Distribution of the invasive pathogenic isolates in blood culture with their antimicrobial susceptibility pattern in diagnostic lab in Karachi

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Abstract

Objective: To determine the frequent bacterial pathogens causing blood stream infections in various age brackets, and to discover their antibiotic susceptibility pattern.

Method: The retrospective, descriptive, observational, cross-sectional study was conducted at the microbiology laboratory of Patel Hospital, Karachi, and comprised positive blood culture bacterial isolates analysed between July 1, 2018, and June 30, 2019. Standard microbiological techniques were employed for the identification and antimicrobial susceptibility testing. Data was analysed using SPSS 20.

Results: Of the 3450 specimen, 1243(36%) were positive; 668(53.7%) from male and 575(46.3%) from female subjects, and gram-positive 771(62%) and gram-negative 472(38%). Salmonella typhi was the most common pathogen 139(11.1) among gram-negative organisms, followed by acinetobacter species 103(8.2%), escherichia coli 96(7.7%) and klebsiella species 42(3.4%). Among gram-positive bacteria, the predominant isolates were staphylococcus epidermidis 650(52%), staphylococcus aureus 67(5.4%) and enterococci 28(2.3%). Linezolid (99.8%), vancomycin (99%) and chloramphenicol (69%) were found to be the most sensitive antibiotics among
gram-positive cocci. Meropenem (60%), amikacin (46%) and gentamicin (40%) were
the most sensitive antibiotics for multidrug resistant gram-negative bacteria.

**Conclusion:** The identification of frequent bacterial pathogens in blood cultures of
patients may guide clinicians in proper empirical selection of antibiotics in patients
with bacteremia.

**Key Words:** Blood stream infection, BSI, Antibiotic susceptibility, AST,
Bacteriological profile, Multi-drug resistance, MDR.

**Introduction**

Blood stream infection (BSI) is a life-threatening condition despite the availability of
effective antimicrobial therapy and enhancement in substantive care. Early diagnosis
and appropriate treatment can reduce the complications associated with BSIs,
otherwise it would lead to extended hospitalisation, increased medical expenses and
higher mortality rate.\(^1\) The frequency of microorganism isolated and its antimicrobial
susceptibility pattern vary in different geographical areas due to variation in
antimicrobial prescribing and consumption practices.\(^2\) Therefore, awareness about
epidemiological trends and antimicrobial susceptibility patterns of common pathogens
in a given region helps in empirical selection of antibiotics.\(^3\) Antibiotic resistance
among bacteria causing BSIs is alarmingly increasing, as a result of irrational
antibiotic use, making common infections more challenging to treat, and ultimately
resulting in catastrophic outcomes for patients. Therefore, continuous surveillance on
blood culture isolates is required to monitor their susceptibility pattern and to
overcome the growing incidence of resistance to conventional drugs.

Both gram-positive and gram-negative bacteria can cause serious infections, but BSIs
due to gram-negative bacilli are more lethal and cause more serious consequences due
to the prevalence of multidrug resistant (MDR) strains among them.\(^4\) It is reported that
gram-negative bacteria are responsible for 25% of nosocomial and 45% of
community-acquired (CA) BSIs.\(^5\) Out of the gram-negative isolates, salmonella
species, escherichia (E.) coli, klebsiella (K.) species, and pseudomonas (P.) species are
the most commonly identified organisms. Moreover, the incidence of gram-positive organism is also increasing due to device-related infections. Coagulase negative staphylococcus (S.) and S. aureus constitute the most common causal agents of BSIs among gram-positive organisms.\textsuperscript{6}

There is a need to monitor the progress of antimicrobial stewardship programmes, that may assist in making policies to control antimicrobial resistance in a regulated way. Antimicrobial agents administered empirically based on hospital antibiogram can help lessen the spread of multidrug resistance as well as antibiotic abuse.\textsuperscript{8}

The current study was planned to determine the frequent bacterial pathogens causing BSIs in various age brackets, and to discover their antibiotic susceptibility patterns in order to develop an empirical treatment strategy for septicemic patients.

**Materials and Methods**

The retrospective, descriptive, observational, cross-sectional study was conducted at the microbiology laboratory of Patel Hospital, Karachi, and comprised positive blood culture bacterial isolates analysed between July 1, 2018, and June 30, 2019. After approval from the institutional ethics review committee, the sample was raised using non-probability consecutive sampling technique. All positive blood cultures of both inpatients and outpatients with suspected bacteraemia and history of fever were included. No patient age restrictions were imposed although single first positive sample taken from one patient were excluded. The data was gathered using Oracle software.

