BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BLOOD CULTURE ISOLATES AMONG FEBRILE PATIENTS IN A TERTIARY CARE HOSPITAL, BANGLADESH

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Abstract

Background: *Bacterial bloodstream infections (BSI) are a major problem for health care personnel's, which leads to high morbidity and mortality of patients. Early and timely diagnosis and appropriate medication will be the best way to save the lives of affected ones.*

Aim: *The aim of the present study was to determine the bacterial profile of bloodstream infections and their antibiotic susceptibility pattern.*

Methods: *This descriptive cross-sectional study was carried out at the Microbiology Laboratory, Popular diagnostic Ltd, Dhanmondi, Dhaka over a three months periods, from November' 2017 to January' 2018. A total 822 blood culture samples were screened. The positive blood cultures were examined and the organisms were identified as per standard procedures. Antimicrobial susceptibility testing was performed for all isolates by using disk diffusion technique, according to CLSI guidelines 27.*

Results: *From total blood culture samples, 105 (12.77%) were positive. The most common isolated pathogens were Salmonella Typhi, 59 (56.19.5%). Other isolates are* Salmonella paratyphi *A & B, 11(10.47%);* Eschericia coli *14 (13.33%); Klebsiella spp, 05(04.76%); Acinetobacter spp.11 (10.47%) Enterococcus spp. 02 (01.90%);* Staphylococcus aureus*, 02 (01.90%) and one Candida spp (0.95%). S. Typhi showed 100% sensitivity against Ceftriaxone and Cefixime, and also more than 80% sensitive against first-line drugs (Chloramphenicol and Co-trimoxazole). Almost all the strains were found resistant towards Nalidixic acid (sensitivity 05.71%). Most of the* Staphylococcus aureus *and Enterococcus spp were susceptible to Vancomycin and Linezolid. More than 80% of* E. coli *and Klebsiella spp.are sensitive to Imipenem and Meropenem and least sensitivity show against Ciprofloxacin, Cephradine and Ceftriaxone.*

Conclusion: *Ongoing surveillance for antimicrobial susceptibility remains essential, and will enhance efforts to identify resistance and attempt to limit its spread.*

Key words: *Bloodstream infections, Automated blood culture, Antimicrobial susceptibility.*

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Introduction

Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease[1]. It remains one of the most important causes of morbidity and mortality globally[2] and is the most common healthcare-associated infections[3].

The blood culture represents a critical tool for the detection of bloodstream infections. Despite its limitations, the blood culture remains the "gold standard" for the detection of bacteremia[4]. It also provides essential information for the evaluation of a variety of diseases like

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endocarditis, pneumonia, and pyrexia of unknown origin and particularly, in patients with suspected sepsis[5]. An accurate interpretation of culture results is critical not only from the perspective of individual patient care but also from the standpoint of hospital epidemiology and public health[4].

Many bacteria have been reported which cause bacteraemia with variation in distribution from place to place[6]. *Salmonella enterica* serotype Typhi, *Staphylococcus aureus*, *Escherichia coli*, and other Gram-negative organisms are regarded as the leading causes of BSI[7]. Among them, antibiotic resistant strains are emerging with great speed, causing a deep concern to the medical fraternity, and present therapeutic challenges[8]. Such infections result in longer hospital stay, higher costs and death as compared to antibiotic susceptible bacteria[9]. Appropriate antimicrobial therapy has been shown to reduce mortality among patients with gram negative bacteremia and, when initiated early, in critically ill patients with bacteremia[10].

Researches in various countries revealed that there is high bacterial drug resistance to commonly used antibiotics mainly due to the lack of national guideline for antibiotic use in some developing countries. There is also absence of good laboratory facilities to do antimicrobial drug susceptibility test. As a result clinicians use empirical way to treat their patients. There is also high self treatment of humans, and animals without prescription of doctors. These all lead to emergence and rapid dissemination of resistance[2].

In Bangladesh there are only a few studies on organisms involved in bloodstream bacterial infection and their susceptibility pattern. Since the antibiotic resistance pattern can vary with the geographical region, this study is undertaken to determine the common bacterial agents associated with bacteraemia and their antimicrobial susceptibility patterns in febrile patients attending Popular Medical College.

Methods

Study design, study area and sampling process
This descriptive cross-sectional study was carried out at the Microbiology Laboratory, Popular diagnostic Ltd, Dhanmondi, Dhaka over a three months periods, from November'2017 to January' 2018. During this period, total 822 blood samples with suspected bacteremia and history of febrile illness from adult and children (below 18 years) were included.

