

Bacterial Aetiology of Bloodstream Infection and Antimicrobial Resistance Pattern in a Tertiary Hospital of Northern Bangladesh: Analysis of Current Situation

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ABSTRACT

Background: Bloodstream infection (BSI) is one of the major causes of morbidity and mortality worldwide. **Objective:** The objective of the present study was to identify the causative organisms of bloodstream infection (BSI) and their resistance pattern to different antibiotics as well as prevalence of multi drug resistant (MDR) organisms in this region. **Methodology:** This retrospective study included blood culture reports from 1899 suspected bacteraemia patients. Culture was done using BACT/Alert machine followed by culture on MacConkey (MC) agar; chocolate agar and blood agar plates. Isolated organisms were identified using standard laboratory procedures. **Results:** Total 383 bacterial isolates were yielded (rate of positive culture 20.2%). *Staphylococcus aureus* (41.8%) and *Escherichia coli* (41.8%) were most frequently isolated gram positive and gram-negative organisms respectively. Other commonly isolated organisms were *Salmonella typhi* (10.7%) and coagulase negative *Staphylococci* (CoNS) (3.9%). More than 90% isolated organisms were multidrug resistant. *Salmonella typhi* (95.1%) and *Staphylococcus aureus* (91.2%) showed most frequently isolated MDR strains. All the organisms showed high resistance rate against commonly used antibiotics like azithromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole. Amoxycillin and clavulanic acid combination, cloxacillin and linezolid were sensitive against *Staphylococcus aureus*. Ceftriaxone as well as amikacin remained a sensitive drug to treat *Salmonella typhi*. Carbapenems and nitrofurantoin were mostly sensitive against all isolated organisms. **Conclusion:** Rational use of antibiotics based on regional epidemiology of causative organisms and sensitivity pattern can preserve the potentiality of available antibiotics and reduce the burden of MDR pathogens.

Keywords: Bloodstream infection, Septicaemia, Antibiogram, MDR, Bangladesh

Introduction

Bloodstream infection (BSI) is one of the major causes of morbidity and mortality worldwide. The

incidence of both community and hospital acquired BSI is increasing day by day, especially in lower- and

middle-income countries.^{1,2} It prolongs the hospital and intensive care unit stay which leads to increased healthcare cost.³

Epidemiology of causative agents of septicaemia mostly depends on geographic location. Commonly isolated organisms associated with septicaemia includes *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella* species and *Escherichia coli*.^{4,5} These organisms were reported to be highly resistant against commonly used antimicrobials.⁶ This high burden of antimicrobial resistance of the organisms causing BSI has made the therapeutic treatment more challenging.

Different medical and socioeconomic factors are responsible for this high level of antibiotic resistance including irrational use of antibiotics and inadequate empirical therapy without the evidence of culture and sensitivity results.⁷ Lower- and middle-income countries are more vulnerable to this problem, as irrational prescription, over-the-counter availability of antibiotics, self-administration and environmental exposure to antibiotics from animal and plant sources are common practices in these countries. Moreover, health facilities are not well equipped to carry out laboratory tests for susceptibility pattern of different organisms. So, there is a place for empirical antibiotics based on clinical judgment rather than diagnostic results for the early treatment of bloodstream infection in these countries. Therefore, local epidemiology of causative organisms and their resistance pattern to different antimicrobials plays an important role in choosing suitable antibiotic therapy.

In Bangladesh *Salmonella typhi*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* species (CoNS) and *Pseudomonas* species were reported as commonly isolated organisms from blood samples.^{8,9} However, these reports were based on the data of blood stream infection of Dhaka city only, so cannot be generalized for the entire country. Moreover, antibiotic resistance pattern is not homogenous all over the country. There is still no evidence according to our knowledge on the causative organisms of bloodstream infection and their antimicrobial resistance pattern in northern Bangladesh. Hence, the present study aimed at identifying the causative organisms of BSI and their resistance pattern to different antibiotics as well as prevalence of multi drug resistant (MDR) organisms in this region.

