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Bacteriologic Profile and Antibiogram of the Blood Culture Isolates in Febrile Children

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Abstract:

Bacteraemia is a common condition in children with a resultant high morbidity and mortality. There are limited data on the etiology and characteristics of bloodstream infections in children.

Objectives: *The aim of this study was to determine the bacteriological profile and antibiotic sensitivity pattern of blood culture isolates.*

Methods: *Blood cultures from 2831 febrile children below 15 years of age presenting to Kanti Children Hospital, Kathmandu; Nepal from June 2011 to April 2012 were examined. The growths from subcultures were identified by conventional biochemical tests and serological tests. Antibiotic susceptibility testing to antibiotics was performed by Kirby Bauer disc diffusion method.*

Results: *The positivity of blood culture was 5% (142/2831). Gram positive and gram negative bacteria constituted 59.1% and 40.9% respectively. The most prevalent bacterial isolates were Staphylococcus aureus (52.1%) and Escherichia coli (23.2%). The study showed that the most effective drugs against S. aureus were Aamikacin and Chloramphenicol and against E. coli were Amikacin, Chloramphenicol and Ciprofloxacin. The most sensitive and preferable among the tested antibiotics was Chloramphenicol and Cephalosporins were most ineffective drugs.*

Conclusion: *This study highlights the variable nature of antibiotic susceptibility patterns and stresses the need for continuous screening and surveillance of antibiotic resistance in pediatric care unit.*

Keywords: ***Bloodstream infection, S. aureus, Multidrug resistance, Children.***

Introduction

Infants and children are among the most vulnerable population groups to contract illnesses.[1] In paediatric age group, bloodstream infections are very common and these are one of the common causes of mortality and morbidity in children and neonates. In developing countries the rate of blood stream infections is about 20%-50%.[2,3] Microorganisms present in the circulating blood whether continuously intermittently are threat to every organ in the body. Early diagnosis and appropriate treatment of these infections can make the difference between life and death.[4] The etiology of bloodstream infection in febrile patients is poorly characterized in Nepal, mainly due to limited laboratory resources, a poor recording system and an inadequate number of trained personnel.[5,6] Studies in different regions have suggested the varying microbiological pattern of bloodstream infections in children which warrants the need for an ongoing review of the causative organisms.[1] Blood cultures are the "gold standard" of BSI diagnosis and are based on the detection of viable microorganisms in the blood.[7] Due to evolving antimicrobial resistant patterns there is the need for constant antimicrobial sensitivity surveillance. The determination of the bacterial profile and their antibiotic sensitivity pattern will guide in the infection control and rational use of antibiotic. This will help clinicians provide safe and effective empirical therapies, develop rational prescription programs and make policy decisions and finally assess the effectiveness of all.[8,9]

As bacteriological profile of bloodstream infection in children is poorly characterized in Nepal. Moreover, antibiotic sensitivity pattern to common bacterial pathogen has been changing. It has been necessary to study about antibiotic sensitivity pattern in periodic intervals in each region for choosing appropriate antibiotic therapy. Therefore, the purpose of this study was to identify the bacteria causing bloodstream infections bacteremia in children and to determine and analyses their antibiotic sensitivity pattern.

Materials and Methods

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This prospective study was carried at Kanti Children Hospital, Kathmandu; Nepal from June 2011 to Apri 2012. Blood for culture was collected from 2832 clinically suspected cases following strict aseptic precautions. 1 ml (neonates) and 2ml (children) blood was collected and inoculated into 10 ml and 20ml respectively, of brain heart infusion (BHI) broth. The culture bottles (BHI) were incubated at 37oC for 5 days aerobically and subculture was done onto MacConkey agar, Blood agar and Chocolate agar after each 24 hours of incubation for up to 5 days. The bacteria were identified by conventional biochemical tests and serological tests.

