ORIGINAL ARTICLE

Anaerobic Bacteremia Spectrum, Risk Factors and Susceptibility Pattern from a Clinical Laboratory in Pakistan

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Abstract

Introduction and Objectives
Patients with malignancy, immunosuppression and recent surgery are particularly at risk of developing bacteremia with anaerobic organisms. Accurate and early identification of anaerobic bacteremia is crucial as these infections are associated with high mortality. In this study we have assessed the spectrum of bacteremic anaerobic isolates and compared their associated risk factors and susceptibility pattern with non-bacteremic anaerobic strains. These associations were also determined in Bacteroides vs. non-Bacteroides bacteremia.

Material and Methods
This study was conducted in Aga Khan University Clinical Laboratory. All the anaerobes isolated from blood and other sources during two study periods (2010-2011) and (2014-2017) were included. Anaerobes were identified and MICs of metronidazole were determined. Association of risk factors such as intra-abdominal infections, malignancy, liver disease, diabetes mellitus and infective endocarditis with bacteremic and non bacteremic anaerobes was evaluated. Similar associations were also determined for *Bacteroides* and non *Bacteroides* bacteremia.

Results
A total of 72 isolates were isolated from blood and 155 from other sources during the two study periods. Over all 29% (21/72) bacteremic and 16.1% (25/155) of non-bacteremic isolates were resistant to metronidazole (*p* value = 0.239). Odd of developing anaerobic bacteremia was significantly higher in patients with intra-abdominal infections and liver disease. The odds of developing bacteremia were 3.6 times higher in elderly patients. Patients with anaerobic bacteremia had almost 7 times higher mortality rate than non-bacteremia cases. Metronidazole resistance was found to be significantly associated with *Bacteroides* bacteremia.

Conclusion
This study shows that anaerobes are an important cause of bacteremia especially in cases with intra-abdominal infections, with liver disease and old age. It is associated with higher rates of mortality if not treated appropriately. Proper empirical therapy should be given to patients with these risk factors.

Key Words
anaerobic bacteremia, risk factors, *Bacteroides* bacteremia, non-*Bacteroides* bacteremia

Introduction
Anaerobic bacteremia usually occurs secondarily to intra-abdominal, pelvic or skin and soft tissue infections.[1] Patients with malignancy, immunosuppression and recent surgery are particularly at risk of developing bacteremia with anaerobic organisms.[1] Accurate and early identification of anaerobic bacteremia is crucial as these infections are associated with high mortality.[1-6] Studies have suggested selective use of anaerobic blood culture bottles for diagnosis, source control and empirical management based on clinical judgment to decrease morbidity and mortality associated with these infections.[7-8]

Isolation and susceptibility testing of anaerobic organisms in laboratories with limited resources is challenging.[9] Since anaerobic bacteremia is associated with mortality; early diagnosis and prompt appropriate empirical therapy is necessary. For the start of adequate empirical therapy, it is essential to identify the patients at risk for anaerobic bacteremia and *Bacteroides* bacteremia.

Limited data on anaerobic infections including bacteremia from Pakistan is available. Previous data from our laboratory based in Karachi reported high metronidazole and clindamycin resistance in anaerobic organisms. The resistance rate to of metronidazole resistance was even higher in cases of bacteremia.[10] Most of the time in Pakistan, patients at risk of anaerobic infections are treated empirically with metronidazole. As survival rates of patients with anaerobic bacteremia treated with inappropriately treatment are reported to be significantly worse, empirical treatment with metronidazole could potentially lead to poor outcome in our setting.[11]

In this study we have assessed the spectrum of bacteremic anaerobic isolates and compared their associated risk factors and susceptibility pattern with non-bacteremic anaerobic strains.

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These associations were also determined in *Bacteroides* vs. non-*Bacteroides* bacteremia.

Material and Methods

Setting: This study was conducted in Aga Khan University Clinical Laboratory based at Karachi, Pakistan.

Specimen selection: All the anaerobes isolated from blood culture and other sources from two periods (2010-2011) and (2014-2017) were included in the study. Duplicate isolates from the same patient were excluded.

