BACTERIOLOGICAL PROFILE OF BLOOD STREAM INFECTIONS AMONG FEBRILE PATIENTS ATTENDING A TERTIARY CARE CENTRE OF WESTERN NEPAL

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"Ciprofloxacin and gentamicin can be used as empirical therapy for blood stream infections."

ABSTRACT

Background

Blood stream infections (BSI) are significant cause of morbidity and mortality in the world. This study was conducted to determine the common bacterial agents associated with BSI with their antimicrobial susceptibility patterns in a tertiary care centre in the Western region of Nepal.

Method

This cross-sectional study was conducted for a period of two years from May 2010 to May 2012. All patients with fever (temperature $\geq$ 38°C) were included in the study.

Results

A total of 4,145 patients with febrile illness were included in this study, bacterial agents were isolated in 251 (6%) cases. Common bacterial isolates were *Salmonella spp., Klebsiella pneumoniae, Escherichia coli, Pseudomonas* species*, Acinetobacter* species, *Staphylococcus aureus* and Coagulase negative *Staphylococci*. Paratyphoid fever (Caused by *S. Paratyphi A*) is more common than typhoid fever. The members of Enterobacteriaceae were found to be resistant to ampicillin and cefazolin. Majority of the non-fermenters were found to be sensitive to most antibiotics. Gentamicin and Ciprofloxacin were sensitive to majority of gram positive bacteria.

Conclusion

Gram-negative bacteria were the predominant causes of BSIs. The occurrence of drug resistance among the isolated bacteria is of great concern. Imipenem showed 100% sensitivity against *Pseudomonas aeruginosa* indicating lack or low level of MBL activity.

Key Words: Blood stream infections, enteric fever, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella paratyphi A.*

INTRODUCTION

Blood is sterile body fluid and its sterility is maintained by various antimicrobial substances present in it. Presence of microorganisms in the circulating blood is threat to every organ. Various infections at different primary anatomical sites such as genitourinary tract, respiratory tract, surgical sites and abscesses often result in BSI and fever is the commonest presentation.

BSIs are significant cause of morbidity and mortality worldwide[1]. Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50 % [2]. It has been estimated that, in United States, 2 million patients every year acquire infections during their hospital stay; approximately 350,000 (10–20%) of these infections involve the bloodstream and 90,000 (4.5%) are fatal [2,3]. In a study from Nepal, gram-negative bacteria were found to be predominant causes of BSIs. *Salmonella spp.*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the common etiological agents of BSIs [4].

BSIs can be healthcare associated or community-associated. BSIs are defined as health care associated if it occurred more than 48 hours after hospital admission or was associated with the presence of an indwelling medical device or it occurred within thirty days of a surgical procedure (where the bloodstream infection was related to the surgical site infection). BSIs were defined as community-associated if it manifested within 48 hours after admission to the hospital unless an organism with a long incubation period was isolated [1].

Increasing resistance among pathogens is worrisome for the clinicians to start empirical therapy especially in the developing countries like Nepal, where enough laboratory data are not available on regular basis for monitoring and formulating antibiotic policies. Isolation of the pathogen by blood culture, identification and antibiotic susceptibility pattern of the isolate are important steps in the diagnosis and management of the BSI. Rapid clinical diagnosis and early empirical antibiotic therapy can significantly reduce the mortality rate in BSI. Selection of antibiotic for empirical therapy by a clinician requires adequate knowledge about the etiological agents as well as their antimicrobial susceptibility pattern in various geographical areas. In developing countries like Nepal, many of the infections including BSI are poorly diagnosed because of limited diagnostic resources.

Febrile illness is one of the most common complain among patients for medical attention in our hospital but information regarding frequency of specific infections is limited. Therefore, we conducted this study which was mainly focused to determine various bacterial agents and to get the currents updates of antimicrobial resistance pattern in a tertiary care centre of Western Nepal. Results of this study would be helpful for clinician of this particular area to start early empirical therapy for the management of BSI and also in minimizing the spread of antimicrobial resistance among the blood pathogens.

MATERIALS AND METHODS

This retrospective study was conducted between May 2010 to May 2012 at Manipal Teaching Hospital (MTH), Pokhara, Nepal. MTH is 825 bedded multispecialty teaching hospital of Manipal College of Medical Sciences (MCOMS), situated in the western region of Nepal. The hospital is a major healthcare provider and referral centre for the region. The institution caters to the population of ten of the fifteen districts of western development region of Nepal. The population of these ten districts was approximately 2 million as per 2001 census [5]. The hospital has an average daily patient load of 700 outpatients and 300 inpatients though it shows seasonal variations.

All the patients (outpatient and inpatient) with febrile illness (temperature ≥ 38°C) were included in this study. Various information were collected from the patients including demographic details, clinical diagnosis at presentation, history of acute and chronic symptoms, past medical history, recent antimicrobial therapy and physical examination findings. Blood samples were collected and inoculated aseptically into brain heart infusion (BHI) biphasic medium (Hi Media Laboratories, India) and incubated at 37ºC for 7 days. Routine sub-cultures were performed after 24 hours, 48 hours, and one week on blood agar (BA), MacConkey agar (MA) and chocolate agar (CA). The bacterial isolates were identified based on standard bacteriological methods[6]. Agglutination with specific antisera was used for identification of different *Salmonella spp.*

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by Kirby Bauer's disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines [7]. Ampicillin (10μg), Carbenicillin (100 μg), cefazolin(30 μg), Ceftazidime (30 μg), Ceftriaxone (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), gentamicin(10 μg), tobramycin (10 μg), netilmicin (30 μg), piperacillin (100 μg) and imipenem (10 μg) were tested for gram-negative bacteria. Penicillin (10 μg), ampicillin (10 μg), cefazolin (30 μg), erythromycin (15 μg), gentamicin (10 μg), Netilmicin (30 μg), ciprofloxacin (5 μg), oxacillin (1μg) and vancomycin (30 μg) were tested for *Staphylococcus aureus*.

