 and 2004, with an increase of 0.2% (P <0.01).

The most frequent anaerobic isolates were the *Bacteroides fragilis* group (n = 328; 45%), *Clostridium* species (n = 89; 12%), *Propioniobacteria* species (n = 80; 11%) and *Fusobacterium* species (n = 42; 6%). The proportion of bacteraemia cases caused by the *B. fragilis* group decreased over the 10-year period, from 44% to 49% of all cases in the first 7 years to a low of 35% in 2010 (Table 2). The proportion of *Clostridium* species and *Fusobacterium* species stayed relatively constant over the study time period. The last 3 years of the study seemed to indicate a greater diversity of anaerobic species, with other species (*Veillonella*, *Peptoniphilus*, *Eggerthella*, *Actinomyces*, *Bifidobacteria*, *Porphyromonas* and *Eubacterium*) accounting for 16% to 17% of annual anaerobic bacteraemias.

Table 1. Anaerobic Bacteraemia Over Time

Year	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Anaerobes										
Number of anaerobes	31	54	72	72	78	94	83	93	75	75
By all blood cultures	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
By anaerobic blood cultures	0.1%	0.3%	0.4%	0.4%	0.4%	0.4%	0.3%	0.4%	0.3%	0.3%
As percentage of all blood culture isolates	2.1%	3.8%	5.0%	4.7%	4.6%	4.8%	4.4%	4.2%	3.6%	3.4%
Rate /1000 admissions	0.84	1.33	1.75	1.84	1.83	2.19	2.02	2.44	1.93	1.85
Aerobic Bacteria										
Number of anaerobes	1419	1368	1378	1459	1622	1853	1787	2105	1980	2102
Rate/1000 admissions	38.65	33.78	33.57	37.29	37.95	43.22	43.41	55.16	50.84	51.79
***Candida* Species**										
Number of aerobes	14	27	20	19	25	33	27	25	18	18
Rate/1000 admissions	0.38	0.67	0.49	0.49	0.58	0.77	0.66	0.66	0.46	0.44

Table 2. Frequency of Anaerobic Species from Blood Cultures

Year of Analysis	Number of Isolates* (% of Total for the Year)										
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Bacteroides fragilis group	19 (61%)	24 (44%)	35 (49%)	33 (46%)	38 (49%)	46 (49%)	42 (51%)	33 (35%)	28 (37%)	30 (40%)	328
Clostridium species	4 (13%)	6 (11%)	8 (11%)	10 (14%)	7 (9%)	12 (13%)	7 (8%)	16 (17%)	11 (15%)	8 (11%)	89
Propionibacterium species	2 (6%)	4 (7%)	8 (11%)	6 (8%)	11 (14%)	12 (13%)	9 (11%)	8 (9%)	6 (8%)	14 (19%)	80
Fusobacterium species	-	8 (15%)	6 (8%)	-	4 (5%)	4 (4%)	6 (7%)	4 (4%)	6 (8%)	4 (5%)	42
Anaerobic Gram-positive bacilli (others)	-	4 (7%)	4 (6%)	5 (7%)	6 (8%)	5 (5%)	7 (8%)	6 (6%)	3 (4%)	-	40
Peptostreptococcus species	2 (6%)	3 (6%)	4 (6%)	10 (14%)	2 (3%)	6 (6%)	3 (4%)	4 (4%)	1 (1%)	-	35
Prevotella species	-	-	3 (4%)	5 (7%)	1 (1%)	-	1 (1%)	-	4 (5%)	2 (3%)	20
Other anaerobes†	4 (13%)	5 (9%)	4 (6%)	3 (4%)	9 (12%)	9 (10%)	8 (10%)	18 (19%)	16 (21%)	17 (23%)	93

*As % of total for the year.

†*Lactobacillus* spp. (n = 20), anaerobic Gram-negative bacilli (not identifiable) (n = 15), *Veillonella* spp. (n = 15), *Peptoniphilus* spp. (n = 11), Anaerobic Gram-negative cocci (not identifiable) (n = 8), *Eggerthella* spp. (n = 8), anaerobic Gram-positive cocci (not identifiable) (n = 7), *Actinomyces* spp. (n = 5), *Porphyromonas* spp. (n = 2), *Eubacterium* spp. (n = 1), and *Bifidobacterium* spp. (n = 1).

