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Original Article

Blood Stream Infection: Identification & Antibiotic Sensitivity Pattern of Microorganisms in a Tertiary Care Hospital in Dhaka City

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Abstract

Background: Blood stream infections are an important cause of mortality & morbidity in all age group worldwide. **Aim & Objective:** The aim of this study was to perform bacteriological analysis and assess antibiotic sensitivity patterns of isolates from bloodstream infections in a tertiary care hospital in Dhaka city. **Material & methods:** The study is a retrospective observational analysis of blood culture isolates received in the Department of Microbiology, Holy Family Red Crescent Medical College & Hospital from January to December, 2021. A total sample size was 2371, out of them positive aerobic bacterial growth was observed in 85 (3.58%) isolates. Sampling technique was used to collect data from the laboratory records and written consent was taken from concerned authority collected data were compiled and analyzed with the help of SPSS version 20. All samples were collected in BacT/ ALERT 3D 60 aerobic bottles irrespective to antibiotics administration. One to five (1-5) ml blood for children and 5-10 ml blood for adult were collected respectively. Samples were incubated in the automated BacT/ ALERT 3D. Subculture was done on blood agar, chocolate agar media & MacConkey's agar media. Identification of organisms by Gram stain & biochemical tests were done as per the standard methods. Negative signal blood culture bottles were kept in machine for up to 5 days. No mixed cultures (the association of two microorganisms) were identified. All the isolates were tested for their antimicrobial susceptibility pattern by Kirby-Bauer's disk diffusion technique according to the Clinical and Laboratory standards institute guideline. **Results:** Overall, 45/874 (5.14%) positive blood cultures were isolated from adult followed by neonate 31/709(4.37%), children 6/390(1.54%) & infant 3/398(0.75%). Bacteremia was more or less equal in both sexes, female 44 (51.77%) & male 41(48.23%). Trend of pathogens recover in highest in November 13(15.29%). The most common organism isolated was *Staph. aureus* 26(30.58%) followed by *Klebsiella* species 15(17.64%), *Acinetobacter* species 13(15.29%), *Pseudomonas* species 11(12.94%), *Salmonella* Typhi 10(11.76%) and *E. coli* 10(11.76%). In *Staph. aureus*, linezolid and vancomycin were 100% sensitive. In *Klebsiella* spp. meropenem was 87% & in *E. coli* 80% sensitive. In *Acinetobacter* spp. amoxicillin/ clavanic acid 85% & colistin 70% sensitive. In *Pseudomonas* spp., meropenem and piperacillin tazobactum 100% sensitive followed by ciprofloxacin 82%, ceftazidime 82% & amikacin 81.8% sensitive. In *Salmonella* Typhi, ceftriaxone was 100% sensitive. **Conclusion:** Drug resistant bacteria are increasing over the years, so, antibiotic policy which formulates according to local sensitivity pattern will be implemented effectively.

Key words: Blood stream infection, antibiotic sensitivity
Short title: Blood stream infection in a tertiary care hospital……. Begum T et al.

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Introduction:

Blood stream infections (BSIs) are an important cause of mortality and morbidity in people of all ages worldwide. BSI associated illness ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment.[1] BSI affects about 30 million people leading to 6 million deaths globally,[2] with 3 million

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newborns and 1.2 million children suffering from sepsis annually.[3]

BSIs are characterized by the presence of viable microorganisms in blood. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body.[4] Clinical presentation of BSIs ranges from benign transient bacteremia with little or no symptoms to fulminant septic shock with high mortality.[5] Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella species*, *Neisseria meningitis*, *Haemophilus influenzae*, and Gram-positive bacteria such as coagulase-negative *Staphylococci* (CoNS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Enterococcus faecium* have been reported as a cause of bacteremia in different countries.[6]

BSIs have serious consequences such as shock, disseminated intravascular coagulation, multiple organ failure, and even death. Early diagnosis of BSI plays a crucial role for management & micro biology laboratories also plays a role for prompt detection of organisms. As blood culture is the gold standard for detection of bacteremia, knowledge of local pathogens and sensitivity pattern of microorganism is essential to start prompt and appropriate empirical therapy. Increasing rates of antimicrobial resistance, changing patterns of antimicrobial usage, and the wide use of indwelling catheters may change the epidemiology and outcome of bloodstream infection. Irrational use of drugs has led to an increase of multidrug-resistant bugs and thus worsened the condition.[4] The rate of blood stream infections in children is about 20-44% in developing countries.[7,8] In adult BSI was identified 567 (9.94%) out of 5706 samples in one year study.[9] Blood culture remains the highly specific indicator of bacteremia and early administration of adequate antibiotic therapy is essential to improve patient outcome and should be based on accurate knowledge of local bacterial pathogens and their sensitivity patterns.[10] The aim of this present study was to perform bacteriological analysis and assess antibiotic sensitivity patterns of isolates from bloodstream infections in a tertiary care hospital in Dhaka city.