RESULTS

A total of 4145 patients with fever were included in the study. In 4145 blood cultures, 2010 (48.5%) were received from pediatrics/ neonatology unit and remaining 2135 (51.5%) were received from adult and elderly age patients. Out of 4145 patients with febrile illness, only 251 (6%) were

diagnosed to have BSIs. The demographic details of those 251 patients with BSIs are summarized in Table 1.

Age group of 15 to 38 years accounted for 56 % of all positive cases of BSIs. The male to female ratio was 1.3:1. PUO was the most common clinical diagnosis at presentation, followed by enteric fever, neonatal sepsis, pneumonia and urinary tract infection. The etiological agents of BSIs in our hospital are summarized in Table 2. Majority of the isolates (65%) were gram-negative bacteria, while the remaining (35%) were gram-positive bacteria.

The causative agents of enteric fever such as *Salmonella typhi*, *Salmonella Paratyphi A* and other *Salmonella* were isolated from 12% of the patients with BSIs. The *Salmonella species* causing enteric fever were significantly associated with community-acquired BSIs ((P value 0.0360)). Members of Enterobacteriaceae other than *Salmonella spp.* were responsible for 32% of BSIs.

The resistance patterns of the gram-negative bacteria (Enterobacteriaceae) isolated from blood is shown in Table 3. Out of 9 *Salmonella typhi*, 5 isolates were found susceptible to all the routine antibiotics tested while 4 were resistant to ampicillin. One isolate was resistant to chloramphenicol and one to ceftriaxone.one isolate of *S. typhi* was resistant to ciprofloxacin. However, resistance of *S. paratyphi A* to all routine antibiotics is higher than *S. typhi*. Only 6 out of 14 *Salmonella Paratyphi A* isolates were sensitive to all routine antibiotics. One isolate was resistant to chloramphenicol and two isolates were resistant to ceftriaxone. Ciprofloxacin was found to be sensitive in all the cases. However, Percentage of resistance pattern of other Salmonella species was found higher than *S. typhi* and *S. paratyphi A*. Majority of the *Klebsiella pneumoniae Escherichia coli* and *Enterobacter species* were found resistant to ampicillin and cefazolin.

Majority of the *P. aeruginosa* isolates were suscep-

-tible to gentamicin,ciprofloxacin, amikacin and imipenem. None of the non-fermenters were resistant to imipenem. Out of 49 *Staphylococcus aureus* isolates, 24.5% were identified as MRSA. Resistance of *Staphylococcus aureus* to penicillin, erythromycin, gentamicin and ciprofloxacin was 90%, 35%, 12% and 12%, respectively. Majority of the coagulase negative staphylococci were sensitive to all routinely used antibiotics.

DISCUSSION

BSIs are among the most severe manifestations of bacterial disease. Patients can present to hospital with a bloodstream infection or may develop as a result of healthcare interventions. Interesting finding in the Subcontinent related to etiological agents of BSI is the isolation of more Gram negative bacteria than Gram positive bacteria. This is seen in the present analysis as shown in Table 2. Similar findings have also been reported in other studies in Pakistan, India and Nepal [8, 9, 10, 12]. However, in a study from Iran, both gram-positive and gram-negative bacteria were almost equally responsible for BSIs [11]. Therefore, the relative predominance of the etiological agents of BSIs appears to vary according to the place of study and the population.

Salmonella spp., K. pneumoniae, E. coli, Pseudomonas spp. and *S. aureus* were the most common etiological agents of BSIs in our study. In other similar studies, *Acinetobacter spp., P. aeruginosa, S. aureus, K. pneumoniae* and *Enterobacter spp.* were the frequent causes of BSIs [4, 12].We isolated *Salmonella* from 12% of the patients with BSIs, while in another recent study from Nepal; *Salmonella* were isolated from 51.7% of the positive blood cultures of patients with BSIs [13]. Interesting finding in our study is that *S. Paratyphi A* and other *Salmonella species* were responsible for 70% of the enteric fever cases while remaining cases were caused by *S. typhi*. This report may have an important implication

on the vaccine strategies, as the current vaccines used in this region do not confer protection against paratyphoid fever.

In the present study Salmonella spp. (12%) were the most commonly isolated among Enterobacteriaceae which is similar to findings of another study from Nepal [13], indicating that enteric fever is endemic in various places of Nepal. *S. aureus* was the most common gram-positive bacteria associated with BSIs in the present study which is similar to studies [4, 12].

In present study, majority of cases of enteric fever were found to be community-acquired and were susceptible to many of the routinely used antibiotics. However, 57% of *S. Paratyphi A* isolated in our study were resistant to ampicillin, while 7% resistant to chloramphenicol. