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Bacteriological profile and resistance pattern of the microorganisms causing bacteremia with special emphasis on unusual blood isolates

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Abstract

Introduction: Bacteremia is a serious infection with high morbidity and mortality. Knowledge of the etiological agents of blood-stream infections and their antibiotic resistance pattern could help in the choice of empirical antibiotics.

Aim: To determine the causative agents of blood stream infection along with their antibiotic resistance pattern in our hospital between 2013 and 2016 and also to identify the uncommon blood culture isolates.

Materials and Method: A retrospective study that included isolates obtained from blood culture between January 2013 and December 2016. The identification and drug resistance pattern of the organisms were performed using standard microbiological techniques. For those patients whose blood yielded uncommon isolates, the medical records were looked into to identify the risk factors and outcome.

Results: Of a total of 9,926 blood samples received for culture, 534 (5.38%) yielded positive results, of which 277 were Gram-positive organisms and 257 were Gram-negative organisms. The most common etiological agents of bacteremia in the present study were Coagulase Negative Staphylococcus species (37.27%) followed by Salmonella typhi (10.11%), Escherichia coli (9.36%) and Staphylococcus aureus (8.05%), of which 53.49% were MRSA.

Conclusion: The most effective antibiotics against Gram positive organisms were vancomycin, teicoplanin and linezolid. The Gram negative organisms showed least resistance to antibiotics like amikacin, tobramycin and carbapenems. The uncommon isolates were all non-fermenting Gram negative bacilli and among these Burkholderia species and Stenotrophomonas maltophila were the most frequently isolated.

Keywords: Bacteremia, Blood culture, Pathogens, Automated blood culture, Antibiotic resistance, Unusual isolates

Introduction

Bacteremia refers to the presence of bacteria in blood and is generally considered a serious infection with high morbidity and mortality rates.(1) The number of Multi-drug Resistant (MDR) organisms responsible for bacteremia has been on the rise in recent years.(2) Bacteremia caused by unusual pathogens is also being increasingly reported due to better identification tools and newer culture techniques. These uncommon isolates add to diagnostic dilemma due to their widespread distribution in the environment and drug resistance pattern.(1) As the treatment in most cases is started empirically before the confirmation by culture, knowledge of the type of organisms isolated in blood culture along with the sensitivity pattern could serve as a useful guide for clinicians in management of patients with bacteremia. Hence this study was undertaken to determine the causative agents of blood stream infections along with their antibiotic resistance pattern in our hospital between 2013 and 2016. The study would also help to identify the uncommon blood culture isolates obtained in the last four years and the epidemiology, risk factors and outcome of these patients.

Materials and Method

The study was a retrospective, descriptive study that included the isolates obtained from blood culture during a three year period from January 2013 to December 2016 at Chettinad Hospital and Research Institute, a tertiary care teaching hospital in the Kanchipuram district of Tamil Nadu in India. Blood from clinically suspected cases of bacteremia was sent to the Microbiology lab and was inoculated into either the conventional Brain Heart Infusion broth or into the automated BACTEC 9050 method (Beckton Dickinson BD) as per the request of the clinician. In the conventional method, blood culture broth was subcultured on Blood agar and MacConkey agar after 24 hours, 72 hours and after 7 days of incubation at 37^0C. If growth was obtained in the subculture plates, the organisms were identified by standard biochemical tests and Gram staining of the broth. The antimicrobial sensitivity pattern was determined by the Kirby Bauer disc diffusion method and interpreted according to the Clinical Laboratory and Standards Institute (CLSI) 2013 guidelines.(3) Methicillin Resistant Staphylococcus aureus (MRSA) was detected by Cefoxitin disc (30µg disc) method. For detection of ESBL (Extended Spectrum Beta Lactamase), double disc method using Ceftazidime (30 µg) and Ceftazidime /Clavulanic Acid (30 µg/10 µg) and Cefotaxime (30 µg) and Cefotaxime/ Clavulanic Acid (30 µg/10 µg) was used. For carbapenem resistant Gram negative bacilli, Modified Hodge test was performed to detect Carbapenemase production. Minimum Inhibitory

Concentration (MIC) was determined using E-strip for Vancomycin, Imipenem, Meropenem, Colistin and Polymyxin B.