Isolation and identification of anaerobes

Anaerobic organisms were isolated from blood cultures using BACTEC 9240 system. Anaerobic bottles that flagged positive in BACTEC 9240 were inoculated on sheep blood agar with 50 µg diagnostic metronidazole disk (Oxoid). Anaerobic organisms from samples other than blood were isolated directly from clinical specimens using standard method. The sample or broth was inoculated on sheep blood agar with a disk of diagnostic metronidazole (50µg Oxoid). Culture plates were incubated in an anaerobic chamber (Concept plus RUSKINN) for 48 h at 36 ± 1° C. Anaerobic organisms were identified by gram stain and colony morphology, aerotolerance, esculin hydrolysis, and API® 20A system (BioMerieux®, Marcy l' Etoile, France).

Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using Clinical Laboratory Standards Institute (CLSI) recommended agar dilution method during (2010-2011) and metronidazole MIC evaluator strips, E-test (Oxoid™, Thermo scientific™, Basingstoke, Hants, UK) during 2014-2017. MICs were read after 24 hours and at 48 hours in case of slow growing organisms. MICs were interpreted in both methods as sensitive (≤8 µg/ml) and resistant (≥16 µg/ml) using CLSI breakpoints.[12] *Bacteroides fragilis* ATCC 25285 was used as control.

Clinical data

Clinical details were collected as part of laboratory protocol and included patient demographics and presence of co-morbids such as intra-abdominal infections, malignancy, liver disease, diabetes mellitus and infective endocarditis. Clinical details of 56 cases of bacteremia and 155 cases of anaerobic infection from non-bacteremic sources (mostly intra-abdominal infections, brain abscess and skin and soft tissue infections) were used for comparison of risk factors and metronidazole susceptibility.

Statistical analysis

The data obtained was entered into the statistical software SPSS version 19.0 (SPSS, Inc., Chicago, IL). For descriptive analysis, mean and standard deviation of continuous variables such as age and MICs were determined. For categorical variables, e.g. gender and antibiotic resistance, frequencies and percentages were calculated. Association of risk factors with anaerobic bacteremia was determined using binomial logistic regression to obtain odds ratios with 95% confidence intervals (95%CI). Similarly, association of risk factors was also seen with *Bacteroides* and non-*Bacteroides* bacteremia using binomial logistic regression. A p-value less than 0.05 was considered significant.

Ethical Review

The study was provided an exemption of ethical approval by the Institutional Ethical Review Committee.

Results

A total of 72 anaerobes were isolated from blood cultures during the two study periods. Out of which 45 (62.5%) isolates were from male and 27 (37.5%) from female patients. There were 18 (25%) isolates from patients aged <18 years, 39 (54.2%) isolates from patients aged 18-60 years and 15 (20.8%) isolates from patients aged >60 years. Most commonly isolated organisms were *Bacteroides* species (n=37) and second most common isolated species were *Clostridium* species (n=25) (Table 1).

A total of 155 of anaerobes were isolated from sources other than blood. 67.1% (104/155) were male and 32.9% (51/155) were female. 17.4% (27/155) isolates were isolated from patients under 18 years, 67.1% (104/155) in between 18 and 60 years and 15.4% (24/155) more than 60 years. Most commonly isolated organism was *Bacteroides* species (n=126).

Antimicrobial susceptibility data

Over all 29% (21/72) anaerobic blood isolates and 16.1%. (25/155) of non-bacteremic isolates were resistant to metronidazole (Table 1). This resistance was higher in *Bacteroides* species where 18/38 (47.4%) strains were resistant compared with 3/34 (8.82%) of non-*Bacteroides* species.

