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Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Tertiary Care Hospital in Kathmandu, Nepal

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Keywords: blood stream infections, multi-drug resistant, salmonella paratyphi A, janamaitri hospital.

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Abstract- Bacterial blood stream infections can lead to life threatening sepsis that requires rapid antimicrobial treatment otherwise may lead to morbidity and mortality of patients. Blood culture is gold standard technique which provides essential information for the diagnosis and appropriate medication to save life of affected patients. Present study was conducted to determine the bacteriological profile of blood stream infections and their antibiotic susceptibility pattern in patients visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal. A total of 838 blood samples were collected from the clinically suspected cases of bacteremia and septicaemia. Isolates were identified by standard biochemical tests, and antibiotic susceptibility test was performed by using CLSI guidelines. Positive blood culture was obtained in 61/838 (7.28%) where gram negative accounted for 48/61 (78.69%) in which *Salmonella* Paratyphi A was leading organisms and gram positive accounted for 13/61(21.31%) in which *Staphylococcus aureus* was leading organisms. The most effective antibiotics for gram negative organisms was imipenem followed by amikacin whereas for gram positive was vancomycin whereas imipenem and amikacin showed wide range of effectiveness for both organisms. All isolates of *Escherichia coli*, *Morgenella morganii*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptoccus pyogenes* were multidrug-resistant. *Salmonella* Paratyphi B does not showed resistance to more than one drug and found most sensitive organisms among isolated ones.

Keywords: blood stream infections, multi-drug resistant, salmonella paratyphi A, janamaitri hospital.

I. Introduction

Bacteremia is the presence of viable bacteria in the circulating blood. The detection of bacteria in blood is always abnormal. A minor injury occurring during tooth brushing, tooth extraction, abscesses, infected wound or boils, insertion of intravenous of bladder catheter, surgery and existing infections like lung infection, Urinary tract infection (UTI), gastrointestinal tract (GTI), burns or bedsores or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis are the sources of bacteremia and blood culture is required for the detection of it [1]. Bloodstream infection (BSI) is one of the most important causes of morbidity and mortality globally [2]. Detection of bacteremia by rapid and reliable method is by culturing blood. The blood should be collected aseptically before the administration of antibiotics [3]. Septicaemia is a clinical term used to describe severe life-threatening bacteremia in which multiplying bacteria release toxins into the blood stream and trigger the production of cytokines, causing fever, chills, toxicity, tissue anoxia, reduced blood pressure and collapse. Septic shock is usually a complication of septicemia with Gram-negative bacilli, and less frequently, Gram-positive organisms and prompt treatment is essential [4]. Continuous septicemia occurs primarily in patients with intravascular infections like endocarditis, septic thrombophlebitis, infections associated with intravascular catheter, septic shock whereas intermittent septicemia occurs in patients with localized infections like lung, urinary tract, soft tissues infections [5].

Bloodstream infections are potentially life-threatening and require rapid identification and antibiotic susceptibility testing of the causative pathogen. Both Gram positive and Gram negative bacteria causes bacteremia and septicemia. Gram negative septicemia, also known as endotoxic shock, which is more severe than Gram positive septicemia [6].

If the infection is caused by multidrug resistant (MDR) bacteria morbidity and mortality will increase which leads to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them. The infections caused by MDR organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics [7].

In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available by keeping in mind that high mortality and

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morbidity are associated with septicemia and right choice of empiric therapy is of importance [6]. The increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired infections is making numerous classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance [7, 22]. For the treatment, the isolation of bacterium from blood is valuable, but there is also urgent need of antimicrobial therapy, so sample is taken and treatment is started and after blood culture result, patient is treated as redirected by in vitro antibiotic sensitivity test [8, 9]. Therefore, we conducted this study to determine the common bacterial agents associated with bacteremia and their antimicrobial susceptibility patterns in febrile patients visiting in Janamaitri Hospital, Balaju, Kathmandu, Nepal.