All samples had been withdrawn after stringent aseptic measures by thorough cleaning of the venous site with 70% alcohol, followed by povidone iodine and finally with alcohol (Triple Swab technique) from two different locations (20 min apart) and before the initiation of antibiotics.\textsuperscript{9} The collected blood samples about 1 ml per site were immediately inoculated into commercially supplied BacT Alert bottles. All BacT Alert positive samples were subjected to gram staining followed by inoculation on chocolate agar, 5% sheep blood agar (SBA), and MacConkey agar
plates and incubated at 37 degrees C for 18-24 hours. Identification of isolates was done in accordance with the standard bacteriological technique, and were distinguished based on gram stain, colony morphology and biochemical tests, such as catalase, coagulase, bile esculin (BE), optochin (OP), Bacitracin (BC) discs for gram-positive isolates, and oxidase, urease, citrate, indole, triple sugar iron tests for gram-negative isolates with serological identification for salmonella species. Analytical Profile Index-20E (API-20E) was further used to distinguish gram-negative rods (GNRs). Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar by Kirby-Bauer Disk Diffusion method in accordance with the Clinical Laboratory Standard Institute (CLSI) guidelines. For gram-positive bacteria, ciprofloxacin (CIP) (5µg), erythromycin (E) (15µg), penicillin (P) (10U), ampicillin (30µg), chloramphenicol (30µg), vancomycin (VA) (30µg), gentamicin (CN) (10µg), linezolid (LZD) (30µg), oxacillin (1µg), sulphonamethoxazole/trimethoprim (SXT) (25µg), and clindamycin (DA) were used. For gram-negative bacteria, ampicillin (AMP) (10µg), CIP (5µg), ceftriaxone (CRO) (30µg), ceftazidime (CAZ) (30µg), CN (10µg), tobramycin (10µg), amikacin (30µg), SXT (25µg), azithromycin (AZT), amoxicillin-clavulanic acid (AMC) (30/10µg), tazobactum-piperacillin (TZP) (100/10µg), and meropenem (30µg) were used. The susceptibility breakpoints were interpreted according to CLSI guidelines 2020. E. coli (ATCC25922), S. aureus (ATCC25923) and P. aeruginosa (ATCC27853) were used as quality control strains for culture and susceptibility testing.

Data was analysed using SPSS 20. For variables like microorganism, gender and antibiotic susceptibility, frequencies and percentages were calculated. For quantitative variable age, mean and standard deviation (SD) was considered.

Results

Of the 3450 specimen, 1243(36%) were positive; 668(53.7%) from male and 575(46.3%) from female subjects. The overall age range was 1-99 years and the
distribution of bacteria in different age groups was noted (Figure). There were 771(62%) gram-positive and 472(38%) gram-negative isolates. Salmonella typhi was the most common pathogen 139(11.1) among gram-negative rods (GNRs), followed by acinetobacter (A.) species 103(8.2%), E. coli 96(7.7%) and K. species 42(3.4%). Among gram-positive cocci (GPC), the predominant isolates were staphylococcus epidermidis 650(52%), S. aureus 67(5.4%) and enterococci 28(2.3%). Linezolid (99.8%), vancomycin (99%) and chloramphenicol (69%) were found to be the most sensitive antibiotics among gram-positive cocci. Meropenem (60%), amikacin (46%) and gentamicin (40%) were the most sensitive antibiotics for multidrug resistant gram-negative bacteria.

The antibiotic sensitivity patterns of GPC (Table 1) and GNRs (Table 2) were noted separately.

Discussion

Periodic surveillance of blood culture isolates with their antimicrobial susceptibility pattern is needed to assess the prevalence of bacterial pathogens in blood culture. This enables the clinicians to be aware of developing resistant strains, and to implement effective empirical therapy. Furthermore, results from blood culture surveillance can be used effectively in infection prevention and control. Among developing countries, like Pakistan, where high-tech diagnostic facilities are sparse, newer mutant bacterial strains are also emerging, making it more difficult to control the infections. Moreover, physicians prescribe antimicrobial agents more frequently and all classes of antibiotics are readily available over the counter (OTC) which further contributes to the emergence of resistant bacteria.

