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High rate of non-susceptibility to metronidazole and clindamycin in anaerobic isolates: Data from a clinical laboratory from Karachi, Pakistan

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ABSTRACT

Due to increasing resistance amongst anaerobic pathogens periodic surveillance of resistance has been recommended in regional/local settings. Anaerobic antimicrobial susceptibility testing is not routinely performed in many laboratories in Pakistan, hence absence of local data may lead to inappropriate empirical therapy in serious cases. 121 clinically significant anaerobic strains (26/121; 21% bacteremic isolates) were isolated and saved from 2010 to 2011. Susceptibility testing against metronidazole, clindamycin, co-amoxiclav, meropenem, piperacillin/tazobactam, linezolid and gatifloxacin was performed by determining minimum inhibitory concentrations (MICs). A high proportion of non-susceptible strains to metronidazole (10% of 121 isolates) and clindamycin (12% of 121 isolates) was seen, most noticeable in *Bacteroides fragilis*. Three *Bacteroides species* strains were non-susceptible to both metronidazole and clindamycin. One strain of *Clostridium species* was fully resistant to metronidazole and had intermediate resistance to clindamycin. No resistance to any of the other tested antibiotics was seen. Resistance to metronidazole was higher in bacteremic vs. non bacteremic isolates (p = value 0.07). In our setting where there is a high usage of empirical metronidazole and clindamycin for the treatment of serious anaerobic infections clinicians should be aware of increased resistance to these agents. Periodic surveillance of resistance to anti-anaerobic drugs especially metronidazole and clindamycin should be performed to generate antibiogram and guide appropriate empiric therapy.

1. Introduction

Anaerobic bacteria result in a variety of serious and life threatening infections [1]. The most commonly reported anaerobes are *Bacteroides* spp., *Fusobacterium spp., Porphyromonas spp., Prevotella* spp and *Clostridium* spp [2]. The prevalence of antimicrobial resistance amongst anaerobic organisms has been identified as a "worrisome development" since last two decades [3]. Recent reports of emergence of carbapenem resistance in anaerobic bacteria are even more alarming [4,5]. Resistance rates to various antimicrobials used to treat anaerobic infections vary widely among different geographic regions and institutions [4–8]. This emerging resistance has been reported to be associated with treatment failure and increased mortality [9]. Due to difficulty in the isolation and complexity of antimicrobial susceptibility, many clinical laboratories either do not routinely perform antimicrobial susceptibility testing of anaerobic organisms or use inaccurate methods like disc diffusion [6,10]. Surveillance of resistance in these settings is therefore problematic. Therefore the available data on antimicrobial resistance of anaerobic organisms is limited and often is not available to clinicians to guide patient's management. Clinical and Laboratory Standards Institute (CLSI) working group on anaerobic susceptibility testing recommends periodic monitoring of resistance from different setting and generate antimicrobial resistance data in various geographic areas to determine changing susceptibility profiles [11]. It is also recommended to test individual patient isolate in invasive life threatening infections, treatment failure cases or when a prolonged therapy is anticipated. This data will be critical in resource limited setting where inappropriate use of anti-anaerobic antimicrobials is also common.

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We therefore, determined antimicrobial resistance pattern of anaerobic organisms isolated from patients with bacteremia and other clinically significant infections to establish resistance data for local isolates. This is the first study from Pakistan performed to determine resistance in anaerobic organisms using a standard method.

2. Material and methods

This study was conducted in clinical microbiology laboratory of the Aga Khan University (AKU), Karachi Pakistan. AKU is a tertiary care center and its microbiology laboratory receives clinical samples from both hospitalized and outpatients through satellite collection points located in all major cities and towns of Pakistan.

During the study period anaerobic organisms were isolated by direct inoculation of specimen on sheep blood agar (SBA) as well as after 24 hours enrichment in cooked meat broth. Anaerobic culture plates were incubated in an anaerobic chamber (Concept plus RUSKINN) for 48 h at 36 ± 1 °C with a disk of diagnostic metronidazole (50ug Oxoid). Identification of each isolate was confirmed using API 20A system (bioMérieux).

