Antibiogram of Blood Culture Isolates of Patients from a Hospital in Dhaka, Bangladesh

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Abstract

Background: In Bangladesh, bloodstream infection is a significant cause of morbidity and mortality and empirical treatment based on clinical symptoms. Patient’s final outcome might be improved with detailed and organized surveillance studies on bloodstream isolates and their resistance. Materials and Methods: Our study was conducted with a total of 520 suspected bacteremia patients from December 2017 to November 2018. Results: Approximately 60% and 49% of the suspected cases were male and in the age group 17–50 years, respectively, with increasing prevalence found from May 2018 to November 2018, whereas, highest was found in July 2018. Only 11.15% of the patients showed blood culture positive outcomes with 74% were Gram-negative and 26% were Gram-positive. Highest drug resistance was found with azithromycin against all the isolates, except for Staphylococcus aureus that showed 50% resistance. Among 58 isolates, 57 and 56 isolates were found sensitive to imipenem and amikacin, respectively. However, all the tested isolates were found 100% sensitive against fourth generation, cefepime, and piperacillin/tazobactam. There were no isolates completely resistant to all the antibiotics tested. It is alarming that 22.41% of the isolates were found multidrug resistant. Conclusion: We expect our present work will be helpful for health-care personnel to provide improved treatment, as well as the researcher and policymakers from hospital and government to take a step in reducing the irrational antibiotic practice.

Keywords: Antimicrobial susceptibility, Bangladesh, bloodstream infection, Gram-negative bacteria, Gram-positive bacteria, multidrug resistance

INTRODUCTION

Bloodstream infection has self-limiting to life-threatening consequences with a mortality rate ranging from 4% to 41.5% depending on severity, age, sex, and other risk factors.[1] Bacteremia is a global concern, and rapid increases of community-acquired and nosocomial bloodstream infections have been reported.[2,3] Although blood culture results not always come positive for bacteremia or septicemia patients, it remains the gold standard to diagnose infection in blood.[4] It is reported that Gram-negative bacteria are the cause of nosocomial bacteremia for around 25% and community-acquired bacteremia for 45%.[5,6] Commonly involved Gram-negative bacteria responsible for bacteremia are Escherichia coli, Pseudomonas, Klebsiella, Serratia, Salmonella, Enterobacter, etc.[7] Bacteremia is a growing concern due to Gram-positive organisms.[8] Most common Gram-positive bacteria that enter into bloodstream are Staphylococcus, Streptococcus, and Enterococcus species.[9] In the perspective of Bangladesh, people are taking medication without consulting with a physician, and this is a true reason in the way of emerging drug resistance. Similar antimicrobial abuse is also commonly observed in surrounding regions like India and Pakistan.[10,11] In contrast, drug resistance is less in Europe and America due to less antimicrobial abuse.[11] Therefore, it is reported that 30% of bloodstream infections do not get empirical therapy that leads to their poor outcome and increasing drug resistance.[12,13] Incidence of both Gram-positive and Gram-negative bacterial strains are increasing day by day.[14] The emerging of single, multi, and extensively drug resistance bacteria is alarming and a matter of huge concern worldwide.[15]

The epidemiology of blood culture infection as well as their antimicrobial resistance varies with different geographic

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Salmonella enterica is frequently isolated from blood culture isolate in African and Asian area but S. paratyphi is the principal bacteria of Salmonella group found from blood culture isolate in Africa whereas, S. typhi mostly predominates in Asian population. Therefore, regional surveillance on blood culture isolates and their resistance pattern have a pivotal importance in treatment management. Those studies are not only important to be aware of the growing resistance of selected isolates but also help in providing effective empirical treatment. It has particular importance in countries like Bangladesh, where early treatment is based on patient’s clinical symptoms rather than the diagnostic results. Therefore, the patient’s final outcome might be improved with kind of those regional studies. Detailed and organized surveillance studies on bloodstream isolates and their resistance are little in Bangladesh. Hence, the present study deals with the isolation of blood culture isolates from patients of a hospital in Dhaka, Bangladesh, and their antibiotic susceptibility pattern.

Materials and Methods

The study was carried out in a hospital in Dhaka, Bangladesh, over a period from December 2017 to November 2018. A total of 520 samples of inward and outward patients clinically suspected as having bacteremia were evaluated for our study. The standard microbiological methods were used in this study. Blood samples of 5–10 mL were collected and directly incorporated into blood culture bottles (Microbiotech, Dhaka, Bangladesh). The bottles were incubated at 37°C aerobically for visible growth to come. Following visible growth, 2–3 drops of the blood culture were inoculated on blood agar (Oxoid) and MacConkey agar (Oxoid) media. Blood culture bottles that do not show any significant growth till 7th day of incubation were reported as culture negative. The culture-positive samples were identified by colony morphology, microscopy, and conventional biochemical tests as per the standard protocol followed in microbiology laboratory.