Material & methods:

The study is a retrospective observational analysis of blood culture isolates received in the Department of Microbiology, Holy Family Red Crescent Medical College & Hospital from January to December, 2021. Records of all such cases were collected from microbiology laboratory and analyzed for inclusion in the study. Written consent was taken from the concerned authority. Data were analyzed with the help of SPSS version 20.

Sample collection & identification of microorganisms:

All samples were collected in BacT/ ALERT 3D 60 aerobic bottles irrespective to antibiotics administration. One to five (1-5) ml blood for children and 5-10 ml blood for adult were collected respectively with all aseptic precautions describe in the manufacturer.[11] Samples were incubated in the automated BacT/ ALERT 3D. Preliminary signal of bacterial growth was detected & displayed on the LED monitor. The BacT/ALERT Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced an organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas – permeable sensor installed in the bottom of each culture bottle

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changes from blue-green to yellow. The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes. Culture positive samples were accomplished by subculture on blood agar, chocolate agar media & MacConkey's agar media for specific identification of organisms. Inoculated media were incubated aerobically at 37^0C. The Chocolate agar plates were incubated at 37^0C under 5-10% CO_2 condition (candle jar) and examined after 18-24 hours of incubation. Identification of organisms by Gram stain & biochemical tests were done as per the standard methods.[12] Negative signal blood culture bottles were kept in machine for up to 5 days. No mixed cultures (the association of two microorganisms) were identified.

Antibiotic susceptibility test:
All the isolates were tested for their antimicrobial susceptibility pattern on Mueller Hinton agar media by Kirby-Bauer's disk diffusion technique according to the Clinical and Laboratory standards institute guideline.[13] The routine antimicrobial susceptibility tests were put for following antibiotics: amoxicillin/ clavulanic acid (30µgm), ampicillin ((10µgm), cloxacillin (5 µgm), cefuroxime (30µgm), ceftriaxone (30µgm), ceftazidime (30µgm), azithromycin (15 µgm), ciprofloxacin (5µgm), chloramphenicol (30µgm), nalidixic acid (30µgm), trimethoprime /sulfamethoxazole (25µgm), gentamicin (10µgm), amikacin (30 µgm), meropenem (10µgm), linezolid (30µgm), vancomycin (300µgm), piperacillin/tazobactum (100µgm), colistin (10µgm). The zone sizes were measured and interpreted according to the CLSI standard.[13]

Results:
A total of 2371 samples were analyzed; of them positive aerobic bacterial growth was observed in 85 (3.58%) isolates.

Table 1: Age & gender distribution of culture positive patients

Age group	Total sample	Culture positive		Percentage
		Male	Female	
Neonate	709	12	19	4.37%
Infant	398	3	0	0.75%
Children up to 12 years	390	2	4	1.54%
Adult	874	24	21	5.14%
Total	2371	41(1.73%)	44(1.85%)	3.58%

Age & gender distribution of culture positive patients is described in Table 1. Overall, 45/874 (5.14%) positive blood cultures were isolated from adult followed by neonate 31/709(4.37%), children 6/390(1.54%) & infant 3/398(0.75%). Bacteremia was more or less equal in both sexes, female 44 (51.77%) & male 41(48.23%).

Table 2: Trend of pathogens recovered in each month (n=85)

Month	Number of isolates	Percentage
January	8	9.41%
February	7	8.23%
March	7	8.23%
April	4	4.70%
May	6	7.05%
June	11	12.94%

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July	3	3.52%
August	6	7.05%
September	6	7.05%
October	10	11.76%
November	13	15.29%
December	4	4.70%

Trend of pathogens recover in highest in November 13(15.29%) then June 11(12.94%), October 10(11.76%) & January 8(9.41%).