Clinical data

Clinical details for 56/72 bacteremic patients are shown in table

Table 1: Frequency of anaerobes isolated from blood and their susceptibility pattern

Organisms	Frequency (n=72)	MIC Range	Metronidazole susceptible n = 51	Metronidazole resistant n=21
Bacteroides species	38	0.06->256	20	18
Clostridium species	25	0.06-36	24	1
Fusobacterium species	2	<0.015-0.12	2	0
Propionibacterium species	3	8- >256	2	1
Peptostreptococcus species	4	0.25- >256	3	1

2. Case fatality rate was 10/56 (17.8%). Despite a small sample size, anaerobic bacteremia was found to have significantly greater odds of occurring in patients with intra-abdominal infections and liver disease than in those without it (Table 2). The odds of developing bacteremia were 3.6 times higher in elderly patients. Patients with anaerobic bacteremia had almost 7 times higher mortality rate than non-bacteremia cases. Association of anaerobic bacteremia with metronidazole resistance was not statistically significant.

We also performed univariate and multivariate analysis of associated risk factors, outcome and metronidazole resistance with *Bacteroides* and non-*Bacteroides* bacteremia (Table 3). After adjusting for gender, metronidazole resistance was found to be significantly associated with *Bacteroides* bacteremia, with four times the odds compared with non-*Bacteroides* bacteremia. Association of other factors with *Bacteroides* bacteremia did not reach statistical significance.

Discussion

This study evaluated spectrum, associated risk factors and outcomes of anaerobic bacteremia and their susceptibility to metronidazole, one of the commonest agents used to treat anaerobic infections in Pakistan. In addition, comparison of *Bacteroides* versus non-*Bacteroides* bacteremia was also performed.

Our findings are consistent with studies that have shown association of anaerobic bacteremia with intra-abdominal infections, liver disease and advanced age.[6,13,14] However as previously reported in several studies, we could not determine significant association between malignancy and anaerobic bacteremia.[2] As depicted in other studies, current study showed a higher mortality rate in patients with anaerobic bacteremia versus non-bacteremia cases.[1,2,4,6,15]

The rates of anaerobic bacteremia vary regionally; the odds

Table 2: Univariate and multivariate analysis of demographics and associated risk factors with anaerobic bacteremia

			Univariate analysis		Multivariate analysis	
Risk factors	**Total (211) N**	**Anaerobes isolated**	**OR (95% CI) from blood (56)**	**p value**	**OR (95% CI)**	**p value**
Age						
<18years	32	5 (15.6)				
18-60 years	139	35 (25.2)	1.82 (0.65- 5.08)	0.255		
>60 years	40	16 (40.0)	3.60 (1.15-11.3)	**0.028**	3.41 (0.98-11.8)	0.053
Gender						
Male	137	33 (24.1)				
Female	74	23 (31.1)	1.42 (0.76-2.67)	0.273		
Diabetes Mellitus	29	08 (27.6)	1.06 (0.44-2.56)	0.891		
Malignancy	13	05 (38.5)	0.55 (0.174-1.77)	0.321		
Intra-abdominal infections	74	28 (37.8)	0.41 (0.22-0.76)	**0.005**	2.375 (1.20-4.689)	**0.013**
Infective endocarditis	02	02 (100)	*	0.999		
Liver disease	05	04 (80)	11.85 (1.29-108.4)	**0.029**	11.5 (1.18-112.1)	**0.035**
Expired	15	10 (66.7)	6.55 (2.12-20.1)	**0.001**	6.81 (2.10-22.0)	**0.001**
Metronidazole resistance	38	13 (34.2)	0.63 (0.29-1.35)	0.239		

*Value too small. Not computed

being higher in patients having intra-abdominal infections and undergoing gastrointestinal surgery.[6] The patients who present with intra-abdominal infections should be treated empirically with anti-anaerobic antimicrobial agent till susceptibility is available, to reduce the rate of anaerobic bacteremia and the mortality associated with it. However, our study lacks data of empirical and targeted therapy and their associated outcomes. As recommended by the IDSA guidelines for the diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the surgical infection society and infectious diseases society of America, patients admitted with complicated intra-abdominal infections should be given appropriate antimicrobial therapy with adequate anaerobic coverage and the choice of therapy should be based on regional antibiogram.[16] Bacteremia with *Bacteroides* was found to be associated with metronidazole resistance (table 3), while overall anaerobic bacteremia was not (table 2). Bacteroides bacteremia was not associated with mortality (p=0.174). Our data shows association of anaerobic bacteremia with metronidazole resistance and mortality, so a second line agent should be ideally recommended.