Imipenem, piperacillin-tazobactam, amoxicillin-clavulanate and metronidazole remained the most active antibiotics, with in vitro susceptibility rates of 95.0%, 93.3%, 90.8% and 90.8% respectively (Table 3). Susceptibilities to penicillin (30.3%), clindamycin (53.8%) and moxifloxacin (51.3%) were much lower. Within these overall results, there was individual variation in antibiotic susceptibilities for specific anaerobic species. *Bacteroides* spp. were uniformly resistant to penicillin, and susceptibilities to moxifloxacin (44.1%) and clindamycin (51.5%) were variable. The most effective in vitro antibiotics for *Bacteroides* spp. were metronidazole, imipenem and the beta-lactam/beta-lactamase inhibitors. *Clostridium* spp. demonstrated lowest susceptibility to moxifloxacin (44.1%), clindamycin (51.5%) and variable susceptibility to metronidazole (78.6%), but were uniformly susceptible to the beta-lactam/

Table 3. Antibiotic Susceptibilities of Selected Anaerobes

Antibiotic	Genus	R (%)	I (%)	S (%)	MIC90 (mg/L)
Penicillin	*Bacteroides* spp. (n = 69)*	98.5	0	1.5	>256
	Clostridium spp. (n = 28)†	14.3	7.1	78.6	96
	Fusobacterium spp. (n = 9)‡	11.1	0	88.9	>256
Amoxicillin/Clavulanic acid	*Bacteroides* spp.	5.9	5.9	88.2	6
	Clostridium spp.	0	3.6	96.4	2
	Fusobacterium spp.	11.1	0	88.9	>256
Piperacillin/Tazobactam	*Bacteroides* spp.	5.9	1.5	92.6	24
	Clostridium spp.	0	3.6	96.4	6
	Fusobacterium spp.	11.1	0	88.9	>256
Imipenem	*Bacteroides* spp.	1.5	0	98.5	0.75
	Clostridium spp.	10.7	0	89.3	32
	Fusobacterium spp.	22.2	0	77.8	64
Moxifloxacin	*Bacteroides* spp.	25	30.9	44.1	64
	Clostridium spp.	28.6	3.6	67.9	64
	Fusobacterium spp.	44.4	11.1	44.4	64
Clindamycin	*Bacteroides* spp.	42.6	5.9	51.5	>256
	Clostridium spp.	35.7	10.7	53.6	>256
	Fusobacterium spp.	33.3	11.1	55.6	12
Metronidazole	*Bacteroides* spp.	2.9	0	97.1	1.5-
	Clostridium spp.	17.9	3.6	78.6	>256
	Fusobacterium spp.	0	0	100	0.38

**Bacteroides fragilis* (n = 40), *Bacteroides thetaiotaomicron* (n = 12), *Bacteroides distasonis* (n = 7), *Bacteroides vulgatus* (n = 4), *Bacteroides ovatus* (n = 3), *Bacteroides uniformis* (n = 2), *Bacteroides stercoris* (n = 1).
†*Clostridium perfringens* (n = 11), *Clostridium* spp. (n = 8), *Clostridium ramosum* (n = 4), *Clostridium difficile* (n = 1), *Clostridium bifermentans* (n = 1), *Clostridium tertium* (n = 1), *Clostridium clostridioforme* (n = 1), *Clostridium glycolium* (n = 1).
‡*Fusobacterium varium* (n = 4), *Fusobacterium mortiferum* (n = 2), *Fusobacterium necrophorum* (n = 2), *Fusobacterium nucleatum* (n = 1).

Table 4. Antibiotic Susceptibilities for Bacteroides Species

	Susceptible (%)			
	Bacteroides Fragilis **(n = 40)**	***Bacteroides Thetaiotaomicron*** **(n = 12)**	***Bacteroides Distasonis*** **(n = 7)**	**Other *Bacteroides* Species* (n = 10)**
Penicillin	0	0	0	10
Amoxicillin/Clavulanic Acid	87.5	91.7	71.4	100
Piperacillin/Tazobactam	95	100	71.4	90
Imipenem	97.5	100	100	100
Moxifloxacin	52.5	25	57.1	20
Clindamycin	60	41.7	14.3	50
Metronidazole	100	100	71.4	100

**B. vulgatus* (n = 4), *B. ovatus* (n = 3), *B. uniformis* (n = 2), *B. stercoris* (n = 1)

beta-lactamase inhibitors. Metronidazole and penicillin remained highly active against *Fusobacterium* species, with susceptibilities of 100.0% and 88.9% respectively. Interestingly, the lowest susceptibility to imipenem was seen in *Fusobacterium* spp. (77.8%).