Table 3: Distribution of bacterial pathogen in blood culture (n=85)

Organisms	Number	Percentage
Staph. aureus	26	30.58%
Klebsiella spp.	15	17.64%
Acinetobacter spp.	13	15.29%
Pseudomonas spp.	11	12.94%
Esch. coli	10	11.76%
Salmonella Typhi	10	11.76%
Total	85	100%

In Table 3, the most common organism isolated was *Staph. aureus* 26(30.58%) followed by *Klebsiella* species 15(17.64%), *Acinetobacter* species 13(15.29%), *Pseudomonas* species 11(12.94%), *Salmonella* Typhi 10(11.76%) and *E. coli* 10(11)

Table 4: Antibiotic sensitivity pattern of the isolates (n=85)

Drugs	***Staph. Aureus* (n=26)**	***Klebsiella* spp. (n=15)**	***Acinetobacter* spp. (n=13)**	***Pseudomonas* spp. (n=11)**	***Salmonella* Typhi (n=10)**	***E. coli* (n=10)**
AMC	20(77%)	3(20%)	-	-	-	4(40%)
AMP	-	-	-	-	7(70%)	-
CX	11(42%)	-	-	-	-	-
CU	20(77%)	8(53.3%)	8(62%)	0(0%)	-	6(60%)
CTR	-	6(40%)	7(54%)	-	10(100%)	3(30%)
CAZ	-	11(73%)	7(54%)	9(82%)	-	4(40%)
AZM	20(77%)	-	-	-	9(90%)	-
CIP	16(62%)	11(73%)	8(62%)	9(82%)	5(50%)	5(50%)
C	-	-	-	-	8(80%)	-
NA	-	-	-	-	2(20%)	-
SXT	15(58%)	9(60%)	-	-	9(90%)	5(50%)
GN	17(65%)	9(60%)	4(30%)	8(73%)	-	6(60%)
AK	17(65%)	7(47%)	5(38%)	9(81.8%)	-	6(60%)
MEM	-	13(87%)	8(62%)	11(100%)	-	8(80%)
LZ	26(100%)	-	-	-	-	-
VA	26(100%)	-	-	-	-	-
PIT	-	-	8(62%)	11(100%)	-	-
Cl	-	-	9(70%)	8(73%)	-	-

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AMC – Amoxicillin/clavulanic acid, AMP-Ampicillin, CX-Cloxacillin, CU-Cefuroxime , CTR-Ceftriaxone, CAZ – Ceftazidime, AZM - Azithromycin, CIP - Ciprofloxacin, C - Chloramphenicol, NA - Nalidixic acid, SXT - Trimethoprime/ Sulfamethoxazole, GN - Gentamycin, AK - Amikacin, MEM - Meropenem, LZ - Linezolid, VA - Vancomycin, PIT - Piperacillin tazobactum, , Cl – Colistin.

In *Staph. aureus*, linezolid and vancomycin were 100% sensitive. In *Klebsiella* spp. meropenem was 87% sensitive, ciprofloxacin 73%. In *Acinetobacter* species amoxicillin/ clavanic acid 85%, colistin 70%, meropenem and piperacillin tazobactum 62% sensitive. In *Pseudomonas* species, meropenem and piperacillin tazobactum 100% sensitive followed by ciprofloxacin 82%, ceftazidime 82% & amikacin 81.8% sensitive. In *Salmonella* Typhi, ceftriaxone was 100% sensitive, followed by azithromycin and co-trimoxazole 90% sensitive. In *E. coli* meropenem was found 80% sensitive, followed by amikacin and gentamycin 60% sensitive.

Discussion:

Blood stream infection may be a life-threatening and challenging problem, so identification of organism and its sensitivity testing is the most important work of microbiology laboratory.[4] A strong interaction between delay in effective initiation of therapy and in-hospital mortality of septic shock. Each hour of delay in therapy initiation is associated with an average decrease in survival of 8%.[14] Therefore, it is important to identify microorganisms & assess their antibiotic sensitivity pattern for better outcome of BSIs.

In our study, we observed 85 (3.54%) culture positive out of 2371 samples. This study showed higher positive blood in adult 45/874 (5.14%) followed by neonate 31/709(4.37%), children 6/390(1.54%) & infant 3/398(0.75%). Bacteremia was more or less equal in both sexes, female 44 (51.77%) & male 41(48.23%) (Table 1). In a study, bloodstream infection rates as published in hospital and laboratory surveillance reports are estimated at 9.3%.[15] The most common sources of bacteremia in adults were the lung (27%), abdomen (25%), and urinary tract (23%).[16] Higher BSI rate in young patients particularly neonates include immature immune system, poor skin integrity, low socioeconomic status of parents, poor hygiene practices, bottle feeding, high incidence of delivery at home & lots of nosocomial organisms in hospital environments could be render neonates more susceptible to bloodstream infections.[17,18] Blood stream infections are considered one of the most important infections in neonate was 28.4%.[19]

Seasonal variation is an important determinant of bloodstream infection burden.[20] In Ghana, the months of December-January and May and October coincide with the dry and wet seasons, respectively.[21] In the current study, an upward trend during the rainy season and in the dry season, with the highest culture positive cases recorded in the months of June (12.94%) and November (15.29%) (Table 2). It was previously reported that higher rates of bloodstream infection directly correlate with increasing temperature.[20] Individuals' susceptibility to bacterial pathogens due to climate changes may be a probable reason.