Methods

Study population and bacterial isolates: This was a cross-sectional study based on retrospective data that included the records of 1899 clinically suspected patients of bacteraemia, for whom blood culture was carried out routinely in the Microbiology laboratory of Rajshahi Medical College Hospital (RMCH) between July 2018 and December 2019. Blood sample was collected from each patient (1–3 ml for paediatric patients and 8–10 ml for adults) and directly inoculated into paediatric (up to 12 years of age) or adult (more than 12 years of age) FAN blood culture bottle. Collected samples were incubated in the BACT/Alert machine for up to 5 days. For identification of organism's positive culture samples were directly inoculated onto MacConkey (MC) agar, chocolate agar and blood agar (5% sheep blood) plates. MC plates were then incubated at 35°C in aerobic condition. Chocolate and blood agar plates were incubated at 35°C in microaerophilic condition (containing 5% CO₂). Isolated organisms were identified using standard laboratory procedures.¹⁰ API 20E identification strips (bioMérieux, France) were used for further identification.

Antimicrobial susceptibility testing: Antimicrobial susceptibility test (AST) was carried out by Kirby–Bauer disc diffusion method and susceptibility patterns were determined following CLSI guidelines.^{10,11} Antimicrobial susceptibility was tested for a panel of antibiotics (Oxoid, UK) including Ceftriaxone (30µg), Cefixime (5µg), Ceftazidime (30µg), Cefepime (30µg), Cefuroxime (30µg), Imipenem (10µg), Meropenem (10µg), Azithromycin (15µg), Ciprofloxacin (10µg), Levofloxacin (5µg), Gentamicin (120µg), Amikacin (30µg), Trimethoprim/Sulfamethoxazole (25µg), Amoxycillin/ Clavulanic Acid (30µg), Doxycycline (5µg) and Nitrofurantoin (300µg). For gram positive organisms Cephadrine (30µg), Amoxicillin (10µg), Cloxacillin (5µg) and Linezolid (30µg) were additionally used, while Ceftriaxone and Cefixime were not used for these organisms. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Non-susceptibility to at least one agent in three or more antimicrobial categories was defined as multi drug resistance (MDR).¹²

Statistical analysis: All statistical analyses were carried out using SPSS version 22.0 SPSS for Windows (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY: IBM Corp.) software.. Frequencies

with percentage were used to present categorical data such as, type of bacterial isolates and resistance to specific antibiotics. Chi-square (χ^2) test or Fisher's exact test was used to determine the difference of rate of isolation between different age and sex groups. p-value <0.05 was considered as significant.

Results

A total of 1899 blood samples from suspected bacteraemia patients were cultured during the study period. Patients' median age was 25 years (range 1 day to 95 years) and 57.3% of them were male. Total 383 bacterial isolates were yielded from 1899 cultures (rate of positive culture 20.2%). Rate of isolation was higher among female (23.2%) compared to male (17.9%). No significant difference was observed among different age groups.

Table 1: Age and sex distribution of the patients.

Characteristics	Total, n (%) n:1899	Culture positive, n (%) n: 383	Culture negative, n (%) n: 1516	p-value
Age in years				
<1	239 (12.6)	46 (12.0)	193 (12.7)	0.463
1-5	80 (4.2)	14 (3.7)	66 (4.4)	
6-18	351 (18.5)	77 (20.1)	274 (18.1)	
19-30	496 (26.1)	111 (29.0)	385 (25.4)	
31-40	211 (11.1)	41 (10.7)	170 (11.2)	
41-50	151 (8.0)	23 (6.0)	128 (8.4)	
51-60	185 (9.7)	40 (10.4)	145 (9.6)	
>60	186 (9.8)	31 (8.1)	155 (10.2)	
Gender				
Male	1088 (57.3)	195 (50.9)	893 (58.9)	0.005
Female	811 (42.7)	188 (49.1)	623 (41.1)	

All infections were due to single organism. Predominant gram-positive organism was *Staphylococcus aureus* (41.8%) and Predominant gram-negative organism was *Escherichia coli* (41.8%). Coagulase negative *Staphylococci* (CoNS) (3.9%) among gram positive organisms and *Salmonella typhi* (10.7%) among gram negative organisms were also commonly isolated. Overall, more than 90% isolated organisms were multidrug resistant. Prevalence of MDR was more commonly demonstrated among *Salmonella typhi* (95.1%) and *Staphylococcus aureus* (91.2%).

Table 2: Isolated organisms from the blood samples

Organism	No of isolates; n (%) (n: 383)	No of MDR isolates; n (%) (n:345)
<i>Staphylococcus aureus</i>	160 (41.8)	146 (91.2)
Coagulase negative <i>Staphylococci</i> (CoNS)	15 (3.9)	13 (86.7)
<i>Escherichia coli</i>	160 (41.8)	141 (88.1)
<i>Salmonella typhi</i>	41 (10.7)	39 (95.1)
<i>Salmonella paratyphi</i>	5 (1.3)	4 (80)
<i>Klebsiella Pneumonia</i>	1 (0.3)	1 (100)
<i>Pseudomonas aeruginosa</i>	1 (0.3)	1 (100)
Total	383 (100)	345 (90.1)

The antimicrobial resistance patterns of commonly isolated bacteria are demonstrated in Table 3. Both gram positive and gram-negative organisms were highly resistant against azithromycin (60 to 78%), ciprofloxacin (60 to 76%), and trimethoprim/sulfamethoxazole (45.6 to 79%). Resistance rate of gram-positive organisms (*Staphylococcus spp.*) was very high against amoxicillin, cephradine, and ceftazidime, however, CoNS was quite sensitive to cephradine (resistance rate 27%). Common gram-negative organisms (*Escherichia coli* and *Salmonella spp.*) were moderate to highly resistant against cefixime, gentamicin and doxycycline (53 to 80%). Carbapenem group of drugs (imipenem and meropenem) were mostly sensitive against all organisms followed by nitrofurantoin. However, amoxicillin and clavulanic acid combination and linezolid were sensitive against gram positive organisms, while ceftriaxone was quite sensitive against *Salmonella spp.*

Table 3: Antimicrobial resistance pattern (percentage of resistance) of the isolated organisms

Antibiotics in %	<i>S. aureus</i>	CoNS	<i>E. coli</i>	<i>S. typhi</i>	<i>S. paratyphi</i>
Cephradine	51.9	26.7			
Ceftriaxone		33.3	46.9	34.1	20.0
Cefixime			53.1	53.7	80.0
Ceftazidime	63.7	46.7	33.1	34.1	40.0
Cefepime	31.9	33.3	36.9	43.9	80.0
Cefuroxime	36.9	13.3	69.4	68.3	80.0
Imipenem	10.0	13.3	13.8	4.9	20.0
Meropenem	31.9	20.0	20.0	7.3	20.0
Azithromycin	74.4	66.7		78.0	60.0
Ciprofloxacin	63.7	46.7	76.2	75.6	60.0
Levofloxacin	58.1	40.0	35.6	31.7	40.0
Gentamicin	40.0	33.3	45.6	65.9	60.0
Amikacin	34.4	60.0	43.8	24.4	0.0
Trimethoprim/ Sulfamethoxazole	78.8	53.3	45.6	78.0	20.0
Amoxicillin	73.8	80.0			
Amoxycillin+ clavulanic acid	25.6	26.7	39.4		
Cloxacillin	38.8	26.7			
Linezolid	13.8	26.7			
Doxycycline	36.9	33.3	59.4		
Nitrofurantoin	15.0	20.0	35.0		

Discussion

Comprehensive understanding of epidemiology of causative organisms of bloodstream infection and their trending pattern of resistance to different available antibiotics is necessary for choosing effective antimicrobial therapy, especially in a limited resource

country like Bangladesh, where diagnostic facilities are not widely available and empirical antibiotics based on best clinical judgment plays an important role in primary patient management. The present study provides an overview for the very first time about the epidemiology and antimicrobial resistance pattern of the organisms causing BSI among the patients of northern Bangladesh.