Antibiotic sensitivity testing was performed by Kirby-Bauer's disc diffusion technique using on Muller Hinton Agar (MHA). Antibiotic discs used in this study included Amikacin (10µg), Amoxycillin (25µg), Eyrthromycin (5µg), Cefixime (10µg), Ceftriaxone (10µg), Ceftazidime (10µg), Chloramphenicol (10µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg), Nalidixic acid (30µg), Ofloxacin (30µg) and Cloxacillin (10µg). Statistical analysis was done by using SPSS 16.0. The variables investigated were age, sex of patients, microbial species and drug sensitivity pattern.

Results

Among a total of 2831 blood samples cultured 142(5%) were positive samples. The total culture positive females were 53 (37.3%) and males were 89 (62.7%). Out of 142 isolates 84 (59.1%) were gram positive bacteria and 58 (40.9%) were gram negative bacteria. Six different species of gram positive isolates were identified. The major gram positive isolate was *Staphylococcus aureus* (52.1%) followed by *Streptococcus pyogenes* (2.8%) and Coagulase Negative Staphylococci (2.1%). A single isolate each of viridans Streptococci, *Streptococcus pneumoniae and Streptococcus fecalis* was isolated. Seven different species of gram negative bacteria were isolated. Among them the major gram negative isolate was *Escherichia coli* (23.2%) followed by *Salmonella typhi* (7.9%), *Salmonella* paratyphi A (3.5%), *Klebsiella spp* (2.8%),*Enterobacter spp* (2.1%), *Pseudomonas aeruginosa* (0.7%) and *Proteus vulgaris* (0.7%).

Table 1: Distribution of growth positive cases by sex

Gender	No. of cases		Culture positive cases	
	Number	%	Number	%
Female	1039	36.7	53	37.3
Male	1792	63.3	89	62.7
Total	2832	100	142	100

The rate of isolation was highest among neonates (65/142, 45.8%) followed by 1yr -5yr age group 29/142, 20.6%). The most common isolate among infants was *Staphylococcus aureus* (36/65, 55.3%), followed by *E. coli* (20/65, 30.3%) *and Klebsiella spp.* (2/65, 3%). The overall rate of isolation reduced with increasing age and the overall growth positive rate was relatively higher in males 62.7% as compared to females 37.3%.

Table 2: Isolated organisms and age wise distribution of the isolates

Age	Ec	Sa	K	St	Spa	Vs	SP	Sf	Spy	Pa	E	Pv	Cons	Total
<1m	20	36	2	0	0	1	1	1	1	0	1	1	1	65
1m-1yr	6	17	0	0	0	0	0	0	0	0	2		1	26
1-5yr	4	12	1	7	2	0	0	0	2	1	0	0	0	29
5-15yr	3	9	1	4	3	0	0	0	1	0	0	0	1	22
Total	33	74	4	11	5	1	1	1	4	1	3	1	3	142

Ec- *E. coli;* Sa- *Staphylococcus aureus; K- Klebsiella spp.;* St-*Salmonella* typhi; Spa-*Salmonella* paratyphiA; Vs-Viridans Streptococci; SP- *Streptococcus pneumoniae*; Sf-*Streptococcus fecalis*; Spy- *Streptococcus pyogenes*; Pa-*Pseudomonas aeruginosa*; E- *Enterobacter spp.*; Pv-*Proteus vulgaris*; Cons- Coagulase Negative Staphylococci

Table3: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotics Used	Antibiotic susceptibility pattern						Total Isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amikacin	65	87.8	4	5.4	5	6.8	74
Cefixime	11	14.9	3	4.1	60	81.1	74

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Ceftazidime	9	12.2	2	2.7	63	85.1	74
Chloramphenicol	57	77	4	5.4	13	17.6	74
Ciprofloxacin	41	55.4	9	12.2	24	32.4	74
Cloxacillin	22	29.7	0	0	52	70.3	74

Table 4: Antibiotic susceptibility pattern of *Escherichia coli*

Antibiotics Used	Antibiotic susceptibility pattern						Total Isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amikacin	24	72.7	4	5.4	5	15.2	33
Cefixime	7	21.2	2	6.1	24	72.7	33
Cefotaxime	12	36.4	2	6.1	19	57.6	33
Ceftazidime	4	12.1	3	9.1	26	78.8	33
Chloramphenicol	22	66.6	4	12.1	7	21.2	33
Ciprofloxacin	20	60.6	7	21.2	24	32.4	33

Table 5: Multidrug resistant isolates

Organisms isolated	Total isolates	Multidrug resistant isolates	
		Number	%
Escherichia coli	33	6	18.2
Staphylococcus aureus	74	11	14.9
Total	107	17	11.9

S. aureus was found to be most sensitive to Amikacin and Chloramphenicol with their efficacy rate of 87.8% and 77% respectively and least sensitive to Ceftazidime (12.2%), Cefixime (14.9%) and Cloxacillin. *E. coli* was found to be most sensitive to Amikacin (72.7%), Chloramphenicol (66.6%) and Ciprofloxacin (60.6%) and least sensitive to Ceftazidime (12.1%). (Table 3, 4 and 5)

All of the *Salmonella* typhi isolates were sensitive to Chloramphenicol and Amoxycillin. 27.3% (3) of the total isolates were resistant to Nalidixic acid, which were also resistant to Ciprofloxacin and Ofloxacin. Similarly, all of the *Salmonella* paratyphiA isolates were susceptible to Chloramphenicol and 80% (4) of the isolates were sensitive to Amoxycillin. However 80% of the isolates were resistant to Nalidixic acid, 60% isolates (3) were resistant to Ciprofloxacin and 40% (2) of the isolates were resistant to Ofloxacin. The Cephalosporins were least effective drugs against them. (Table 6 and 7)

Table 6: Antibiotic susceptibility pattern of *Salmonella* typhi

Antibiotics Used	Antibiotic susceptibility pattern						Total Isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	11	100	0	0	0	0	11
Ceftazidime	4	36.4	2	18.2	5	45.5	11
Chloramphenicol	11	100	0	0	0	0	11
Ciprofloxacin	8	72.7	0	0	3	27.3	11
Nalidixic Acid	8	72.7	0	0	3	27.3	11
Ofloxacin	8	72.7	0	0	3	27.3	11

Table7: Antibiotic susceptibility pattern of *Salmonella* paratyphi A

Antibiotics Used	Antibiotic susceptibility pattern						Total Isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	4	80	1	20	0	0	5
Ceftazidime	2	40	1	20	2	40	5
Ceftriazone	3	60	1	20	1	20	5
Chloramphenicol	5	100	0	0	0	0	5
Ciprofloxacin	2	40	0	0	3	60	5
Nalidixic Acid	1	20	0	0	4	80	5
Ofloxacin	3	60	0	0	2	40	5

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Klebsiella spp. was found to be most sensitive to Amikacin (100%), Ofloxacin (100%) and Chloramphenical (100%). All of the 4 isolates of *Klebsiella spp.* were resistant to Cephlaosporins-Cefixime, Cefotaxime and Ceftazidime. *Streptococcus pneumonia* was found to be most sensitive to Penicillin, Chloramphenicol (100%) followed by Ampicillin and Erythromycin (83.3%). Viridans Streptococci was found to be most sensitive to Chloramphenicol (100%) followed by Erythromycin (80%) and Penicillin (75%).