II. Materials and Methods

The study was conducted in Microbiology Laboratory of Janamaitri Hospital, Kathmandu, Nepal from March 2014 to April 2015. Written informed consents were obtained from patients prior to their inclusion in the study. A total of 838 blood samples were processed during the research period. Five ml blood sample was collected from each adult, 2-5ml from each child and 0.5-2ml from infant's aseptically using 70% alcohol and 2% tincture of iodine and inoculated immediately into 50ml Brain Heart Infusion (BHI) Broth with 0.025% of sodium polyanethol sulphonate as anticoagulant. In pediatrics cases, 1-2ml of blood was inoculated in 5-10ml of BHI broth. Negative result was followed by examining the broth daily for the sign of bacterial growth (turbidity, haemolysis, clot formation) and by doing final subculture at the end of seventh day. Bottles that showed sign of growth were further processed by Gram stain, followed by subculture on Blood agar, Mac Conkey agar, Manitol salt agar and examined after 18-24 hrs of incubation. Bacterial isolates were identified by colony morphology, Gram staining, catalase test, coagulase test, oxidase test, methyl red/voges-proskauer test (MR-VP), Triple sugar iron agar test, citrate utilization test, Urease test and Sulfur Indole Motility (SIM) test using standard procedure for bacterial identification [10].

a) *Antibiotic susceptibility test*

Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion method following guidelines of Clinical and Laboratory Standard Institute 2012 [11]. The inoculums used for susceptibility testing was prepared in nutrient broth by touching 5/6 colony and matched to 0.5 McFarland standard (1.5 X 10^8 CFU/ml). Within 15 minutes, a sterile cotton swab was dipped into the inoculums suspension and pressed inside the wall of tube above the fluid level and inoculated at 60°C over the dried surface of Muller-Hilton agar (MHA) plate. After 3-5 minutes of inoculation, the antibiotic discs were applied and gently pressed down to ensure complete contact with agar. Organisms which showed resistance to at least one antibiotic among three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [12, 13, 14].

b) *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 [14, 15].

c) *Data analysis*

The data obtained from the research were analyzed by using statistical tools in SPSS-21 version. The chi-square test was used for statistical analysis of data. A 'P' value less than 0.05 was considered as statistically significant.

III. Results

A total of 838 clinical blood samples were collected from the patients attending Janamaitri Hospital, Kathmandu to study the prevalence of bacteraemia and septicemia from 9th August, 2014 to 8th November, 2015.

a) *Ward wise distribution of positive samples*

There were all together 838 blood samples, out of which, 61(7.28%) samples showed growth and rest 777 (92.72%) showed no growth. Again higher percentage of growth was obtained from OPD (Outpatient Department) followed by emergency and wards (Table 1).

Table 1 : Ward wise distribution of positive samples

Types of patients	Growth		Total samples	
	Number	%	Number	%
OPD	41	67.21	476	56.80
Emergency	14	22.95	261	31.15
Wards	6	9.84	101	12.05
Total	61	100	838	100

b) *Pattern of bacteria isolated from blood culture*

Out of 61 bacterial isolates, 48 (78.69%) were Gram negative and 13 (21.31%) were Gram positive bacteria. Among Gram negative bacterial isolates *Salmonella* Paratyphi A 26/48 (54.17%) was found to be most predominant among all bacterial isolates followed by *Salmonella* Typhi (33.33%), *Escherichia coli* (6.25%), *Salmonella* Paratyphi B (4.17%), *Morganella morganii* (2.08%). In Gram positive isolates *Staphylococcus aureus* 5/13(38.46%) was most predominant followed by coagulase-negative staphylococci (CNS) 4/13 (30.77%), *Streptococcus pyogenes* 2/13 (15.38%) and *Enterococci* 2/13 (15.38%) (Table2).

Table 2 : Pattern of bacteria isolated from blood culture gender wise

Bacteria	Number	Percentage	Male		Female	
			Number	%	Number	%
Gram negative						
Escherichia coli	3	6.25	1	2.08	2	4.17
Morgenella morganii	1	2.08	1	2.08	0	0
Salmonella Paratyphi A	26	54.17	18	37.50	8	16.67
Salmonella Paratyphi B	2	4.17	2	4.17	0	0
Salmonella Typhi	16	33.33	9	18.75	7	14.58
Total	48	78.69	31	64.58	17	35.42
Gram positive						
Staphylococcus aureus	5	38.46	3	23.08	2	15.38
CNS	4	30.77	2	15.38	2	15.38
S. pyogenes	2	15.38	2	15.38	0	0
Enterococci	2	15.38	0	0	2	15.38
Total	13	21.31	7	54.85	6	46.15

c) *Age wise distribution of the isolates*

Out of 61 bacterial isolates, highest number of bacteria was isolated from age group 16-30 years 33(54.10%) followed by the age group 31-45 years 13(21.31%) and age group 76-90 years was found to be least affected 1(1.64%). Age group 16-30 years was most affected by wide range of bacteria (Table3).

Table 3 : Age wise and Ward wise distribution of the isolates

Bacteria	Age group (year)						OPD		Emergency		Wards	
	1-15	16-30	31-45	46-60	61-75	76-90	Number (%)		Number (%)		Number (%)	
Gram Negative Bacteria												
Escherichia coli	0	3	0	0	0	0	1	2.08	2	4.17	0	0
Morgenella morganii	0	1	0	0	0	0	0	0	0	0	1	2.08
Salmonella Paratyphi A	0	16	6	3	1	0	19	39.58	6	12.50	1	2.08
Salmonella Paratyphi B	1	1	0	0	0	0	1	2.08	1	2.08	0	0
Salmonella Typhi	2	7	4	2	0	1	12	25.00	3	6.25	1	2.08
Total	3	28	10	5	1	1	33	68.75	12	25.00	3	6.25
Gram Positive Negative												
Staphylococcus aureus	1	2	1	0	1		3	23.08	1	7.69	1	7.69
CNS	0	1	2	0	1	0	2	15.38	1	7.69	1	7.69
Streptococcus pyogenes	0	1	0	1	0	0	1	7.69	0	0	1	7.69
Enterococci	1	1	0	0	0	0	2	15.38	0	0	0	0
Total	2	5	3	1	2	0	8	61.54	2	15.38	3	23.08

d) *Seasonal variation on the prevalence of bacterial growth*

Out of 48 Gram negative organisms isolated, 20(41.67%) showed growth in summer season and followed by autumn season 20 (41.67%). Growth in winter was 4/48 (8.33) and in spring was 4/48 (8.33%). Out of 13 Gram positive bacterial isolates, 6/13(46.15%) showed growth in summer and followed by spring season 4/13(30.77%). Growth in winter was 2/13(15.38%) and in autumn was 1/13(7.69%). Sample showed highest growth in summer and autumn season (Table 4).

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Table 4 : Seasonal variation on the prevalence of bacterial growth

	Season			
	Summer	Autumn	Winter	Spring
Gram Negative Bacteria				
Escherichia coli	1	0	1	1
Morgenella morganii	0	0	0	1
Salmonella Paratyphi A	9	14	2	1
Salmonella Paratyphi B	0	1	1	0
Salmonella Typhi	10	5	0	1
Total	20 (41.67%)	20(41.67%)	4 (8.33%)	4 (8.33%)
Gram Positive Bacteria				
Staphylococcus aureus	3	1	0	1
CNS	1	0	1	2
S. pyogenes	1	0	0	1
Enterococci	1	0	1	0
Total	6 (46.15%)	1 (7.69%)	2 (15.38%)	4 (30.77)

e) Antibiotic resistance pattern of Gram negative bacteria

Out of 838 blood samples, 48 different Gram negative bacteria were isolated. Among the isolates, *Salmonella* Paratyphi A was most predominant followed by *Salmonella* Typhi. Five different bacteria were isolated during study period. Antibiotic susceptibility was performed on them in which imipenem was found most effective followed by amikacin (Table 5).

Table 5 : Antibiotic susceptibility pattern of Gram negative isolates

Gram Negative Bacteria	Antibiotic susceptibility pattern (%) / number											
	AMX	COT	CN	OF	CIP	C	CFM	NA	CTX	GEN	AK	IPM
Escherichia coli (3)	(33.3) 1	(66.67) 2	(33.33) 1	(66.66) 2	(33.33) 1	(33.3) 1	(33.3) 1	-	(100) 3	(100) 3	(100) 3	(100) 3
Morgenella morganii (1)	-	-	-	-	-	100 1	100 1	-	100 1	100 1	100 1	(100) 1
Salmonella Paratyphi A (26)	(96.15) 25	(50) 13	(88.46) 23	(73.08) 19	(73.08) 19	(84.62) 22	(100) 26	-	(100) 26	(96.15) 25	(96.15) 25	(100) 26
Salmonella Paratyphi B (2)	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	-	(100) 2	(100) 2	(100) 2	(100) 2
Salmonella Typhi (16)	(87.50) 14	(62.50) 10	(81.25) 13	(75.00) 12	(75.00) 12	(75.00) 12	(81.25) 13	-	(93.75) 15	(87.50) 14	(68.75) 11	(100) 16