Data collection and laboratory procedures

About 10 ml of venous blood for adults and 3 ml for children was collected aseptically using 70% alcohol and 2% tincture of iodine and transferred into a BD BACTEC™ bottle. Blood culture broths were then incubated in the automated BD BACTEC™ system at 37°C for 72 hours.

Bacterial identification

The preliminary signal of bacterial growth in BD BACTEC™ bottle was detected. Specific identification of all culture positive samples was accomplished by immediate Gram staining. Then sub-culture was done on Blood agar, Chocolate agar and MacConkeys agar media (OXOID CO. UK). Inoculated Blood agar and MacConkeys agar plates were incubated aerobically at 37^0 C. The Chocolate agar plates were incubated at 37^0 C under 5-10% CO2 condition (Candle jar) and examined after 18-24 hours of incubation.

Bacterial isolates were identified by Colony morphology, Gram staining reaction, biochemical tests using Catalase test, Coagulase test, Oxidase test, Triple Sugar Iron agar (TSI) (OXOID, UK),Citrate utilization test (BBL™),Urease test (BBL™) and Motility Indole Urea (MIU) (BBL™) test and use of antisera for Salmonella for the standard procedure for bacterial identification[11].

Blood culture broths that did not generate any signal within 72 hours of incubation were sub-cultured before being reported as a negative result.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (MHA) media according to Clinical Laboratory Standards Institute (CLSI) guidelines 27[12] and antibiotic disc from OXOID CO. Minimum distance of the disc were 24 mm from center to center. Zone of inhibition were measured in millimeters after 24 hours of incubation.

Based on the zone of inhibition obtained, the isolates were classified into sensitive, intermediate, and resistant pattern.

For each separate group of organisms separate set of antimicrobials were used. The antibiotics discs and their concentrations were as follows:

Amoxyclav (20/10 mcg), Co-trimoxazoleb (1.25/ 23.75 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Nalidixic acid (30 mcg), Ceftriaxone (30 mcg), Cefixime (5 mcg) for S. Typhi and *S. paratyphi.*

Co-trimoxazole (1.25/23.75 mcg), Gentamicin (10 mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Cephradine (30 mcg), Cefoxitin (30 mcg), Ceftriaxone (30 mcg), Ceftazidime (30mcg),

Cefepime (30 mcg), Aztreonam (30 mcg), Imipenem (10mcg), Meropenem (10mcg), Netilmicin (30 mcg) for *E. coli* and Klebsiella spp.

Amikacin (30 mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Ceftriaxone (30 mcg), Ceftazidime (30mcg), Cefepime (30 mcg), Aztreonam (30 mcg), Imipenem (10mcg), Meropenem (10mcg), Piperacillin-Tazobactum(100/10mcg) for Acinetobacter spp.

Ciprofloxacin (5mcg), Levofloxacin (5mcg), Ampicillin (10mcg), Penicillin (10mcg), Gentamicin (10 mcg), Vancomycin (30mcg), Linezolid (30mcg) for Enterococcus spp.

Amikacin (30mcg), Cephradine (30 mcg), Cefoxitin (30 mcg), Cefepime (30 mcg), Cloxacillin (5 mcg), Gentamicin (10 mcg), Vancomycin (30mcg), Linezolid (30mcg) for *S. aureus*.

Quality control

Reference strains *E. coli* (ATCC 25922) and *S. Aureus* (ATCC 25923) were used as a control reference strains for identifications and drug susceptibility testing. Negative control was done by randomly taking the prepared culture media and incubating over night to see for any growth.

Data analysis

SPSS version 20 software was used for statistical analysis. Chi square test ($\div^2$) was used to determine relationship between dependent and independent variable. P value <0.05 was used to indicate significant association.

Results

From the total 822 febrile patients, 105 (12.77 %) were culture positive, 717 (87.22 %) were negative (Table I). Among 105 culture positive patients 68 (71.40%) were males and 37 (38.85%) were females. Their age ranges from 1day – 80 years [mean 26.326 ± 19.506 (SD)]. Predominant isolates were Salmonella Typhi, 59(56.19%) followed by *S. paratyphi A & B* 11(10.47%), *Escherechia coli* 14(13.33), Acinetobacter spp 11(10.47), Klebsiella spp 05(04.76), *Staph aureus* 02(01.90), and Enterococcus spp 02(01.90), Candida spp 01(00.95). (Table IV).

In culture positive samples, 60 (57.14%) were adult and 45 (42.85%) were children (Table II). There were 68 (71.40%) males and 37 (38.85%) females, as shown in (Table III).

Antibiotic susceptibility patterns of bacterial isolates are elaborated in (Table V), (Table VI), (Table VII) (Table VIII) and (Table IX).

Table-I

Rate of culture positive and negative samples

Results of culture	Frequency (N=822)	Percentage (%)
Growth of bacteria (Positive)	105	12.77
No growth (Negative)	717	87.22
Total	822	100

Table - II

Distribution of children and adult in culture positive specimens

Age	Frequency (n=105)	Percentage (%)
Adult	60	57.14
Children*	45	42.85
Total	105	100

* Age below 18 years

Table - III

Distribution of sex in culture positive specimens

Sex	Frequency(n=105)	Percentage (%)
Male	68	71.40
Female	37	38.85
Total	105	100

Table - IV

Distribution of isolated pathogens

Pathogens	Frequency (n=105)	Percentages (%)
Salmonella typhi	59	56.19
Salmonella paratyphi A&B	11	10.47
Escherichia coli	14	13.33
Staphylococcus aureus	02	01.90
Acinetobacter spp.	11	10.47
Klebsiella spp.	05	04.76
Enterococcus	02	01.90
Candida species	01	00.95
Total	105	100

Table V

Antibiotic sensitivity of Salmonella (S. Typhi and S. paratyphi)

Antibiotic	Disc content mcg	S. Typhi (59) Sensitive	S. Typhi (59) Resistant	S. paratyphi A & B (11) Sensitive	S. paratyphi A & B (11) Resistant	Sensitive ≥ mm	Inter mediate (mm)	Resistance ≥ mm
Amox-clav	20/10	39 (66.10%)	20 (33.89%)	11 (100%)	------	18	14-17	13
Co-trimoxazole	1.25/ 23.75	50 (84.74%)	09 (15.25%)	11 (100%)	------	16	11-15	10
Chloramphenicol	30	50 (84.74%)	09 (15.25%)	11 (100%)	------	18	13-17	8
Ciprofloxacin	5	*45 (76.27%)	14 (23.72%)	11 (100%)	------	31	21-30	20
Levofloxacin	5	*45 (76.27%)	14 (23.72%)	11 (100%)	-------	31	21-30	20
Nalidixic acid	30	06 (5.71%)	53 (89.83%)	------	11 (100%)	19	14-18	13
Ceftriaxone	30	59 (100%)	------	11 (100%)	-------	23	20-22	19
Cefixime	5	59 (100%)	------	11 (100%)	------	19	16-18	15

* I=Intermediate sensitive

Table VI

Antibiotic sensitivity of E.coli and Klebsiella spp

Antibiotic	Disc content mcg	E.coli (14) Sensitive	E.coli (14) Resistant	Klebsiella spp (05) Sensitive	Klebsiella spp (05) Resistant	Sensitive ≥ mm	Inter mediate (mm)	Resistance ≤ mm
Co-trimoxazole	1.25/23.75	06(42.85%)						
	08(57.14%)	01(20%)	04(80%)	16	11-15	10		
Gentamicin	10	09(64.28%)	05(35.71%)	01(20%)				
	04(80%)	15	13-14	12				
Ciprofloxacin	5	04(28.57%)	10(71.42%)	02(40%)	03(60%)	21	16-20	15
Levofloxacin	5	04(28.57%)						
	10(71.42%)	03(60%)						
	02(40%)	17	14-16	13				
Cephradine	30	02(14.28%)	12(85.71%)	------	05(100%)	18	15-17	14
Cefoxitin	30	09(64.28%)	05(35.71%)	03(60%)	02(40%)	18	15-17	14
Ceftriaxone	30	06(42.85%)	08(57.14%)	01(20%)	04(80%)	23	20-22	19
Ceftazidime	30	11(78.57%)	03(21.42%)	03(60%)	02(40%)	21	18-20	17
Cefepime	30	11(78.57%)	03(21.42%)	04(80%)				
	01(20%)	25	---	18				
Aztreonam	30	10(71.42%)						
	04(28.57%)	01(20%)	04(80%)	21	18-20	17		
Imipenem	10	13(92.85%)						
	01(7.14%)	05(100%)	-------	23	20-22	19		
Meropenem	10	13(92.85%)	01(7.14%)	05(100%)	-------	23	20-22	19
Netilmicin	30	09(64.28%)	05(35.71%)	05(100%)	-------	15	13-14	8