Very few studies have evaluated association of metronidazole resistance to bacteremia cases, possibly due to low resistance rates. In one study only 2% of 51 blood isolates had metronidazole resistance.[17] In a previous study conducted in Pakistan the rate of metronidazole non-susceptibility of bacteremic anaerobic isolates was higher than non-bacteremic isolates.[10] Our study showed that 29% of the bacteremia isolates were resistant to metronidazole, mandating regular susceptibility testing of anaerobes. Also, the rising resistance trends of metronidazole warrant exploration of other safe therapeutic options.

One of the major limitations of our study is that we have analyzed a small number of anaerobes isolated from blood. Since anaerobic bacteremia is rare, data from previous study was also included to increase sample size, which might have contributed to some bias as the methodology of susceptibility testing during the two study periods differed. Previously we

Table 3: Univariate and multivariate analysis of demographics, associated risk factors and metronidazole resistance with *Bacteroides* and non *Bacteroides* bacteremia. Adjusting for gender, metronidazole resistance was significantly associated with *Bacteroides* bacteremia, despite the small sample size.

		Univariate analysis			Multivariate analysis		
Risk factors	**Total (56) N (%)**	***Bacteroides* Bacteremia (28)**	**OR (95% CI)**	**p value**	***Bacteroides* Bacteremia OR (95% CI)**	**Non *Bacteroides* Bacteremia OR (95% CI)**	**p value**
Age							
<18years	5 (8.9)	2 (7.14)					
18-60 years	35 (62.5)	18 (64.3)	0.67 (0.09-5.13)	0.697			
>60 years	16 (28.6)	8 (28.6)	1.06 (0.32-3.45)	0.925			
Gender							
Male	33 (58.9)	20 (71.4)					
Female	23 (41.1)	8 (28.6)	0.35 (0.11-1.05)	0.061	0.37 (0.12-1.16)-1.30)	2.47 (0.77-7.97)	0.088
Diabetes Mellitus	8 (14.3)	3 (10.7)	0.55 (0.12-2.57)	0.449			
Malignancy	5 (8.93)	2 (7.14)	0.64 (0.09-4.12)	0.641			
Intra-abdominal infections	28 (50)	17 (60.7)	2.25 (0.76-6.61)	0.141			
Infective endocarditis	2 (3.57)	1 (3.57)	1.00 (0.06-16.8)	1.000			
Liver disease	4 (7.14)	3 (10.7)	3.24 (0.32-33.2)	0.322			
Expired	10 (17.8)	7 (25)	2.78 (0.64-12.1)	0.174			
Metronidazole resistance	13 (23.2)	10 (35.7)	4.63 (1.11-19.2)	**0.035**	4.38 (1.01-18.82)	0.23 (0.052-0.99)	**0.047**

used agar dilution while this time we used MIC evaluator strips; however, both were standardized methodologies according to CLSI guidelines and internal validity ensured by concurrent use of quality controls.

Secondly, this is a laboratory based data from a single center and the risk factors and rates will differ with the type of facility, such as the chances will be higher in health care facilities dealing with abdominal surgeries or cases with skin and soft tissue infections rather than those dealing with orthopedic surgeries may have lesser cases. Thirdly we have not discussed empirical and targeted therapy outcomes.

In summary, this study shows that anaerobic bacteremia is of grave clinical concern due to its higher occurrence in frail populations including elderly, liver disease patients and those with intra-abdominal infections. It is associated with greater mortality if not treated appropriately. Higher rate of metronidazole resistance has been observed in *Bacteroides* bacteremia. The findings of this study need to be confirmed by prospective cohort studies. The results if corroborated may provide enough evidence to warrant empirical therapy with second line anti-anaerobic agents such as imipenem, in elderly patients with intra-abdominal infections and liver disease, resulting in a decrease in mortality.

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