Antibiotic resistance was lower in *B. fragilis* than for other *Bacteroides* species in the *fragilis* group (Table 4). Moxifloxacin resistance was higher in *B. thetaiotaomicron*, while *B. distasonis* was more likely to be resistant to the beta-lactam/beta-lactamase inhibitors and clindamycin. Two strains of *B. distasonis* were resistant to metronidazole; one with high-level resistance (minimum inhibitory concentration (MIC) >256 mg/L) and the other with low-level resistance (MIC = 32 mg/L). One multiresistant isolate of *B. fragilis* was present, with high-level resistance to all tested antibiotics except for metronidazole. Two unusually multiresistant isolates of *Fusobacterium* were also detected, with one strain of *F. mortiferum* showing high-level resistance to all tested beta-lactams but remaining susceptible to metronidazole.

Discussion

This is the first study to report on anaerobic bacteraemia with antibiotics susceptibilities in Singapore. The data suggest that anaerobes comprise 2.1% to 5.0% of all blood culture isolates, and this proportion has remained constant over the past decade. In comparison to other reported studies,[8,10] the rate of positivity for anaerobic vials was at the lower end of the reported range of 0.5% to 7.0%.[11] *Bacteroides* spp. accounted for nearly half of all bacteraemias, followed by *Clostridium* spp. and propionibacteria. Both *Bacteriodes* and *Clostridium* spp. are considered to represent clinically significant bacteraemia, which may be associated with crude mortalities of 20% to 30%.[19,20] However, propionibacteria are much less likely to be associated with clinically significant infections.[21] Over the 10-year period, our data suggests a reduction in bacteraemia caused by *Bacteroides* species. The increasing diversity of anaerobes in this study contrasts with results from other investigators, which report an increase in the proportion of bacteraemia caused by *Bacteroides* species.[10,11]

Susceptibility testing indicates that the carbapenems, beta-lactam/beta-lactam inhibitors and metronidazole generally retain excellent in vitro activity against anaerobes. Conversely, resistance to penicillin, clindamycin and moxifloxacin are high, which suggests that these antibiotics are no longer suitable for empiric treatment of anaerobic bacteraemia. A prospective study in the United States demonstrated similar trends in clindamycin and moxifloxacin resistance over a 3-year period, with differentially higher rates of resistance in non-*Bacteroides fragilis* isolates.[22] A Taiwan study reported higher rates of carbapenem resistance (up to 12% in *B. fragilis*) over a 10-year period, but conversely, lower rates of resistance to moxifloxacin.[13] As with antibiotic resistance in aerobes, this serves to highlight the importance of local epidemiology and data, as regional differences in susceptibility are inevitable.

There are several limitations to this study. Firstly, the data is generated from a single institution and may not be representative of other healthcare centres. This institution does not provide solid organ transplant, haematology, oncology, paediatric or obstetrics/gynaecology services, while provision of outpatient renal dialysis only commenced in 2010. The relative lack of severely immuno-compromised patients may account for the lower rate of anaerobic bacteraemia reported in our patient population. Secondly, phenotypic identification of anaerobes was performed by different test methods over the study period. However, the performance of the test methods used has been reported to be roughly equivalent, based on existing data.[23,24] Phenotypic identification of anaerobic bacteria by conventional methods remains challenging. Most commercial kits reliably identify anaerobic Gram-negative bacilli, including *Bacteroides* spp.,[25,26] but generate less reliable results for non-perfringens clostridial species and anaerobic Gram-positive bacilli. Finally, antibiotic susceptibility testing was performed using a gradient-diffusion method (Etest) rather than the reference agar dilution method. However, there is evidence to suggest that results by Etest are comparable to the reference test method.[27,28]

In summary, this study demonstrated a low but constant rate of anaerobic bacteraemia over a 10-year period, with the *Bacteroides fragilis* group as the predominant pathogen. Overall antibiotic resistance was high for penicillin, clindamycin and moxifloxacin but there were species-specific resistance patterns.

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