Chloramphenicol was most effective antibiotic (efficacy 100%) against *Streptococcus pyogenes* and *Enterobacter spp.* and the Cephalosporins were least effective (efficacy 0%). All of the Coagulase Negative Staphylococci (CoNS) isolates were sensitive to Amikacin, Ciprofloxacin and Cefotaxime. The efficacy of Chloramphenicol against Coagulase Negative Staphylococci was 66.7%. Except for the isolates of *Streptococcus fecalis* and *Pseudomonas aeruginos* all of the other isolates- Virdans Streptococci, *Streptococcus pneumoniae, Proteus vulgaris and Serratia marcescens* were sensitive to Chloramphenicol. CoNS, *Pseudomonas aeruginosa, Streptococcus pneumoniae, Proteus vulgaris* and *Enterobacter spp* isolates in majority were found sensitive to Fluroquinolones. The efficacies of different Cephalosporins, Cefixime, Cefotaxime, Ceftazidime and Ceftriazone among the tested organisms were found to be 17.4%, 34.8%, 14.9% and 46.7% respectively and were least effective drugs among tested organisms. In this study the incidence of Multi Drug Resistance (MDR) was 11.9% (17/142). MDR strains were observed only among *E.coli* strains (6/33, 18.2%) and *Staphylococcus aureus*(11/74, 14.9%).

Discussion

Among the 2831 blood specimen collected from suspected febrile patients who presented persistent fever, 5% (142) cases were culture positive. The overall incidence of confirmed bacteraemic cases was 50.15 per 1000 cases. This overall positive rate was relatively low but similar results were observed in different studies conducted in Nepal. Similar culture positivity results were obtained in other studies conducted in Nepal. Culture positivity observed in suspected cases of bacteraemia as in different studies as 6.9%, 4.2% and 7.5% which was similar to present study.[1, 2 &10] The low incidence of positive cases is quite common in such studies because of our reliance upon a single blood culture for diagnosis, due to the likely possibility of self-medication before presentation for physician's care prior to sample collection or it may be because of low concentration of circulating bacteria in blood. The present study included children from the neonatal to 14 years of age. In this study, the rate of isolation was found highest among newborns (45.8%) followed by children of 1yrs-5yrs age group (20.4%) and the overall rate of isolation was reduced with increasing age. Similar results were reported in a study done in Nigeria where the rate of isolation was 50.8% among newborns[8] and also study done in Kathmandu, Nepal where the incidence of bacteraemia was 64% among neonates.[2] On statistical analysis, culture-confirmed bacteraemia and age were found significantly associated which means the culture positivity is dependent on age groups. In the neonates, under-developed immune system and lack of hygienic practices during delivery may account for higher incidence of bloodstream infections among them. The overall growth positive rate was relatively higher in males 63.3% as compared to females 36.7%. However, on statistical analysis culture positive and culture negative rate was found independent of gender. This gender wise difference in the prevalence of bacteraemia may be because of higher number of female patients or it may be due to comparatively small sample size. The gram positive bacteria accounted for 59.1% of the total cases while gram negative bacteria accounted for 40.9% of the total blood culture isolates. Among all of the isolates, *Staphylococcus aureus* (51.7%) and *E. coli* (23.1%) were predominant.

Study conducted in Nigeria found that *Staphylococcus aureus* was isolated in 48.7% and *E. coli* in 23.4% as the most frequent isolates.[8]This suggests that infections by these agents constitute a significant threat to child survival in developing countries. However, *Salmonella* typhi and *Salmonella* paratyphi A was found as the most common blood culture isolate.[2,11] Group A streptococci, coliform organisms, and *Staphylococcus aureus* have dominated during different periods but studies have shown major changes in the etiology of bacteraemia.[12]

The different blood culture isolates showed varying degree of resistance and susceptibility to the tested antibiotics. A re-emergence of sensitivity to the classical first-line agents (eg Amoxycillin and Chloramphenicol) has been seen due to their restricted use in the "Ciprofloxacin era" of the 1990s. It suggests that the effectiveness of such preliminary drugs is increasing along with improvement in susceptibility of isolates towards Chloramphenicol and create a path for them to be re-considered as one of the drugs of choice for treatment. This study highlights the variable nature of antibiotic susceptibility patterns Therefore; it is advisable to continuously evaluate the sensitivity-resistance pattern of isolates so as to make a rational use of antibiotics.

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