

## Original Article

# Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among bloodstream infection suspected patients attending in a referral hospital

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### Abstract

Bloodstream infections (BSIs) are the leading cause of morbidity and mortality in people of all ages. On time diagnosis and appropriate antimicrobial treatment is of utmost important to save the lives of affected ones. The present study is aimed to determine the bacterial profile and antimicrobial susceptibility patterns of blood culture isolates among BSI suspected patients attending in Uttara Adhunik Medical College and Hospital from 1<sup>st</sup> July to 31<sup>st</sup> December, 2020. This cross sectional study was conducted among 1,675 BSI suspected patients. About 10 ml of venous blood for adults and 2-3 ml for children was collected aseptically and transferred into an automated blood culture bottle. The BD BACTEC FX40 automated blood culture method was used to isolate bacterial pathogens and antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method following CLSI guidelines. The rate of bacteriologically confirmed cases was 222/1675 (13%). Majority of BSI were caused by gram negative bacteria predominantly *Salmonella* Typhi (51%) followed by *Salmonella paratyphi* (15%), *Escherichia coli* (15%), *Klebsiella* spp. (7%), *Staphylococcus aureus* (6%), *Pseudomonas aeruginosa* (4%). *Salmonella* Typhi and *Salmonella* Paratyphi isolates showed 100% susceptibility to meropenem and ceftriaxone. A higher percentage of strains of *Salmonella* Typhi (80%,84%,85%) and *Salmonella* Paratyphi (93%,95%,95%) were found sensitive to amoxiclav, chloramphenicol and cotrimoxazole whereas only 4% isolates were sensitive to ciprofloxacin which is an alarming situation. None of the antibiotic was 100% effective against *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*. Amikacin and meropenem were found more effective against gram negative bacteria. In this study, none of the *S. aureus* strains showed resistance to cloxacillin, amoxiclav, gentamicin and doxycycline and sensitivity to azithromycin and clindamycin were found 40% and 50% respectively. Multidrug resistance among blood stream isolates are increasing in a threatening way which need to be addressed through effective surveillance and infection control strategies.

### Introduction

The term bloodstream infection (BSI) generally refers to the growth of a microorganism from a blood culture obtained from a patient with clinical signs of infection and where contamination has been ruled out<sup>1</sup>. It is characterized by the presence of viable bacterial or fungal microorganisms in the bloodstream that elicit inflammatory response and often accompanied by alteration of clinical, laboratory and hemodynamic parameters<sup>2</sup>. Bacterial bloodstream infection (BSI) is a global concern. It may range from self-limiting infections to life-threatening sepsis that

requires rapid and aggressive antimicrobial treatment<sup>3</sup>. It is often associated with increased length of hospital stay which ultimately leads to significant amount of healthcare related costs and a high rate of morbidity and mortality<sup>4</sup>.

Many bacteria such as *Staphylococcus aureus*, alpha-hemolytic *Streptococci*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* Typhi and *Acinetobacter* species have been reported as a cause of bacteremia with variation in distribution from place to place.<sup>5</sup> As an example, *Salmonella enterica* is a frequently isolated pathogen from blood samples in both African and Asian regions, however their serotypes differ substantially. *Salmonella* Paratyphi is the predominant organism in the *Salmonella* group in Africa whereas

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*Salmonella Typhi* is the most frequently isolated organism in Asia. Besides their isolation rate and their antibiotic susceptibility pattern varies substantially<sup>6</sup>.

Depending on the age, the severity of infection, other risk factors and the mortality rate for BSI varies between 4.0 and 41.5%. Globally, bloodstream infection affects about 30 million people leading to 6 million deaths<sup>7</sup>, with 3 million newborns and 1.2 million children suffering from sepsis annually<sup>8</sup>. Changing patterns of epidemiology, lack of proper antimicrobial guidelines in the locality, the emergence of antimicrobial resistance and paucity of good diagnostic facilities are connected to the surge in BSI associated morbidity and mortality<sup>9</sup>.

Increasing antimicrobial resistance is a worldwide concern. It is a serious challenge for health care professionals in prescribing suitable antimicrobial therapy as many bacterial pathogens have developed resistance to most of the antibiotics<sup>6</sup>. This is specially true in countries like Bangladesh due to lack of national guideline for antibiotic use, absence of good laboratory facilities to do antimicrobial drug susceptibility test and overuse or misuse of antibiotics without any rational prescription. In most of the cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Selection of right antibiotic for empiric therapy is of utmost importance. Continuous monitoring trends in the microbiology of BSI pathogens and their antibiotic susceptibility patterns are therefore important to guide empiric antibiotic treatment strategies and infection control programs.

The present study is aimed to determine the bacterial profile and antimicrobial susceptibility patterns among BSI suspected patients in a referral hospital to assist the health care professionals to choose an appropriate antimicrobial therapy for treatment of BSIs.

### Materials and Methods

For this cross sectional study, a total of 1,675 samples from clinically suspected cases of blood stream infection were collected at Uttara Adhunik Medical College and Hospital, Dhaka for a period of six months from 1<sup>st</sup> July 2020 to 31<sup>st</sup> December 2020. All the samples were collected from inpatient's and outpatient's department of our hospital during the study period and processed in Microbiology laboratory. About 10 ml of venous blood for adults and 2-3 ml for children was collected aseptically using 70% alcohol and 2% tincture iodine and transferred in to

automated blood culture bottles. The BD BACTEC FX40 automated blood culture method was used. In case of a positive growth, the BD BACTEC FX40 automatically gives an alert. Blood culture bottles with no alert signal of bacterial growth after recommended days of incubation is considered culture negative. The positive bottles were sub cultured on MacConkey's agar, blood agar and chocolate agar media. The chocolate agar plates were incubated inside a candle jar to provide 5-10% CO<sub>2</sub>, whereas the other two agar plates (blood agar and MacConkey's agar) were incubated aerobically for 18-24 h at 37°C.

Isolates were further processed according to standard operating procedure (SOP) of the laboratory for its complete identification. Pure cultures of bacterial isolates were subsequently subjected to species identification and confirmation. gram positive isolates were identified using catalase and coagulase tests. Isolates of members of *Enterobacteriaceae* family were identified biochemically by means of a series of tests: catalase, indole, citrate, urease, H<sub>2</sub>S production and triple-sugar iron. Non lactose fermenting Gram negative bacteria were identified by indole, triple-sugar iron, urease, oxidase and catalase tests. Antimicrobial susceptibility tests were performed by using the Kirby-Bauer disc diffusion method and susceptibility patterns were determined following CLSI guidelines<sup>10</sup>. Diameters of the zone of inhibition were measured to the nearest millimeter and categorized as sensitive, intermediate and resistant according to CLSI guidelines<sup>10</sup>. Isolates were classified as either susceptible or resistant to an antibiotic and all the isolates with intermediate resistance were classified as resistant. Culture media and antibiotic discs used in the study were obtained from OxoidLtd., UK.

### Quality control

Reference strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were used as a control reference strains for identification and drug susceptibility testing. Quality control for media was done by randomly taking the prepared culture media and incubating overnight to see for any growth. In this study multi-drug resistance (MDR) was defined as simultaneous resistance to more than two antimicrobial agents. Isolates of *Staphylococcus aureus* were further tested for methicillin resistance according to the CLSI guidelines by using cefoxitin disc.

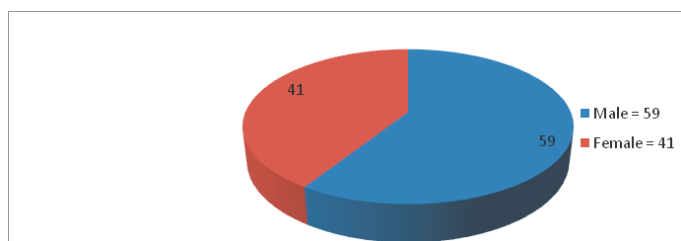
**Results**

**Table-1: Distribution of bacterial growth among all participants (n=1,675)**

Bacterial growth	Number	Percentage
Growth	222	13
No growth	1453	87
<b>Total</b>	<b>1675</b>	<b>100</b>

During the study period, 1,675 blood cultures were analyzed, of which 222 (13%) bacteria were isolated. Of the total positive cases, 132 (59%) were males and 90 (41%) were females giving a male: female ratio of 1.5: 1 (Figure -1).

**Figure-1: Gender variation regarding growth positive cases (n=222)**



**Table-2: Distribution of growth positive cases by age group (n=222)**

Age	No. of growth positive cases (%)
Up to 18	62 (28%)
> 18	160 (72%)

Among 222 blood culture positive cases, 62 patients were up to 18 years of age and 160 patients were above 18 years of age. The rate of isolation was noted higher among age group above 18 years of age when compared to the age group up to 18 years.

**Table-3: Types of bacteria isolated in blood culture (n=222)**

Gram negative organisms	Types of bacteria isolated	No. of isolates (%)
Gram negative organisms	<i>Salmonella Typhi</i>	114 (51%)
	<i>Salmonella Paratyphi</i>	34 (15%)
	<i>Escherichia coli</i>	34 (15%)
	<i>Klebsiella spp.</i>	15 (7%)
	<i>Pseudomonas aeruginosa</i>	9 (4%)
	<i>Acinetobacter spp.</i>	2 (1%)
Gram positive organisms	<i>Staphylococcus aureus</i>	12 (6%)
	<i>Streptococcus pneumoniae</i>	2 (1%)
	<b>Total</b>	<b>222</b>

Table-3 shows the distribution of organisms found throughout this study period. *Salmonella Typhi* was the most frequently isolated blood borne bacterial pathogen in this study, accounting for 51% of the total isolates. Other frequently isolated pathogens were *Salmonella Paratyphi* (15%), *Escherichia coli* (15%), *Klebsiella* species (7%), *Staphylococcus aureus* (6%), *Pseudomonas aeruginosa* (4%), *Acinetobacter* species (1%) and *Streptococcus pneumoniae* (1%).

**Table-4: Antibiotic susceptibility pattern of isolated *Salmonella* species (n=148)**

Organisms	AMC %	CIP %	CFM %	CRO %	C %	SXT %	AZM %	MEM %
<i>Salmonella Typhi</i> (n=114)	80	4	96	100	84	85	83	100
<i>Salmonella Paratyphi</i> (n=34)	93	4	95	100	95	95	68	100

AMC-Amoxiclav, CIP-Ciprofloxacin, CFM-Cefixime, CRO-Ceftriaxone, C-Chloramphenicol, SXT-Cotrimoxazole, AZM-Azithromycin, MEM-Meropenem

*Salmonella Typhi* and *Salmonella Paratyphi* isolates showed 100% susceptibility to meropenem and ceftriaxone. A higher percentage of strains of *Salmonella Typhi* (80%,

84% and 85%) and *Salmonella* Paratyphi (93%, 95% and 95%) were found sensitive to amoxiclav, chloramphenicol and cotrimoxazole indicating that the resistance trend might have started reversing. On the other hand, 83% *Salmonella* Typhi and 68% *Salmonella* Paratyphi were susceptible to azithromycin. Only 4% isolates of *Salmonella* Typhi and *Salmonella* Paratyphi were sensitive to ciprofloxacin which is an alarming situation (Table-4).

**Table-5: Antibiotic susceptibility pattern of isolated *S. aureus* (n=12)**

Organisms	AMC %	DOX %	CIP %	SXT %	CN %	AZM %	CD %	CLOX %
<i>S. aureus</i>	100	100	70	70	100	40	50	100

AMC-Amoxiclav, CIP-Ciprofloxacin, DOX-Doxycycline, CD-Clindamycin, SXT-Cotrimoxazole, CN-Gentamycin, AZM-Azithromycin, CLOX-Cloxacillin

All the isolates of *S.aureus* were sensitive (100%) to amoxiclav, doxycycline, cloxacillin and gentamicin. *S.aureus* isolates were 70% responsive to ciprofloxacin and cotrimoxazole. On the other hand, sensitivity to azithromycin and clindamycin were found 40% and 50% respectively (Table-5).