The current study showed the occurrence of BSI, and evaluated the sensitivity profile to main antibiotics used to treat such infections. The positivity rate was 36% which is comparable to a study conducted at the Armed Forces Institute of Pathology, Rawalpindi (27%). Another study in Pakistan reported BSIs as the second most
frequent health-associated infection (21.5%). Culture positivity is slightly higher in this part of world compared to studies done globally, reporting 31.2% and 11.2%. Being a third world country having poor healthcare strategies are valid reasons, but different countries also have different blood culture systems, research populations, study designs, geographical locations, aetiological agents and antibiotic stewardship policies.

In the current study, GPC rate (62%) was higher than GNR (38%). Conversely, some studies have revealed a greater occurrence of GNR than GPC.

In the current study, men had higher levels of cultural positivity (53.7%) than women (46.3%). The findings were consistent with earlier findings.

Among the gram-positive isolates (766), the predominant isolates were S. epidermidis (650; 52%), S. aureus (67; 5.4%) and enterococci (28; 2.3%). Over the past two decades, S. species (coagulase-negative staphylococci), the normal skin flora are progressively being considered bloodstream pathogens in select settings. Similar observations were found in most studies.

Among the gram-negative bacteria (477), the major isolates were salmonella species (139; 11%), followed by A. species (103; 8.2%), E. coli (96; 7.7%), K. species (42; 3.4%) and P. aeruginosa (40; 3.2%) These findings were compatible with earlier studies.

The age group of 61-70 years yielded the greatest number of positivity in blood culture, with S. epidermidis being the most common pathogen, followed by E. coli, which is in line with a study in Bangladesh.

Methicillin-resistant S. aureus (MRSA) and MDR against both gram-positive and gram-negative bacteria were found to be excessive in the current study, which is consistent with earlier findings.

It Majority of S. aureus and coagulase-negative staphylococci isolates were resistant to cefoxitin (67% and 73% respectively) (Table 2). Cefoxitin is preferred to oxacillin as it is a better inducer for mecA gene (MRSA). This is consistent with the findings reported in Ethiopia in 2018 which reported MRSA to be 72%. Another study in
India in 2018-2019 reported MRSA to be 97.5%. MRSA BSIs remain associated with worst clinical outcomes as they cause metastatic infections, such as osteomyelitis, infective endocarditis and sepsis. Prevalence of vancomycin-resistant enterococci (VRE) in the current study is estimated to be 25%. This is higher than that reported in various regional studies. The management of VRE infections poses a real challenge for the clinicians, as the therapeutic option is often limited to linezolid. Current findings showed linezolid, vancomycin and chloramphenicol as the most sensitive antibiotics among gram-positive bacteria. The outcomes were in line with earlier studies.

The high occurrence of antibiotic resistance in GNRs linked to BSIs is concerning. This occurrence is due to production of extended spectrum beta lactamases (ESBLs) and carbapenemase resistant enterobacteriaceae (CRE) by gram-negative bacteria. These enzymes hydrolyze beta lactam antimicrobials, making them inefficient as well as causing cross-resistance to other antimicrobials. Meropenem, amikacin, and gentamicin appear to be the last-resort antimicrobials among gram-negative bacteria. This is consistent with earlier findings. It is important to consider the importance of carbapenem resistance as it is the main contributing factor for MDR and usually the definitive step before pandrug resistance (PDR). In the current study, the highest meropenem resistance was found in A. species (85%), followed by K. species (71%) and P. aeruginosa (67%). E. coli consistently had the lowest rate of meropenem resistance (20%). Carbapenem resistance been reported earlier as well. Compared to previous studies cited above, the antibiotic sensitivity profile of various organisms isolated in the current study was diverse. This could be because the sensitivity of organisms to antibiotics varies depending on the prevalence of strains, antibiotic use, and resistance profile in a specific region.

In the light of the findings, appropriate analysis of positive blood culture pathogens and surveillance of their antibiotic susceptibility profile are recommended for reducing the frequency of BSIs. Rational antibiotic prescribing policies and strategies to enhance infection control practices must be implemented. The contribution of
physicians in antibiotic administration workshops can also help decrease such resistance.