In the BACTEC method, a positive beep was taken as an indication of growth in culture and the BACTEC negative bottles were also routinely subcultured after 5 days for confirmation. The positive BACTEC bottles were removed from the machine and smears made from the broth and were stained by Gram staining. Then subcultures were made on blood agar, chocolate agar and MacConkey agar. After primary isolation of the organism, the emulsified colonies were inoculated into Vitek-2 Compact (bioMerieux), an automated system designed to provide identification upto the species level and antimicrobial sensitivity testing results along with Minimum Inhibitory Concentration (MIC) values of the recommended antibiotics. The VITEK system accommodates colorimetric reagent cards that are incubated and interpreted automatically. Pure culture of the organism to be tested is suspended in Vitek saline, uniformly vortexed and density adjusted using by Densi check Plus provided by bioMerieux. The inoculum is finally transferred into ID and AST tubes and the inoculum cassette is loaded into the Vitek 2 Compact instrument.

For those patients whose blood yielded uncommon isolates, the medical records of these patients were looked into to give information regarding risk factors and outcome.

Results

A total of 9,926 blood samples were received for culture during the period from January 2013 to December 2016, of which conventional method was used for 7,229 samples and 2,697 were tested by BACTEC method. Positive cultures accounted for 534 of the total isolates (167 positive by BACTEC method and 367 positive by conventional method), positivity rate overall was 5.38%. The BACTEC had a slightly better rate of identification of positive cultures at 6.19% when compared to conventional cultures at 5.07%. The positive cultures were more among male patients (317) when compared to female patients (217). None of the positive cultures yielded polymicrobial growth. Of the total 534 positive cultures, 277 isolates were Gram-positive organisms and 257 were Gram-negative organisms. Among the 277 Gram positive bacteria, the predominant organisms were Coagulase negative *Staphylococcus* species (CoNS) followed by *Staphylococcus aureus, Streptococcus* species and *Enterococcus* species. The details of the organisms isolated are given in Table 1. All the *Staphylococcus aureus* and CoNS isolates were susceptible to Vancomycin, Teicoplanin and Linezolid. Among the *Staphylococcus aureus* isolates 53.49% were MRSA. (Table 2)

Among the 257 Gram negative isolates, the most common organisms were 175 bacterial isolates of the family Enterobactereciae including *Salmonella typhi*, *Salmonella paratyphi A, Escherichia coli, Klebsiella* species, *Proteus vulgaris* and *Serratia marcesens*. The Non-fermentative Gram negative bacteria accounted for the remaining 82 isolates. Among these, the common non-fermenting Gram negative bacilli were *Pseudomonas* species and *Acinetobacter* species. (Table 1/Fig. 1)

The other uncommon non-fermenting Gram negative bacilli included eight isolates of *Burkholderia* species, four isolates of *Stenotrophomonas maltophila*, two isolates of *Brevundimonas dimunita*, two isolates of *Chryseobacterium* species, one isolate of *Ralstonia picketti* and one isolate of *Sphingomonas* species. The above isolates were identified by standard biochemical tests and confirmed by Vitek 2 compact. The antibiogram of the predominant Gram-positive organisms and Gram-negative organisms are given in Table 2/Fig. 2 and Table 3/Fig. 3 respectively.

Table 1/Fig. 1: Distribution of the organisms isolated in blood culture

Organism	Number	Percentage
Gram Negative Organisms	257	48.13
Salmonella typhi	54	10.11
Escherichia coli	50	9.36
Pseudomonas species	36	6.74
Klebsiella species	33	6.18
Acinetobacter species	28	5.24
Salmonella paratyphi A	27	5.06
Burkholderia species	8	1.50
Enterobacter species	7	1.31
Stenotrophomonas maltophila	4	0.75
Citrobacter species	2	0.37
Brevundimonas dimunita	2	0.37
Chryseobacterium species	2	0.37
Proteus vulgaris	1	0.19
Serratia marcesens	1	0.19
Ralstonia picketti	1	0.19
Spingomonas species	1	0.19
Gram Positive Organisms	277	51.87
CoNS	199	37.27
Staphylococcus aureus	43	8.05
Enterococcus species	11	2.06
Streptococcus species	22	4.12
Streptococcus pneumoniae	1	0.19
Aerococcus species	1	0.19