The antibiotic susceptibility pattern of bacterial isolates was performed by Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates, and the results were recorded following the Clinical and Laboratory Standards Institute 2015 guidelines (CLSI, 2016). Various categories of antibiotics were used in our study, including aminoglycosides (amikacin and gentamycin), beta-lactamases including carbapenems (imipenem), cephalosporins (ceftriaxone, cefixime, cefepime, and ceftalexin), penicillins (co-amoxiclav and piperacillin/tazobactam), fluoroquinolones (levofloxacin and ciprofloxacin), macrolides (azithromycin), and sulfonamides (cotrimoxazole).

Results

A total of 520 patients suspected of bacteremia were subjected to investigate bacteriological profile and antibiogram of blood culture isolates from December 2017 to November 2018. Of all patients, 310 (59.62%) were male and 210 (40.38%) were female. The month-wise distribution shows that the number of male patients was higher in any month than female patients, except for September 2018. The number of male and female patients was highest in July 2018 and October 2018, respectively [Figure 1].

There was no very prominent difference in the age group of patients from December 2017 to February 2018. After that time period, patient number increased along with the apparent differences among their age group. Patients of 0–16 years old were highest in March 2018 and April 2018, whereas middle-aged patients (17–50 years) were found highest for the rest of the months (May 2018 to November 2018). Of a total of 520 patients, 0–16, 17–50, and above 50 years patients were 28.85%, 49.04%, and 22.12%, respectively [Figure 2]. Throughout the months, the percentage of positive patients varied from 2.86% to 23.91%, except for September 2018, when all the 45 patients were found as culture negative. Among 520 suspected patients, we have only 11.15% bacteremia-positive patients showing monomicrobial growth. Table 1 shows the percentage of positive and negative samples over the months of the study period as well as the total samples. Figure 3 depicts both month-wise and total distribution of bacterial isolates. In our study, Gram-negative bacteria were prevailed over Gram-positive bacteria. The frequently isolated species of Gram-negative bacteria belonged to Enterobacteriaceae were Klebsiella species (6.89%), Salmonella species (48.28%), and E. coli (5.17%). Another Gram-negative bacillus found was Pseudomonas species (13.8%). The most frequently isolated Gram-positive cocci include Staphylococcus epidermidis (18.97%), followed by S. aureus (6.9%). The month-wise distribution showed that Salmonella species is the most predominantly found isolate in almost all of the months. The highest count was observed for Salmonella species that was 8 among 14 isolates in July 2018. In September 2018, no isolates were detected, and in November 2018, all the isolates were found except Salmonella species and E. coli.

The antibiotic sensitivity and resistant profile of blood culture isolates is summarized in Table 2. All the Gram-negative
isolates showed the highest resistance against azithromycin as *Salmonella* species showed 35.7%, *Pseudomonas* species showed 87.5%, *E. coli* showed 100%, and *Klebsiella* species showed 50%. Moreover, *E. coli* also showed 100% resistance against ciprofloxacin. Gram-negative isolates showed a high degree of sensitivity against the rest of the antibiotics. All the isolates showed 100% sensitivity against imipenem, except only one *Pseudomonas* isolate. Among 58 isolates, 57 and 56 isolates were found sensitive to imipenem and amikacin, respectively. All the isolates tested with fourth-generation cephalosporin, cefepime, and piperacillin/tazobactam were 100% sensitive. Furthermore, amikacin and gentamycin showed the highest degree of sensitivity (100%) against most of the isolates. Gram-positive *S. epidermidis* showed the highest resistance against azithromycin (81.8%), whereas, *S. aureus* showed the highest resistance against both ceftriaxone (100%) and cefixime (100%). Both *S. epidermidis* and *S. aureus* showed 100% sensitivity against amikacin, imipenem, and cefepime. No isolates were found to be 100% resistance against all the tested antibiotics. Among all the isolates, only 13 multidrug-resistant (MDR) isolates were observed that accounts for 22.41% [Figure 4].