**Table-6: Antibiotic susceptibility pattern of gram negative isolates**

Organisms	AMC %	CIP %	CXM %	CFM %	CRO %	CAZ %	MEM %	AK %	TET %	ATM %	SXT %	CN %	TZP %
<i>Escherichia Coli</i> (n=34)	25	36	30	30	30	32	95	88	42	29	47	76	85
<i>Klebsiella</i> spp. (n=15)	37	56	44	50	50	44	88	70	55	44	50	62	57

AMC-Amoxiclav, CIP-Ciprofloxacin, CXM-Cefuroxime, CFM-Cefixime, CRO-Ceftriaxone, CAZ-Ceftazidime, MEM-Meropenem, SXT-Cotrimoxazole, AK-Amikacin, TET-Tetracycline, ATM-Aztreonam, CN-Gentamycin TZP-Tazobactam-piperacillin

Antibiotic susceptibility pattern of isolated gram negative bacteria is shown in Table-6. The observed sensitivity of *E. coli* was 95% to meropenem, 88% to amikacin, 85% to tazobactam-piperacillin and 76% to gentamicin. On the other hand, about 88%,70% and 62% *Klebsiella* were sensitive to meropenem, amikacin and gentamycin respectively. Lower percentage of susceptibility were seen in case of *E. coli* (25%,30% and 36%) and *Klebsiella*

(37%,50% and 56%) against amoxiclav, 3<sup>rd</sup> generation cephalosporins and ciprofloxacin respectively.

**Table-7: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=9)**

Organisms	AK %	ATM %	CIP %	LEV %	CAZ %	FEP %	CN %	TZP %	MEM %
<i>Pseudomonas Aeruginosa</i>	75	62	62	62	62	75	62	95	75

CIP-Ciprofloxacin, CAZ-Ceftazidime, LEV-Levofloxacin, AK-Amikacin, ATM-Aztreonam, CN-Gentamicin, TZP-Tazobactam-piperacillin, FEP-Cefepime, MEM-Meropenem

Among the isolates of *P. aeruginosa*, 95% were found to be sensitive to tazobactam-piperacillin and on the contrary, 75% of the *P. aeruginosa* isolates were sensitive to meropenem, amikacin and cefepime (Table-7).

## Discussion

Bloodstream infection (BSI) is a challenging problem and sometimes it may be life threatening; therefore, timely detection, identification and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. There exists a strong relationship 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%<sup>11</sup>.

Knowledge of the hospital epidemiology and the antimicrobial susceptibility pattern of blood isolates help physicians to effectively manage blood stream infections. Bacteria isolation rate in this study was 13%, which is comparable with the result done in India (14%) and Germany (13%)<sup>11-12</sup>. It was however, lower than reports from Ethiopia (28%) and Gambia (34%)<sup>13-14</sup>. The reason may be most of the cases must have taken antibiotics before admission in our hospital and also self-medication is very common in Bangladesh as the antibiotics are commonly available over the counter. Such variation in blood culture positivity across the countries can be explained by various factors such as difference in blood culture system, volume or the number of blood culture samples, geographical location, nature of patient population, epidemiological difference of the etiological agents, and difference in infection control policies among nations<sup>15</sup>.

In our study, more frequent bacteremia cases noted in case of males than females. This may be explained as men are involved in more physical activities for livelihood and less frequent hand hygiene practice which could potentially provide environment for large reservoirs of common pathogens responsible for causing blood stream infections.

In this study, *S. Typhi* (51%) was the predominant gram negative bacteria followed by *S. Paratyphi* (15%), *E. coli*, *Klebsiella* spp. and *S. aureus*. Several studies from Bangladesh have identified *S. Typhi* as a common cause of bloodstream infection in this region and reported *Salmonella* species to be responsible for almost half of the disease burden associated with BSI in Dhaka<sup>16</sup>. More or less similar observations have been seen in cases of bacteremia in different countries, though the proportion and prevalence of the bacterial agents varied<sup>8,15</sup>. As the only source of *Salmonella* infection is the infected human and fecal contamination of drinking water and food supplies, the highest percentage of *Salmonella* isolates in this study indicate the necessity of proper waste management and infection control practices.