Antibiotic powders (metronidazole, clindamycin, co-amoxiclav, meropenem, piperacillin/tazobactam, linezolid and gatifloxacin) were obtained from Sigma Chemicals, St. Louis, MO. Antibiotic powders were reconstituted with CLSI recommended diluents to yield stock solutions [11]. Stock solutions once made, were kept frozen. Antimicrobial susceptibility was tested by the CLSI recommended agar dilution method, serial two-fold dilutions of antimicrobial agents were prepared on the day of the test and added to the recommended *Brucella* agar supplemented with Hemin, Vitamin K1 and 5% pooled sheep blood at various concentrations [11]. Antibiotic dilutions were prepared according to break points with at least four fold dilutions below the lower cut off and four fold above the upper cut off. An inoculum of 10^5 cfu/spot was inoculated onto the agar plates with control plates without antimicrobial agents before and after each set of drug-containing plates. Plates were incubated in an anaerobic chamber (Concept plus RUSKINN) for 48 h at 36 ± 1 °C. The control strains tested included *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741. Due to unavailability *Clostridium difficile* ATCC 700057 was not tested. MIC breakpoints were adapted and interpreted as sensitive, resistant or intermediate as per CLSI criteria (except for linezolid and gatifloxacin for which breakpoints are not available). For the purpose of this study strains that fell in either resistant or intermediate category were labeled as non-susceptible strains. The drug dilutions at which 50% and 90% of tested isolates got inhibited were recorded as MIC_{50} & MIC_{90} respectively as per standard methods [17]. The study was approved by Ethical Review Committee of Aga Khan University Hospital Karachi, Pakistan.

2.1. Statistical analysis

The data was entered into SPSS (Statistical Package for Social Sciences) version 19 software for statistical analysis. For descriptive analysis, mean and standard deviation of continuous variable such as age were reported. For categorical variable like gender and antibiotic resistance, frequencies and percentage were calculated. For bacteremic isolates, variable like age, sex, underlying disorder, source of bacteremia were separately analyzed. Resistance pattern and outcome of treatment in bacteremic patient were reported. *p-values* were calculated using chi-square test and a *p-value* of <0.05 was considered as significant.

3. Results

During the study period (2010–2011), a total of 121 isolates of obligate anaerobic bacteria were isolated. Clinical information for the patients was obtained as routine laboratory practice and the isolates obtained from clinically significant cases were included in the study. Non-significant isolates/colonizers/contaminants were excluded. The isolates were yielded from complicated intra-abdominal infections (17 cases), blood (26 cases), brain abscess (3 cases), synovial fluid (1 case), surgically collected sterile tissue (14 cases) and complicated skin and soft tissue infections (60 cases). 60% of the cases were mono-microbial and 40% were poly-microbial. Among co infecting organism *E. coli* was isolated in 7.5% followed by *Streptococcus milleri* in 7.2% of the cases. Of the infected patients, 82 (68%) were male and 39 (32%) were female and mean age of the patients was 36 years (Range 3–89 years). Table 1 shows frequency of various anaerobes isolated during the study period with *Bacteroides species* and *Clostridium species* as frequently isolated organisms.

3.1. Bacteremia patients

Of the 26 patients with a positive blood culture for anaerobic organisms, 16 (61.5%) were male and 10 (38.5%) were female. Mean age of the patients was 44 years (Range 3–84 years). Nine patients had history of admission to a critical care unit. The underlying risk factor in these patients included malignancy (8 cases), recent abdominal surgery or trauma (6 cases), aplastic anemia (3 cases), fracture (2 cases), pneumonia (1 case), puerperal sepsis (1 case). No risk factor was identified in 5 cases. The organisms responsible for bacteremia were *Bacteroides fragilis* (9 cases), other Bacteroides species (5 cases), *Clostridium species* (9 cases) and *Peptostreptococcus species* (3 cases). Overall case fatality ratio was 9/26 (35%).

Table 1
Frequency of isolation of anaerobes during the study period (2010–2011).

Organisms	Frequency (n = 121)
Bacteroides spp	67
- *Bacteroides fragilis*	39
- *Bacteroides ovatus*	5
- *Bacteroides thetaiotaomicron*	3
- *Bacteroides merdae*	2
- *Bacteroides rumin brevis*	1
- *Bacteroides uniformis*	1
- *Bacteroides vulgatus*	1
- *Bacteroides capillosus*	1
- *Bacteroides* spp (Not identified further)	14
Clostridium spp	32
- *Clostridium bifermentans*	7
- *Clostridium perfringes*	3
- *Clostridium butyricum*	2
- *Clostridium septicum*	2
- *Clostridium paraputrificum*	1
- *Clostridium sordelli*	1
- *Clostridium tertium*	1
- *Clostridium* spp (Not identified further)	15
Peptostreptococcus spp	14
Provetella spp	6
- *Provetella bivia*	4
- *Provetella oralis*	2
Fusobacterium varium	1
Eubacterium lentum	1