AMX=Amoxycillin, COT=Cotrimoxazole, CN=Cefalexin, OF=Ofloxacin, CIP=Ciprofloxacin, C=Chloramphenicol, CFM=Cefixime, NA=Nalidixic acid, CTX=Cefotaxime, GEN=Gentamicin, AK=Amikacin, IPM=Imipenem

f) Antibiotic resistance pattern of Gram positive bacteria

Out of 838 blood samples, only 13 samples showed growth of Gram positive bacteria. Isolated bacteria were subjected to antibiotic susceptibility test (Table 6).

Table 6 : Antibiotic susceptibility pattern of Gram-positive isolates

Gram positive bacteria	Antibiotic susceptibility pattern (%) / number											
	P	AMP	C	CIP	GEN	MET	E	VAN	PIP	IPM	AK	CLO
Staphylococcus aureus (5)	0	(20) 1	(40) 2	(40) 2	0	(60) 3	(60) 3	(100) 5	(100) 5	(100) 5	(80) 4	(20) 1
CNS (4)	0	(50) 2	(25) 1	(50) 2	(75) 3	(50) 2	(75) 3	(100) 4	0	(50) 2	(75) 3	(50) 2
Streptococcus pyogenes (2)	0	(50) 1	(50) 1	0	(50) 1	(100) 2	(100) 2	(100) 2	(50) 1	(100) 2	(100) 2	(50) 1
Enterococci (2)	0	0	(50) 1	0	(50) 1	(100) 2	(100) 2	(100) 2	0	(100) 2	(100) 2	0

P= Penicillin, AMP= Ampicillin, C=Chloramphenicol, CIP= Ciprofloxacin, GEN= Gentamicin, MET=Methicillin, E=Erythromycin, VAN=Vancomycin, PIP=Piperacillin, IPM=Imipenem, AK=Amikacin, CLO=Cloxacillin *g) Multi drug resistance of bacterial isolates*

Out of 48 Gram neative isolates 15 strains (31.25%) were resistant to ≥3 antibiotics and were considered as multidrug resistant. It was found that 16 (33.33%) isolates were resistant to 1 antibiotic and 17(35.42%) isolates were resistant to 2 antibiotics. Among the MDR strains, 43.75% (7 out of 16) of *Salmonella* Typhi were found to be MDR. Similarly 15.38% (4 out of 26) of *Salmonella* Paratyphi A were found to be MDR. All the isolates (100%) of *Escherichia coli, Morganella morganii*, and *Staphylococcus aureus* were found to be MDR (Table 7).

Table 7 : MDR pattern of the bloodstream isolates

Organisms	Number	Number of bacteria resistance to			MDR	
Gram Negative Bacteria		1 antibiotic	2 antibiotic	≥3 antibiotics	Number	%
Escherichia coli	3	0	0	3	3	100
Morgenella morganii	1	0	0	1	1	100
Salmonella Paratyphi A	26	9	13	4	4	15.38
Salmonella Paratyphi B	2	2	0	0	0	0
Salmonella Typhi	16	5	4	7	7	43.75
Gram positive						
Staphylococcus aureus	5	0	0	5	5	100
CNS	4	0	0	4	4	100
Streptococcus pyogenes	2	0	0	2	2	100
Enterococci	2	0	1	1	2	50.00

IV. Discussion

Blood culture is a well-established procedure of the standard diagnostic workup for many infectious diseases. In the countries like Bangladesh, where all kinds of drugs including the antibiotics, are sold over the counter, misuse of antibiotics has been found to be responsible for developing pool of resistant bacteria as well as negative results of blood culture [16]. Blood culture is employed for the detection of bacteremia and septicemia in blood. Blood stream infection is one of the main agent causing morbidity and mortality worldwide. Urgent and effective treatment is required to manage blood infections [17].