Among the isolated *Salmonella* species *Salmonella Typhi* was more prominent than *Salmonella Paratyphi*. There is no such well-established reason behind the variation of *Salmonella* serovars; however, it can be assumed that the higher incidence of *S. Typhi* could be achieved via water-borne transmission as requires smaller inoculum than *S. Paratyphi* which requires larger inoculum via a food-borne transmission<sup>17</sup>.

In this study, we found all *Salmonella Typhi* and *Salmonella Paratyphi* isolates were susceptible to ceftriaxone and meropenem, but a very high level of reduced susceptibility exists against ciprofloxacin (4%). A high percentage of *Salmonella Typhi* (>80%) and *Salmonella Paratyphi* (>90%) isolates appeared to be sensitive to first line antityphoid agents like amoxiclav, chloramphenicol and cotrimoxazole, which is consistent with studies carried out in Nepal and Pakistan<sup>17,18</sup>. This might give us some hope that in future we can again start using these antimicrobials for treatment to *Salmonella Typhi* and *Paratyphi*. Our finding shows 83% *Salmonella Typhi* and 68% *Salmonella Paratyphi* were susceptible to azithromycin. A study from Nepal also reported a low rate of azithromycin resistance among tested antibiotics<sup>17</sup>. Several clinical studies have demonstrated good efficacy of azithromycin

for the treatment of uncomplicated enteric fever in clinical and in vitro studies. On the other hand, a study from Netherland shows a high percentage of increased MICs for azithromycin<sup>18</sup>. In our study, we only used disc diffusion method for azithromycin; MIC was not done which is our limitation. From the susceptibility findings of *Salmonella* it is noticed that first line anti-typhoidal drugs were becoming more sensitive and sensitivity to ciprofloxacin is remarkably decreased. Ceftriaxone and meropenem use should be reserved for the treatment of MDR and XDR cases of enteric fever. It is necessary to understand the importance of proper reporting in context of microbiology, evaluation and monitoring of antimicrobial therapy<sup>18</sup>.

In this study all the isolates of *S.aureus* were found to be sensitive (100%) to cloxacillin, amoxiclav, doxycycline and gentamicin.

In the current study, most of the isolates of *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa* showed extended drug resistance. None of the antibiotic was 100% effective against *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*.

*E. coli* isolates showed <30% sensitivity to 2<sup>nd</sup> and 3<sup>rd</sup> generation of cephalosporins. Resistance to fluoroquinolones remains high. The fact that cephalosporins are one of the most commonly used antibiotics for inpatient's as well as for outpatient's could be the reason for such low degree of sensitivity. Hence, rationalization of treatment strategies is very much warranted considering the local trends of BSIs<sup>12</sup>. The observed sensitivity of *E. coli* was 95% to carbapenems, 85% to tazobactam-piperacillin and 88% to amikacin. Similar findings have been observed across Saudi Arabia and China<sup>19,20</sup>. *Klebsiella* isolates showed sensitivity rate of 88% and 70% to meropenem and amikacin. For the *Pseudomonas aeruginosa* isolates tazobactam-piperacillin (95%), amikacin (75%) and meropenem (75%) were found more active. However other studies from Ethiopia and Nepal showed all the isolates of Gram negative bacteria were susceptible to meropenem<sup>21,22</sup>. Our study shows that multidrug resistant gram negative pathogens are on rise in our hospital.

We acknowledge several limitations of our study, like this was single center study with shorter duration and small number of isolates which may not reflect the true status of the antimicrobial pattern of wider community or even other hospitals.

## Conclusion

Bacterial bloodstream infections are of great concern globally. The knowledge of bacteriological profile and antimicrobial sensitivity patterns of hospital is crucial for effective management of blood stream infection. Results of this study will help in providing useful guidelines for choosing an effective antibiotic in our hospital. Robust infection control practices and antibiotic stewardship programs may help to reduce incidence of blood stream infections as well as prevent the emergence of resistance. Furthermore, research should also focus on diagnostic stewardship, to establish newer, rapid, automated bacterial identification method and sensitivity analysis that will not only help early initiation of appropriate antimicrobial therapy for better patient outcome but will also defer bacterial resistance.

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