The rate of bacterial isolation in the blood culture of our patient cohort was 20.2%, which is quite higher compared to a study from Dhaka city that reported positive culture rate as 13.6% as well as some studies from neighbouring India, where the rate was reported as 12.7 to 14%.^{8,13,14} Though proportion of male patients was higher, isolation rate was higher in female patients (23% vs 18%), which is in line with studies from India and Ethiopia.^{15,16}

Staphylococcus aureus and *Escherichia coli* were most frequently isolated gram positive and gram-negative organisms respectively in the present study. *Salmonella typhi* was most commonly isolated in previous study in Dhaka city, followed by coagulase-negative *Staphylococcus* species (CoNS) and *Pseudomonas* species.⁸ Though, CoNS was one of the common gram-positive organisms found in our study, *Pseudomonas spp.* constituted a very minor portion. Non-fermenter gram-negative bacilli like *Pseudomonas* species and *Acinetobacter* species are mostly associated with nosocomial infections.^{17,18} Though we could not identify the samples were whether from inpatient or outpatient department, most probably, most of the samples were referred from outpatient department that reduces the frequency of these organisms. Another notable issue is that blood samples of all febrile patients are not cultured routinely in our study setting, that may cause missing of large number of *Salmonella typhi* and *Salmonella paratyphi* isolates. Our study demonstrated a large variation in case of gram-negative organisms compared to the Indian ones that reported *Acinetobacter* species and *Klebsiella* species as commonest gram negative organisms.¹³⁻¹⁵ The exact cause of this variation is not clear. Further investigation is suggested to find out whether it is an actual scenario or due to laboratory procedure.

Level of multidrug resistance was high among both gram positive and gram-negative bacteria. Similar finding was reported in previous study from Bangladesh, though rate of MDR was decreasing in

case of *Salmonella typhi* as reported in that study.⁸ No such trend was observed in the present study. All the gram positive and gram-negative organisms showed high resistance rate against commonly used antibiotics like azithromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole, which is consistent with the findings of several studies which was conducted both in Bangladesh and neighbouring country India.^{8,13,15} previous ones Amoxycillin and clavulanic acid combination, cloxacillin and linezolid were sensitive against *Staphylococcus aureus*. Linezolid was also reported as sensitive against this organism in a longitudinal study conducted in a tertiary care hospital of Dhaka, Bangladesh during 2005 to 2014 Similar finding in case of linezolid was reported in the previous study and it suggested vancomycin as a potent drug to treat staphylococcal infection, though vancomycin was not used in our antibiogram. Ceftriaxone as well as amikacin remained a sensitive drug to treat *Salmonella typhi* both in our study and previous one.⁸ Nitrofurantoin is a promising choice of drug to treat *E. coli* as well as Staphylococcal infection. Carbapenems were mostly sensitive against all isolated organisms, consistent with previous finding.⁸ However, this should not be prescribed routinely to preserve its efficacy for long term.

The present study has several limitations. First of all, it was a single centre study, so the findings cannot be generalized in national context. Detailed clinical features of the patients could not be included. Moreover, classification of patients based on the sources of infection (community acquired or nosocomial) also could not be mentioned. Despite these shortcomings the study provides a vivid picture of current pattern of antibiotic resistance among the organism causing bloodstream infection in the respective region.

Conclusion

Bloodstream infection caused by MDR organisms is a major therapeutic challenge. Causative organisms of BSI and their resistance pattern vary a lot according to different geographical location. We suggest that, conventional antibiotics like amoxycillin and clavulanic acid combination, cloxacillin and linezolid are still effective against gram positive organisms; ceftriaxone and amikacin are potent drugs against *Salmonella typhi*, while nitrofurantoin can be a suitable choice to treat both *E. coli* and Staphylococcal infection. Newer drugs like carbapenems should be

preserved for special cases only, where treatment with first line therapy fails. Above all, rational prescription of antibiotics based on local epidemiology and resistance pattern can reduce the burden of MDR bacterial infection.

List of abbreviations

BSI: Bloodstream infection, MDR: Multidrug resistant, CLSI: Clinical and Laboratory Standards Institute, MC: MacConkey agar, AST: Antimicrobial susceptibility test, RMCH: Rajshahi Medical College Hospital

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethical Review Committee of Rajshahi Medical College [Ref. RMC/ERC/2017-2019/179 Date: 31/10/2019]. As it was a retrospective study based on recorded data, informed consent of participants was not necessary.

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