Table 2/Fig. 2: Antibiogram of the predominant Gram-Positive isolates (% Resistance)

Antibiotic	CoNS (n=199)	*Staphylococcus aureus* (n=43)	*Enterococcus* species (n=11)	*Streptococcus* species (n=22)
Cefazolin	39.20	53.49	54.55	NT
Chloramphenicol	0.50	0.00	NT	NT
Ciprofloxacin	20.10	90.70	100.00	9.09
Clindamycin	21.61	39.53	NT	4.55
Cloxacillin	39.20	53.49	NT	NT
Cotrimoxazole	42.21	39.53	NT	13.64
Erythromycin	59.30	55.81	NT	22.73
Gentamicin	8.54	39.53	NT	0.00
Gentamicin (HL)	NT	NT	63.64	NT
Linezolid	0.00	0.00	0.00	0.00
Netilmicin	0.00	4.65	NT	0.00
Penicillin	63.82	90.70	54.55	0.00
Teicoplanin	0.00	0.00	9.09	0.00
Tetracycline	9.55	13.95	27.27	22.73
Vancomycin	0.00	0.00	9.09	0.00

(NT- Not tested)

Table 3/Fig. 3: Antibiogram of the predominant Gram-negative isolates (% Resistance)

Antibiotic	*Salmonella typhi* (n=54)	*Salmonella paratyphi* A (n=27)	*Escherichia coli* (50)	*Klebsiella* spp (n=33)	*Pseudomonas* spp (n=36)	*Acinetobac-ter* spp (n=28)
Amikacin	NT	NT	6.00	15.15	13.89	17.86
Ampicillin	1.85	0	86.00	100	NT	NT
Aztreonam	NT	NT	NT	NT	13.89	NT
Cefazolin	NT	NT	82.00	75.76	NT	NT
Cefepime	NT	NT	70.00	45.45	25	35.71
Cefotaxime	NT	NT	76.00	54.55	NT	53.57
Ceftazidime	NT	NT	NT	NT	19.44	NT
Ceftriaxone	0	0	NT	NT	NT	NT
Cefuroxime	NT	NT	82.00	72.73	NT	NT
Chloramphenicol	0	0	NT	NT	NT	NT
Ciprofloxacin	83.33	96.3	74.00	36.36	19.44	25
Colistin	NT	NT	2.00	3.03	2.78	3.57
Cotrimoxazole	3.7	0	64.00	54.55	NT	28.57
Gentamicin	NT	NT	52.00	39.39	30.56	25
Imipenem	NT	NT	2.00	9.09	8.33	21.43
Meropenem	NT	NT	6.00	12.12	8.33	10.71
Ofloxacin	NT	NT	NT	NT	11.11	NT
Piperacillin-Tazobactam	NT	NT	24.00	24.24	11.11	21.43
Polymyxin B	NT	NT	0.00	0	2.78	0
Tetracycline	0	0	NT	NT	NT	NT
Tobramycin	NT	NT	16.00	18.18	5.56	7.14

NT- Not tested

Discussion

In the present study, the positivity rate for blood culture was 5.38%, which is lower than other studies.[2,4] The distribution of Gram positive and Gram-negative isolates was comparable. In studies by Gohel et al.,[2] Karlwosky et al.,[5] and Wasihun et al.,[6] the causative agents of bacteremia were predominantly Gram positive whereas Gram negative organisms accounted for the majority in some other studies.[4,7] In the current study CoNS were the most common blood culture isolate contributing to 37.27%. This is similar to studies by Karlwosky et al.,[5] Wasihun et al.,[6] in which CoNS accounted for 42% and 30.6% respectively. MRSA rate in our study is 53.49% that is in accordance to a study done by Tariq TM[8] and Karlowsky et al.,[5] in which oxacillin resistance rate was 51% and 49.3% respectively. The *Staphylococci* were all susceptible to Vancomycin. All the *Streptococci* were uniformly sensitive to penicillin. There was one isolate of Vancomycin Resistant Enterococci (VRE), which was isolated from a newborn male from the Neonatal Intensive Care Unit (NICU).