The causative agents responsible for BSIs vary from country to country with unique geographical peculiarities.[22,23] BSIs to the predominance of Gram-negative bacteria was identified by some studies.[24,25] However, those results were challenged by some other works where Gram-positive bacteria were predominantly responsible for BSIs. Our study revealed *Staph. aureus* (30.58%) as the leading causative agent of BSI followed by *Klebsiella* spp. (17.64%), *Acinetobacter* spp. (15.29%), *Pseudomonas* spp. (12.94%), *E. coli* (11.76%)

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& *Salmonella* Typhi (11.76%) (Table 3). In a study, *Staph aureus* was recovered as the predominant pathogen (40.7%) of BSIs which correlates with this present study.[26] In contrast, however, our results contradict the findings of others who reported *Esch. coli* as the leading cause for bloodstream infections.[27,28]

In this study, *Staph. aureus* showed 100% sensitivity to linezolid and vancomycin, 77% to amoxyclav, cefuroxime, azithromycin (Table 4). This is inconsistence with a study where they found *Staph. aureus* was sensitive to linezolid 55% and vancomycin 52%. It was noted that in neonatal intensive care unit neonatal pneumonia was empirically treated with broad range antibiotics in all babies without culture and sensitivity, this injudicious use of antibiotics in turn accounts for such high rate of hospital acquired VRSA.[29] *Staph. aureus* was found to be highly sensitive to vancomycin, linezolid, gentamicin and ciprofloxacin as reported by another investigator.[30] This Gram - positive bacteria do not respond to the broad class of beta-lactam antibiotics and acquire resistance to newer antibiotics rapidly. This ultimately complicates the management of such BSIs.

In our study, *Klebsiella* species were sensitive to meropenem 87% followed by ciprofloxacin 73%, gentamicin 60%. In *E. coli*, meropenem was 80%, followed by amikacin 60%, gentamycin 60% sensitive (Table 4). *E. coli* was found 100% sensitivity to ciprofloxacin and highly resistant to cephalosporin group in a study.[31] In *Acinetobacter* species, we found 70% sensitivity to colistin, 62% sensitivity to meropenem and piperacillin tazobactum. In *Pseudomonas* species, meropenem and piperacillin tazobactum were 100% sensitive followed by ciprofloxacin and ceftazidime 82% sensitive, amikacin was 81.8% sensitive (Table 4). The result was inconsistent with a study who reported piperacillin tazobactum was 92.3% resistant to *Pseudomonas* spp. and *Acinetobacter* was 100% resistant to ciprofloxacin and ceftazidime.[31] Amikacin sensitivity 80% was noticed in a study which is consistent with our study.[4]

This study found *Salmonella* Typhi was 100% sensitive to ceftriaxone, followed by azithromycin and co - trimoxazole 90% (Table 4). Ciprofloxacin showed 50% sensitivity. A study showed all *Salmonella* isolates were susceptible to ceftriaxone, but a very high level of reduced susceptibility exists against ciprofloxacin.[32] Studies suggest that the presence of a mutation in the quinolone resistance-determining region (QRDR) of the gyr A gene is responsible for the emergence of this reduced susceptibility.[33] We have observed a higher sensitivity of *Salmonella* Typhi against ampicillin, co-trimoxazole 90% & chloramphenicol 80%. If this kind of sensitivity continues, then using 1st line cheaper antibiotics against *Salmonella* Typhi infection might be possible in future. Chloramphenicol was the original drug of choice for many years. Because of rare serious side effects, chloramphenicol has been replaced by other effective antibiotics now a days.

Conclusion:

Blood stream infection was more common among adult age group (52.94%). BSIs were more or less equal in both sexes. Trend of pathogens recover in highest in November 15.29%. The most common isolated organism was *Staph. aureus* 30.58%. *Staph. aureus* was 100% sensitive to vancomycin & linezolid. *Klebsiella* spp. & *E. coli* were 87% & 80% sensitive to meropenem. *Pseudomonas* spp. was 100% sensitive to meropenem & pipercillin/tazobactum. *Salmonella* Typhi was 100% sensitive to ceftriaxone. These all injectable & reserve antibiotics showed higher sensitivity which is a threat for us because all these drugs are going to be resistant within few years if proper use of these drugs are not done. Drug resistant bacteria are increasing over the years, so, antibiotic policy which formulates according to local sensitivity pattern will be implemented effectively.

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Original Article

Limitation of the study:

Small sample size due to pandemic situation of COVID-19 & potential risk factors could not identify due to unavailability of information.

Conflicts of Interest:

The authors have no conflicts of interest to declare.

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