**Conclusion**

The identification of frequent bacterial pathogens in blood cultures of patients may guide clinicians in proper empirical selection of antibiotics in patients with bacteraemia. There was a high rate of MDR bacterial pathogens.

**Disclaimer:** Kamran Dawood is currently affiliated with Memon Medical Institute, Karachi.

**Conflict of Interest:** None.

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**References**


15. Ghafoor T, Hussanain A, Qureshi MM, Mehmood T, Ali L. Antimicrobial susceptibility pattern of blood culture isolates from patients with suspected neonatal


Table 1: Antibiotic sensitivity pattern of gram-positive cocci (GPC) isolated from blood samples

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>Staphylococcus epidermidis n=650 (%)</th>
<th>Staphylococcus aureus n=67 (%)</th>
<th>Enterococci n=28 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>76 (11.6%)</td>
<td>8 (12%)</td>
<td>5 (18%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>177 (27%)</td>
<td>22 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>454 (70%)</td>
<td>41 (61%)</td>
<td>NT</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>55 (8.5%)</td>
<td>11 (16%)</td>
<td>NT</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>271 (42%)</td>
<td>26 (39%)</td>
<td>NT</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>231 (35.5%)</td>
<td>17 (25%)</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>115 (18%)</td>
<td>11 (16%)</td>
<td>NT</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100%</td>
<td>100%</td>
<td>21 (75%)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>100%</td>
<td>96 (98.5%)</td>
<td>100%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>458 (70%)</td>
<td>45 (67%)</td>
<td>12 (43%)</td>
</tr>
</tbody>
</table>

Table 2: Antibiotic sensitivity pattern of gram-negative rods (GNRs) isolated from blood samples.

<table>
<thead>
<tr>
<th>Antibiotic tested</th>
<th>Salmonella species n=139 (%)</th>
<th>Acinetobacter species n=103 (%)</th>
<th>E. coli n=96 (%)</th>
<th>Klebsiella pneumoniae n=42 (%)</th>
<th>Pseudomonas aeruginosa n=40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>15 (11%)</td>
<td>NT</td>
<td>2 (2%)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>AMC</td>
<td>NT</td>
<td>NT</td>
<td>43 (45%)</td>
<td>6 (14%)</td>
<td>NT</td>
</tr>
<tr>
<td>TZP</td>
<td>NT</td>
<td>13 (12.6%)</td>
<td>52 (54%)</td>
<td>9 (2%)</td>
<td>17 (42%)</td>
</tr>
<tr>
<td>CRO</td>
<td>29 (21%)</td>
<td>5 (5%)</td>
<td>7 (7%)</td>
<td>2 (4%)</td>
<td>NT</td>
</tr>
<tr>
<td>CAZ</td>
<td>NT</td>
<td>7 (6.7%)</td>
<td>NT</td>
<td>NT</td>
<td>23 (58%)</td>
</tr>
<tr>
<td>CFM</td>
<td>27 (19%)</td>
<td>4 (3.8%)</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CFP</td>
<td>NT</td>
<td>7 (6.7)</td>
<td>11 (11.4%)</td>
<td>3 (7%)</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>AK</td>
<td>NT</td>
<td>23 (22%)</td>
<td>71 (74%)</td>
<td>15 (36%)</td>
<td>19 (48%)</td>
</tr>
<tr>
<td>CN</td>
<td>NT</td>
<td>23 (22%)</td>
<td>52 (54%)</td>
<td>11 (26%)</td>
<td>20 (50%)</td>
</tr>
<tr>
<td>MEM</td>
<td>100%</td>
<td>15 (15%)</td>
<td>77 (80%)</td>
<td>12 (29%)</td>
<td>13 (33%)</td>
</tr>
<tr>
<td>CIP</td>
<td>3 (2%)</td>
<td>11 (11%)</td>
<td>17 (18%)</td>
<td>9 (21%)</td>
<td>21 (53%)</td>
</tr>
<tr>
<td>C</td>
<td>16 (11.5%)</td>
<td>NT</td>
<td>57 (59%)</td>
<td>15 (38%)</td>
<td>NT</td>
</tr>
<tr>
<td>SXT</td>
<td>16 (11.5%)</td>
<td>8 (8%)</td>
<td>18 (19%)</td>
<td>6 (14%)</td>
<td>NT</td>
</tr>
<tr>
<td>AZT</td>
<td>100%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Figure: Distribution of bacteria in different age groups.