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Clinical characteristics and antimicrobial susceptibilities of anaerobic bacteremia in an acute care hospital

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ABSTRACT

This study investigated the clinical features of anaerobic bacteraemia in an acute-care hospital, and evaluated the antimicrobial susceptibility of these isolates to commonly available antibiotics.

Microbiological and epidemiological data from 2009 to 2011were extracted from the laboratory information system and electronic medical records. One hundred and eleven unique patient episodes consisting of 116 anaerobic isolates were selected for clinical review and antibiotic susceptibility testing. Susceptibilities to amoxicillin-clavulanate, clindamycin, imipenem, metronidazole, moxifloxacin, penicillin and piperacillin-tazobactam were performed using Etest strips with categorical interpretations according to current CLSI breakpoints. Metronidazole-resistant and carbapenem-resistant anaerobic Gram-negative bacilli were screened for the *nim* and *cfiA* genes. Clinical data was obtained retrospectively from electronic medical records.

During the 3 year period, *Bacteroides fragilis* group (41%), *Clostridium* species (14%), *Propionibacterium* species (9%) and *Fusobacterium* species (6%) were the most commonly isolated anaerobes. Patients with anaerobic bacteraemia that were included in the study were predominantly above 60 years of age, with community-acquired infections. The most commonly used empiric antibiotic therapies were beta-lactam/beta-lactamase inhibitor combinations (44%) and metronidazole (10%). The crude mortality was 25%, and appropriate initial antibiotic therapy was not significantly associated with improved survival. Intra-abdominal infections (39%) and soft-tissue infections (33%) accounted for nearly three-quarters of all bacteraemia.

Antibiotics with the best anaerobic activity were imipenem, piperacillin-tazobactam, amoxicillin-clavulanate and metronidazole, with in-vitro susceptibility rates of 95%, 95%, 94% and 92% respectively. Susceptibilities to penicillin (31%), clindamycin (60%) and moxifloxacin (84%) were more variable. Two multidrug-resistant isolates of *Bacteroides* species were positive for *nim* and *cfiA* genes respectively, while another two imipenem-resistant *Fusobacterium* species were negative for *cfiA* genes.

This study demonstrated that anaerobic bacteraemia in our patient population was predominantly associated with intra-abdominal and soft-tissue infections. Overall antibiotic resistance was high for penicillin and clindamycin, and the presence of emerging resistance to carbapenems and metronidazole warrants further monitoring.

1. Introduction

Anaerobic bacteraemia continues to account for a small, but significant, proportion of bacteraemia (around 7–10% of all bacteraemic episodes) [1,2]. Common sources of infection include the gastrointestinal tract, abscesses, gynaecologic sources and soft tissue infections [3], while reported risk factors for anaerobic bacteraemia include gastrointestinal surgery and haematologic malignancy [4].

Antibiotic therapy for anaerobes is usually empiric. Anaerobic susceptibility testing is not performed routinely, as it is expensive, methodologically demanding and time consuming. Based on

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published reports, the prevalence of antibiotic resistance in anaerobes is increasing [5,6]. Clinical data suggest that antibiotic resistance impacts both microbiological cure and patient mortality for patients with anaerobic bacteraemia [7]. There is little susceptibility data for anaerobic infections in Singapore, and no data exists for the clinical characteristics of patients with 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 clinical data for patients with anaerobic bacteraemia over a three-year period, and to determine antibiotic susceptibilities for these anaerobic isolates.

2. Methods

2.1. Retrospective microbiological data extraction

Bacterial information and epidemiological data were extracted from the laboratory information system for the period 2009–2011. Duplicate isolates from the same patient (defined as similar isolates within a 30 day period) were excluded from analysis. During the study period, routine aerobic and anaerobic blood cultures were performed using BACTEC™ Plus Aerobic/F and Plus Anaerobic/F vials (BD, USA), incubated for five days in a continuous monitoring blood culture system (Bactec 9000 series, BD, USA). Anaerobic vials with positive growth indices were sub-cultured on to trypticase soy agar with 5% sheep blood (BD, USA), MacConkey agar (Oxoid, UK), Chocolate agar (BD, USA) and CDC anaerobe agar with 5% sheep blood (BD, USA). Media were routinely incubated for 2 days, with additional incubation for up to 4 days if a slow-growing anaerobe was suspected. Gram-stain and aero-tolerance testing were performed for suspected anaerobic isolates, with speciation by Vitek® ANC identification cards tested on the Vitek® Compact system (bioMérieux, France), supplemented with API 20A (bioMérieux, France) where necessary.

Detailed clinical and bacteriological information was retrospectively obtained for 111 unique patient episodes. These cases were randomly chosen to represent the most common clinically significant anaerobic isolates from blood cultures. *Propionibacterium* species, anaerobic Gram-positive cocci, and nonsporulating anaerobic Gram-positive rods were excluded from detailed analysis, as previous studies have demonstrated that these genera are much less likely to be clinically significant [3,8,9].