Table 1: Month-wise and total distribution of culture positive and negative samples

| Period          | Positive (%) | Negative (%) |
|-----------------|--------------|--------------|
| December 2017   | 2 (7.41)     | 25 (92.59)   |
| January 2018    | 2 (8)        | 23 (92)      |
| February 2018   | 1 (5)        | 19 (95)      |
| March 2018      | 1 (2.86)     | 34 (97.14)   |
| April 2018      | 7 (17.07)    | 34 (82.93)   |
| May 2018        | 11 (23.91)   | 35 (76.09)   |
| June 2018       | 4 (8)        | 46 (92)      |
| July 2018       | 14 (15.91)   | 74 (84.09)   |
| August 2018     | 4 (9.30)     | 39 (90.70)   |
| September 2018  | 0 (0)        | 45 (100)     |
| October 2018    | 6 (10.71)    | 50 (89.29)   |
| November 2018   | 6 (13.64)    | 38 (86.36)   |
| Total           | 58 (11.15)   | 462 (88.85)  |

Figure 2: The bar graph showing the month-wise distribution of different age group of patients from December 2017 to November 2018. The pie diagram showing the percentage of age-wise distribution of total patients

Figure 3: The bar diagram presented the frequency distribution of blood culture isolates in every month of the study period. The distribution of the total bacterial isolates obtained from positive blood culture samples presented by a pie diagram

Discussion

Our study was designed to investigate bacterial isolates causing bloodstream infection throughout 1 year period from December 2017 to November 2018. Overall male patients suspected with bacteremia were higher (59.62%) in comparison to female patients (40.38%). A multinational population-based surveillance study showed that males have a higher risk of methicillin-resistant *S. aureus* infection and bloodstream infection than females although females have a poorer outcome. It was suggested that hand-hygiene behavior might take part to higher colonization and infection rate. Another study revealed that males are more prone to sepsis than females because of male sex hormone that suppresses cell-mediated immune response. Our findings showed that the higher percentage (49%) of suspected bacteremia patients was belonging to the middle-aged group (17–50 years). This might be due to more exposure to populations in day-to-day life during transportation, gathering, traveling, and professional life than infants and old-aged persons, although they are considered vulnerable to any kind of infection due to their less immunity. Among 520 suspected patients, we have 11.15% bacteremia-positive patients, which very closely corresponds with other earlier findings. This result coincides with previous reports where the low number of positive cases ranged from 5.6% to 8.39%, where the high number of positive cases from 33.9% to 52.10%. In our study, Gram-negative bacteria were prevailed over Gram-positive organisms. Similar observations were found in most of the studies. The commonly isolated Gram-negative bacteria were *Klebsiella* spp., *Salmonella* spp., *E. coli*, and *Pseudomonas* spp. that accounts for approximately 74% whereas the most frequently isolated Gram-positive cocci include *S. epidermidis* and *S. aureus* that accounts for around 26% of the total isolates. Among both Gram-negative and Gram-positive isolates, *Salmonella* spp. was found predominant (48%), which is almost half of the isolates. All the patients included in this study had fever and other symptoms of suspected bacteremia. The bacteria
Table 2: Antibiotic susceptibility of blood culture isolates

| Antibiotics       | Klebsiella sp. (4) | Salmonella sp. (28) | Pseudomonas sp. (8) | Staphylococcus epidermidis (11) | Staphylococcus aureus (4) | Escherichia coli (3) |
|-------------------|--------------------|---------------------|---------------------|-------------------------------|--------------------------|---------------------|
|                    | S/R                | S/R                 | S/R                 | S/R                           | S/R                      | S/R                 |
| Amikacin           | 3 (75) 1 (25)      | 27 (96.4) 1 (3.6)   | 8 (100) 0 (0)       | 11 (100) 0 (0)                | 4 (100) 0 (0)            | 3 (100) 0 (0)       |
| Levofloxacin       | 3 (75) 1 (25)      | 28 (100) 0 (0)      | 7 (87.5) 1 (12.5)   | 11 (100) 0 (0)                | 3 (75) 1 (25)            | 2 (66.7) 1 (33.3)   |
| Ceftriaxone        | 3 (75) 1 (25)      | 25 (88.2) 3 (11.8)  | 5 (62.5) 3 (37.5)   | 11 (100) 0 (0)                | 0 (0) 4 (100)            | 2 (66.7) 1 (33.3)   |
| Cefixime           | 3 (75) 1 (25)      | 26 (92.6) 2 (7.4)   | 5 (62.5) 3 (37.5)   | 7 (63.6) 4 (36.4)             | 0 (0) 4 (100)            | 2 (66.7) 1 (33.3)   |
| Co-amoxiclav       | 3 (75) 1 (25)      | 26 (92.6) 2 (7.4)   | 4 (50) 4 (50)       | 9 (81.8) 2 (18.2)             | 4 (100) 0 (0)            | 3 (100) 0 (0)       |
| Azithromycin       | 2 (50) 2 (50)      | 18 (64.3) 10 (35.7) | 1 (12.5) 7 (87.5)   | 2 (18.2) 9 (81.8)             | 2 (50) 2 (50)            | 0 (0) 3 (100)       |
| Ciprofloxacin      | 3 (75) 1 (25)      | 28 (100) 0 (0)      | 8 (100) 0 (0)       | 9 (81.8) 2 (18.2)             | 3 (75) 1 (25)            | 0 (0) 3 (100)       |
| Gentamycin         | 4 (100) 0 (0)      | 28 (100) 0 (0)      | 5 (62.5) 3 (37.5)   | 11 (100) 0 (0)                | - -                      | 3 (100) 0 (0)       |
| Co-trimoxazole     | 4 (100) 0 (0)      | 22 (78.6) 3 (21.4)  | 5 (62.5) 3 (37.5)   | 9 (81.8) 2 (18.2)             | - -                      | 3 (100) 0 (0)       |
| Imipenem           | 4 (100) 0 (0)      | 28 (100) 0 (0)      | 7 (87.5) 1 (12.5)   | 11 (100) 0 (0)                | 4 (100) 0 (0)            | 3 (100) 0 (0)       |
| Cefepime           | 4 (100) 0 (0)      | 28 (100) 0 (0)      | 8 (100) 0 (0)       | 11 (100) 0 (0)                | 4 (100) 0 (0)            | - -                |
| Piperacillin/tazobactam | 4 (100) 0 (0)    | 28 (100) 0 (0)      | 8 (100) 0 (0)       | 11 (100) 0 (0)                | - -                      | - -                |
| Cefalexin          | 4 (100) 0 (0)      | 28 (100) 0 (0)      | 4 (50) 4 (50)       | - -                           | - -                      | - -                |