Table VII

Antibiotic sensitivity of Acinetobacter spp

Antibiotic	Disc content mcg	Acinetobacter spp (11) Sensitive	Acinetobacter spp (11) Resistant	Sensitive ≥ mm	Intermediate mm	Resistance ≤ mm
Amikacin	30	08(72.72%)	03(27.27%)	17	15-16	14
Ciprofloxacin	5	06(54.54%)	05(45.45%)	21	16-20	15
Levofloxacin	5	07(63.63%)	04(36.36%)	17	14-16	13
Cephradine	30	11(100%)	-------	18	15-17	14
Ceftriaxone	30	05(45.45%)	06(54.54%)	21	14-20	13
Ceftazidime	30	08(72.72%)	03(27.27%)	18	15-17	14
Cefepime	30	08(72.72%)				
	03(27.27%)	18	15-17	14		
Aztreonam	30	06(54.54%)				
	05(45.45%)	22	16-21	15		
Imipenem	10	11(100%)	------	22	19-21	18
Meropenem	10	11(100%)	------	18	15-17	14
Piperacillin-tazobactam	100/10	11(100%)	-------	21	18-20	17

Table VIII

Antibiotic sensitivity of Enterococcus spp

Antibiotic	Disc content	Enterococcus Spp (02)		Sensitive	Intermediate	Resistance
	mcg	Sensitive	Resistant	≥ mm	mm	≤ mm
Ciprofloxacin	5	----	02(100%)	21	16-20	15
Levofloxacin	5	----	02(100%)	17	14-16	13
Ampicillin	10	---	02(100%)	17	----	16
Penicillin	10 units	-----	02(100%)	15	----	14
Vancomycin	30	02(100%)	-----	17	15-16	14
Gentamycin	10	-----	02(100%)	15	13-14	12
Linezolid	30	02(100%)	-----	23	21-22	20

Table IX

Antibiotic sensitivity of Staphylococcus spp

Antibiotic	Disc content	Staphylococcus Spp (02)		Sensitive	Intermediate	Resistance
	mcg	Sensitive	Resistant	≥ mm	mm	≤ mm
Amikacin	30	02(100%)	------	17	15-16	14
Cephradine	30	02(100%)	------	18	15-17	14
Cefepime	30	02(100%)	------	18	15-17	14
Cefoxitin	10	02(100%)	-------	18	15-17	14
Cloxacillin	05	02(100%)	-------	22	----	21
Vancomycin	30	02(100%)	------	15	----	----
Gentamycin	10	02(100%)	------	15	13-14	12
Linezolid	30	02(100%)	-----	21	-----	20

Discussion

Bloodstream infection 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[1].

Due to wide variations in bacterial drug resistance, results of studies and reports in one region or in a period of time are not necessarily true for other regions or periods of time[13]. They are related with a series of social, environmental and technological changes[14].

In the developing countries like Bangladesh, physicians prescribe antimicrobial more than the actual need, all kinds of antibiotics are easily available over the counter and anybody can buy drugs without physician's prescription are responsible for developing pool of resistant bacteria as well as negative results of blood culture[15].

In this study, 12.77% bacteria were isolated, that is almost same (14.38%) found in another private diagnostic centre in Dhaka[16]. In another study from Bangladesh, the recovery rate of microbial pathogens among blood cultures was found to be 11.6%[17]. Isolation rate of 20% was reported from a study done in Nepal[18]. This shows that there may be inter country variation.

In the present study, culture positive rate in adult patients was 57.14% which correlate with the study done by Wadud *et al* in 2009[15] which has revealed significantly high rates of blood culture positivity in adult patients (63.51%).

Slightly higher isolation rate in children was reported from Children's Hospital at Myanmar,

where isolation rate in was found as 54.2%[19]. Similar study from Japan found blood culture positivity among paediatric age group as 53.6%[20], which are contradictory to this study where isolation rate in children was 45(42.85%). The reasons behind this outcome might be the selection criteria of samples. We included all samples (indoor and outdoor) came to the study laboratory irrespective of age or may be due to empirical use of broad-spectrum antibiotics before collection of blood samples.

In this study, men had high culture positivity as compared with women. The result was consistent with the study done by Kaur and Singh (2014)[21] who reported high culture positivity in 65.22% men. The finding was also similar to study by Hussein *et al.* (2005)[22] that reported 66.66% positivity in men and 33.33% in women. Men are the active and are the main earning members of most families, so they are more privileged to visit physician chamber for treatment. However, Zenebe *et al.* (2011)[2] reported more high culture positivity in women, 59.2%, than men, 40.8%, in their study.