Gram-negative organisms were responsible for 48.13% of bacteremia in this study. *Salmonella typhi* was the most common isolate accounting for 10.11% of the total isolates followed by *Escherichia coli* (9.36%), *Pseudomonas* species (6.74%) and *Klebsiella* species (6.18%). In studies by Mehta et al.,[4] Wasihun et al.,[6] and Tiwari et al.,[7] the incidence rate of *Salmonella typhi* was 12.87%, 5.6% and 6.25% respectively. Most of the *Salmonella typhi* (83.3%) and *S.paratyphi* A (96.3%) isolates were resistant to Ciprofloxacin. *Escherichia* coli was the predominant cause of Gram-negative bacteremia in studies by Gohel et al., (15.2%)[2] Wasihun et al.,(10.4%)[6] and Karlowsky et al.(7.2%).[5] The rate of isolation of *Klebsiella* (6.18%) in our study was lower than Gohel et al., (9.8%),[2] Mehta et al., (14.99%)[4] and Tariq TM (16.1%).[8] The isolation rate of *Pseudomonas* species in this study (6.74%) was higher than Tariq TM(5.12%),[8] Gohel et al., (5.3%)[2] and Karlowsky et al (2.5%).[5]

Gram negative organisms in the present study showed low sensitivity to commonly used penicillins, cephalosporins, fluoroquinolones. However, most isolates were sensitive to Amikacin and Carbapenems as reported in other studies.[2,8]

Reports of bacteremia due to unusual pathogens have been on the rise thanks to higher index of suspicion and better diagnostic techniques. All the uncommon blood culture isolates in this study were non-fermenting Gram-negative bacilli and were identified by the Vitek-2 Compact system. A total of eight isolates of *Burkholderia* species were obtained, of which three were ***Burkholderia cepacia*** and five were *B.pseudomallei*. *Burholderia* can be a common contaminant of soil and water and *B.cepacia* is an important opportunistic pathogen of cystic fibrosis patients.[9] *B.cepacia* has been linked to intrinsic contamination of medication vials[10] and even distilled water.[9] In our study, the three isolates of *B.cepacia* were isolated from three middle-aged males with sepsis, of which one patient succumbed to the infection. In one of the patients who survived, the same organism had been previously isolated from the patient's endotracheal aspirate implying spread from the lung. Meliodosis caused by ***B.pseudomallei*** can clinically manifest as simple skin and soft tissue infection or in its more severe form as pneumonia or sepsis.[11] Occurrence of pneumonia or bacteremia due to *Burkholderia pseudomallei* is associated with higher mortality rates.[11] In the present study, all the five *B.pseudomallei* isolates were from male patients including four middle-aged males and one from a young adult. All the isolates were sensitive to ceftazidime, co-trimoxazole and carbapenems and four out of five patients responded favorably to treatment. However, the disease was fatal in one patient who developed Multiorgan dysfunction syndrome.

Four isolates of ***Stenotrophomonas maltophila*** were obtained from blood, of which two were from children, one from a middle-aged female and another from a middle-aged male. *Stenotrophomonas* is a Gram-negative organism that is common present in the environment and its incidence in recent years has been increasing.[12] As the organism is intrinsically resistant to beta-lactams including carbapenems and aminoglycosides,[12,13] it has been known to cause break-through bacteremia[14] in patients already on carbapenems. *Stenotrophomonas maltophila* is a nosocomial pathogen especially in the immunosupressed[15] and the spectrum of infections caused by it includes pneumonia, urinary tract infections, bacteremia, skin and soft tissue infections.[12] Bacteremia due to *Stenotrophomonas maltophila* has been associated with increased morbidity and mortality and the important risk factors are hematological malignancies, neutropenia, central venous catheter, mechanical ventilation, hemodialysis, prolonged ICU stay and exposure to broad spectrum antibiotics like carbapenems.[12,16] Despite reports of drug resistance, the drug of choice remains to be co-trimoxazole.[17] In our study, all the four isolates were sensitive to co-trimoxazole. Both the children who had *Stenotrophomas* bacteremia did not have any of the above risk factors. The female patient whose blood culture yielded *Stenotrophomonas* was a diabetic and hypertensive with chronic kidney disease and had to undergo hemodialysis, was admitted in the ICU, underwent mechanical ventilation and ultimately developed Acute Respiratory Distress Syndrome, pericardial effusion and finally succumbed to the infection. The male patient with *Stenotrophomonas* bacteremia had risk factors of prolonged ICU stay and presence of central venous catheter. The patient however recovered with treatment.