2.2. Antimicrobial susceptibility testing

For the 111 unique patient episodes, 116 anaerobic isolates were retrieved from cryo-storage for antibiotic susceptibility testing. Bacterial identification was re-confirmed by MALDI-TOF testing (Vitek MS database 2.0, bioMérieux, France) prior to susceptibility testing. Susceptibilities to amoxicillin-clavulanate, clindamycin, imipenem, metronidazole, moxifloxacin, penicillin and piperacillin-tazobactam were performed using Etest antimicrobial strips on Brucella agar with 5% sheep blood, vitamin K and hemin (BD, USA). Overnight pre-reduction of testing media was performed prior to testing, and following inoculation, plates were incubated in an anaerobic workstation system at 35 °C (Forma Anaerobic System, ThermoFisher Scientific, USA) in an anaerobic atmosphere comprising 5% hydrogen, 5–10% carbon dioxide and 85–90% nitrogen. Concurrent quality control (QC) testing was performed using *Bacteriodes fragilis* ATCC 25285 according to manufacturer's guidelines, and QC values were within acceptable limits. Etest results were read after 48–72 h incubation, with all except one *Fusobacterium* isolate achieving satisfactory growth endpoints at 48 h. Antibiotic susceptibilities were interpreted according to existing CLSI breakpoints [10]. Metronidazole-resistant and carbapenem-resistant anaerobic Gram-negative bacilli were screened for *nim* and *cfiA* genes encoding 5-nitroimidazole resistance and carbapenem resistance respectively, by conventional PCR [11,12].

2.3. Clinical data extraction and analysis

Clinical data collection for each patient episode of anaerobic bacteraemia was obtained retrospectively from electronic medical records. Data collected included the admitting and final discharge diagnosis, empiric antibiotic prescription, results of clinical investigations and inpatient mortality. The presence of polymicrobial bacteraemia was defined as the presence of other bacterial isolates from blood cultures collected on the same day as the primary anaerobic isolate. The Charlson co-morbidity scores were calculated for each patient using an electronic spreadsheet [13], while statistical analysis and odds ratios were calculated using OpenEpi (online open source statistical software) [14] supplemented with SPSS Statistics (IBM, USA).

3. Results

3.1. Anaerobic bacteraemia over the three year period

During the 3 year period, 250 obligate anaerobes were recovered from blood cultures, representing 4.1% of all positive blood cultures. The most frequent anaerobic isolates were the *Bacteroides fragilis* group (n = 102; 41%), *Clostridium* species (n = 34; 14%), *Propioniobacterium* species (n = 23; 9%) and *Fusobacterium* species (n = 16; 6%). The remainder of the isolates included *Prevotella* spp., *Veillonella* spp., *Eubacterium* spp., *Actinomyces* spp., *Peptostreptococcus* spp., *Lactobacillus* spp., *Parvimonas* and *Porphyromonas* spp. Twenty three isolates could not be identified by phenotypic tests, consisting predominantly of anaerobic Gram-positive bacilli.

3.2. Detailed clinical information on subset of 111 patients

For the detailed subset of 111 study patients with anaerobic bacteraemia, the average age was 73 years (range: 21–99 years). Patients older than 60 years of age accounted for 84% of cases. Most patients (n = 89, 80%) were admitted from home, with a further 21 (19%) patients admitted from a long-term residential care facility. The majority (87%) of anaerobic bacteraemia were reported from blood cultures taken within two days of hospital admission. The majority of patients were reported as independently mobile (n = 70, 63%), while 24 (22%) patients were reported to be bed-bound. The average Charlson co-morbidity score was 2.6 (range 0–12), while the average age-adjusted score was 5.1 (range 0–15). Information on antibiotic therapy was available for 97 patient episodes. The most commonly used empiric antibiotic therapy were beta-lactam/beta-lactamase inhibitor combinations (amoxicillin/clavulanic acid or piperacillin/tazobactam) (n = 49, 44%), metronidazole (n = 11, 10%) and carbapenems (n = 8, 8%). Eighteen patients (16%) were on antibiotics with no activity against anaerobic bacteria, and two patients had no antibiotic coverage. The majority of patients were started on initial appropriate antibiotic therapy (n = 72, 65%). The crude mortality was 25%, with a higher mortality risk for patients with infections due to an extra-abdominal source (odds ratio 0.3, 95%CI 0.12–0.92), increasing age (increased risk of 3.7% per year, 95%CI 0.2–7.3%) and increasing adjusted Charlson co-morbidity score (odds ratio 1.2, 95%CI 1.0–1.3) on univariate analysis. However, on multivariate analysis, the increased risk of mortality remained statistically significant only for the source of