In the countries like Nepal, the overuse of antibiotics, random use of antibiotics as hit and trial method by clinicians without proper sensitivity test, unawareness of people about emergence of antibiotics resistance, random use of antibiotics without advice of physicians, prolonged intensive care unit (ICU) stay, nursing home residency, severe illness, use of instrumentation or catheterization etc. are the major causes of drug resistance in our country. The less growth percentage may due to previous exposure of patients to used antibiotics that hindered their growth or dominance of organism's growth [18].

In the present study the isolation rate was (61/838) 7.28% which was comparable to those study conducted by Karki et al where 4.2% were culture positive [19]. Gram negative bacteria were common organisms isolated during this study accounting 48/61 (78.69%). Among Gram negative isolates the most common was *Salmonella* Paratyphi A 26/48 (54.17%) followed by *Salmonella* Typhi 16/48 (33.33%). Similar finding was seen from the studies in Kathmandu Model hospital Nepal, where 71% of total isolates from blood were *Salmonella* Typhi and 16% of the total isolates were *Salmonella* Paratyphi A [20]. In this study the isolation rate was highest in age group between 16-30 years 33/61 (54.10%) followed by age group 31-45 years 13/61 (21.31%). Similar study conducted in showed that the isolation rate was highest in age group between 21-40 (28%) followed by 41-60 (24%) [21]. In this study, among Gram positive bacteria the most common isolated bacteria was *Staphylococcus aureus* 5/13 (38.46%) followed by CNS 4/13 (30.77%).

The most effective antibiotic in Gram negative bacteria was imipenem followed by amikacin. The others effective drugs were cefotaxime and gentamycin. Similar study conducted in Nepal in KIST Medical College by Surya et al showed that imipenem and amikacin followed by gentamycin were most effective antibiotics for the treatment of *Escherichia coli* and *Klebsiella pneumoniae* [22]. Those organisms which showed resistance to at least one agent in three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [23].

In the present study among Gram negative bacteria, 100% of *E. coli* and *Morgenella morganii* isolates were MDR, followed by *Salmonella* Typhi (43.75%) and *Salmonella* Paratyphi A (15.38%). None of the isolates of *Salmonella* Paratyphi B was found to be MDR. Similar study conducted in Nepal showed that 96.10% of the *E. coli* isolates were MDR whereas 44.8% of the *Salmonella* isolates were MDR in Ghana [22, 24].

In this study, among Gram positive bacteria*, S. aureus*, CNS and *Streptococcus pyogenes* were found 100% MDR whereas 50% of *Enterococci* isolates were MDR. Similar study conducted in Ethiopia showed that 100% of the *S. aureus* and CNS were MDR whereas none of the *S. pyogenes* isolates were MDR [2]. In the present study vancomycin is the antibiotic of choice for the treatment of Gram positive bacterial isolates followed by imipenem and amikacin. Higher rate of infection by *Salmonella* species may be due to unhygienic practices, contaminated food and drinking water. The prevalence of typhoid was higher in summer and autumn season which may be due to contaminated water and food [25, 26].

In this study, Imipenem and Amikacin were found most effective for Gram negative isolates whereas vancomycin was found most effective for Gram positive bacteria followed by imipenem and amikacin. These three antibiotics should not be used indiscriminately and kept as reserve drug because if resistance is developed then treatment will be complicated.

V. Conclusion

Salmonella Paratyphi A and *Salmonella* Typhi was the major Gram negative organisms causing blood stream infections whereas *Staphylococcus aureus* and CNS was the major Gram positive organisms. The antibiotic resistance pattern varies according to geographical location, country to country and even institute to institute in the same country and continuously changes over time so determination of antibiotic sensitivity pattern in periodic intervals is mandatory for choosing appropriate antibiotics for the treatment. The antibiotic susceptibility pattern of this study suggested that vancomycin is the drug of choice for Gram positive bacteria while imipenem and amikacin for Gram negative bacteria but the later drugs showed wide coverage for Gram positive organisms too.

VI. Acknowledgements

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