Brevundimonas dimunita is a rare organism causing bacteremia. Only a few case reports exist and a majority of these infections have occurred in the setting of immunosuppression especially due to hematological malignancies. Very rarely, bacteremia due to *Brevundimonas* can occur without any predisposing factors.[18] In our study, one isolate was from an infant who was admitted with fever, vomiting, loose stools. The other was from a newborn with hyperbilirubinemia. Both babies recovered with antibiotics and supportive therapy. Most cases due to *Brevundimonas* are non-fatal because of susceptibility to most antibiotics including aminoglycosides, co-trimoxazole,[19] piperacillin-tazobactam and carbapenems.[20] They are however intrinsically resistant to fluoroquinolones.[21]

Chryseobacterium meningosepticum is a known cause of neonatal meningitis especially in premature infants and infections in adults comprises of pneumonia, bacteremia, endocarditis and meningitis especially in inmmunocompromised patients.[22,23] *Chyrseobacterium* species have been reported to cause bacteremia in patients with diabetic nephrophathy[23] and in end stage renal disease.[24] *Chyrseobacterium* is known to be a multidrug resistant pathogen with few treatment options. The organism is known to be resistant to beta-lactams including carbapenems, and vancomycin which was previously the drug of choice is no longer considered useful in treatment of this infection.[22,25] Co-trimoxazole and quinolones were found to be better alternatives.[22,25] In our study, one isolate was *Chryseobacterium meningosepticum* and it was isolated from a six month old female child with pneumonia with no predisposing factors. The other isolate was *Chryseobacterium indologenes* which was obtained from a middle aged female who was a diabetic, hypertensive and had chronic kidney disease for which hemodialysis was done. The patient's condition however worsened and she succumbed to the infection. The isolate of *C.meningosepticum* from the infant was a sensitive strain whereas *C.indologenes* in our study was resistant to all beta-lactams, was sensitive to co-trimoxazole and intermediate to ciprofloxacin.

Sphingomonas paucimobilis is a known waterborne pathogen[26] that has been found in natural environment as well as in hospitals.[27] It has been associated with community acquired or nosocomial infections including bacteremia, urinary tract infection, diarrhoea, peritonitis, meningitis, endophthalmitis etc.[26,28] This non-fermenting Gram negative bacteria is usually of low virulence but can cause septic shock in immuno-suppressed patients and may even be rarely fatal.[29] In the present study the organism was obtained from a female patient who was an old case of pulmonary tuberculosis with community-acquired pneumonia. The isolate was susceptible to cefepime, ciprofloxacin, co-trimoxazole, gentamicin, piperacillin –tazobactam and carbapenems and the patient responded favorably to treatment with piperacillin tazobactam.

Ralstonia picketti is another uncommon organism causing bacteremia in patients undergoing hemodialysis as result of contamination of medical solutions.[30,31] Contamination of medical solutions due to Ralstonia is believed to occur due its ability to withstand a wide range of temperatures (15-42^{0}C) and its ability to pass through filters of size 0.2 and 0.45 µm which is used to filter-sterilize solutions for medical use.[30] The major risk factors for *Ralstonia picketti* bacteremia are cystic fibrosis, central venous catheter use and immunosupression.[30] In our study a single isolate of Ralstonia picketti was obtained from a male patient who was a hypertensive with decompensated liver disease and acute kidney injury.

In conclusion, the most common etiological agents of bacteremia in our study were Coagulase Negative Staphylococcus species (37.27%) followed by *Salmonella typhi* (10.11%), *Escherichia coli* (9.36%) and *Staphylococcus aureus* (8.05%) of which 53.49% were MRSA. The most effective antibiotics against Gram positive organisms were vancomycin, teicoplanin and linezolid. The Gram negative organisms showed least resistance to antibiotics like amikacin, tobramycin and carbapenems. The uncommon isolates were all non-fermenting Gram negative bacilli and among these *Burkholderia* species and *Stenotrophomonas maltophila* were the most frequently isolated. A knowledge of the common and uncommon organisms isolated from blood culture and its drug sensitivity pattern would be useful for the clinicians to decide the empirical antibiotics in treatment of bacteremia in hospital ward and ICU settings.

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