Figure 4: The frequency distribution of multidrug-resistant isolates

Salmonella can cause salmonellosis, a food-borne disease by nontyphoidal Salmonella, and is responsible for typhoid fever by S. typhi and S. paratyphi. Moreover, Salmonella for salmonellosis usually only invades gastrointestinal tract, but Salmonella responsible for typhoid fever invades bloodstream, even different organs throughout the body. Typhoid fever is endemic in Bangladesh. Chloramphenicol was routinely used since its introduction until the emergence of drug resistance. However, the situation was handled using trimethoprim–sulfamethoxazole and ampicillin as drugs of choice in 1970. However, MDR emerged against those drugs in Bangladesh, reported in 1992. Those dilemmas were addressed using fluoroquinolones such as ceftriaxone and ciprofloxacin, followed by azithromycin and third-generation cephalosporins such as cefixime, cefotaxime, and ceftriaxone. In our study, levofloxacin, ciprofloxacin, gentamycin, imipenem, cefepime, piperacillin/tazobactam, and cefalexin were showing 100% sensitivity against Salmonella spp.; in addition, increased susceptibility was also found with rest of the antibiotics tested and that were >64.3%. The highest resistance was found with azithromycin and that was 35.7%. In addition, highest resistance was observed with azithromycin against most of the isolates, except S. aureus. It was on the list of essential medicine of the World Health Organization because of its effective and safe use.[29] There were no isolates showing 100% resistance against all the antibiotics. It was found that S. aureus showed 100% resistance against third-generation ceftriaxone and cefixime whereas S. epidermidis showed 100% sensitivity with only ceftriaxone. Whereas, other bacterial isolates showed increasing degree of resistance with both of the third-generation cephalosporin, ceftriaxone, and cefixime, and this is alarming. The reason lied here might be due to their huge and irrational use. All the isolates tested with fourth-generation cephalosporin and cefepime were 100% sensitive. Our data revealed that 22.41% of the isolates were MDR. There is no choice, but to mitigate the indiscriminate use of antibiotics.

Conclusion
The present study emphasizes the age- and sex-wise distribution of suspected patients and the prevalence of bacterial pathogens responsible for bloodstream infection with their antimicrobial resistance throughout the study period. Among the patients, males in the age range of between 17 and 50 years come more frequently with fever and suspected bacteremia infection. Gram-negative isolates prevailed among the isolates and Salmonella spp. was found to be the highest in number, and that is, approximately one-half of the total isolates. The frightening issue is that most of the isolates were showing increasing resistance with third-generation cephalosporin antibiotics. All the isolates tested were found to be sensitive with the fourth-generation cephalosporin, cefepime, and piperacillin/tazobactam. Overall, 22.41% of MDR cases were observed. However, this is not yet late if we can still alleviate the use of antibiotics through their rational use, stringent policy from hospital and government with implementation for effective management and drug resistance policy. Besides,
a routine surveillance study for the baseline drug resistance pattern is simultaneously required to go far in combating drug resistance among pathogens.

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**Conflicts of interest**

There are no conflicts of interest.

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