In the present study most common isolates are Salmonella Typhi (56.19%) followed by *Salmonella paratyphi* (10.47%). In another study in Dhaka, Salmonella spp was the single most common pathogen (72.7%) among the recovered isolates[16]. A Study in BSMMU, Dhaka demonstrates that Salmonella Typhi and *Salmonella paratyphi* isolation rate as 77.97 % and 22.02% respectively[23]. The high rate of isolation of enteric fever in these studies is probably as people consume contaminated water from sewage system. However, the prevalence may be much higher, but empirical use of antibiotic hinders their growth in vivo.

In this study, Salmonelle Spp shows 100% sensitivity to Ceftriaxone and Cefixime. Exceptionally, Nalidixic acid showed low sensitivity (05.31%). In a study in Pakistan show similar sensitivity pattern[24]. These findings were in agreement to a study done in Dhaka Medical College, 2017[25]. Although third generation Cephalosporin, Ceftriaxone is effective, the cost and route of administration makes Ceftriaxone less appropriate for therapeutic use in developing countries such as Bangladesh. Many Salmonella strains seem to be sensitive to fluoroquinolones in vitro (Table V), they do not work as good in vivo because most of them are nalidixic acid resistant due to mutation in QRDR region of gyrA gene[26].

We showed over 80% susceptibility of Salmonella to both Chloramphenicol and Co trimoxazole. Decrease resistance to these antibiotics for Salmonella was similar to studies from India[27] and Nepal[28]. As in many developing countries like Bangladesh conventional first-line drug (Chloramphenicol and Co trimoxazole) have been restricted for almost two decades due to development of resistant strains. This may be due to reduction in the antimicrobial pressure on these organisms cause lost their resistance genes. Studies have shown that if antimicrobial is withheld for a long period the organisms lose their resistance gene. Thus these findings may be helpful to revise current empirical therapy policies for enteric fever. These findings were agreed to a study done in Dhaka Medical College, 2017[24].

In this study, rate of isolation of *Escherechia coli* was 13.33% and Klebsiella spp is 04.76%. . In a study in Myanmar, where *Escherichia coli* was 12.3% that is almost same to our findings[18]. More than 90% *E. coli* and 100% Klebsiella spp. were sensitive to Imipenem and Meropenem. The results for Klebsiella spp. were consistent with the study done by Saghir *et al.* (2009)[29] who reported 96% sensitivity. The results of *E. coli* and Klebsiella spp. were also consistent with the study done by Jyothi *et al.* (2013)[30] who reported sensitivity of 93% for *E. coli* and Klebsiella spp.

In the present study, ceftriaxone showed 42.85% sensitivity to *E. coli*, 20% to *Klebsiella* spp. These findings for *E. coli* and *Klebsiella* spp. were consistent with the studies done by Fayyaz *et al.* (2013)[31] who reported 28% and 22.44% sensitivity respectively. The observation of ceftriaxone resistance pattern is suggestive of the fact that 57.14% *E. coli* and 80% of *Klebsiella* spp. isolates were extended spectrum beta-lactamase (ESBL) producers.

In the present study only 01.90% *Staphylococcus aureus* was isolated. Similar low

(00.28%) isolation rate was observed in another study in Bangladesh[15]. However, in a study in India the isolation rate was high 13 (52%) [1]. The low level of isolation of Staphylococcus may be that most patients start antimicrobials from the very beginning of any symptoms presentation and most of these organisms are sensitive to most of the antimicrobials.

In our study, all the *Staph aureus* were 100% sensitive to Vancomycin, Linezolid, Cefepime, Cefoxitin. This correlates with the sensitivity done in India[1].

Isolation of Enterococcus spp in this study was 01.90% and all Enterococcus spp showed 100% sensitivity to Vancomycin, Linezolid and 100% resistant to Ciprofloxacin, Penicillin. This result is consistant with the sensitivity done by Nikita *et al* (2016)[1].

There was variation in the antibiotic sensitivity rate of various organisms isolated in the present study when compared to different past studies. This may be due to the fact that sensitivity of organisms to antibiotics is variable and depends upon prevalence of strains, antibiotics use, and its resistance patterns in a particular area.

Conclusion

Our study result showed the presence of invasive bacterial pathogens with high rate of resistance to most commonly used antibiotics used to treat bacterial infections. Therefore, timely investigation of bacterial flora of the blood stream infections and monitoring of their antibiotic resistance pattern plays an important role in reduction of the incidence of blood stream infections.

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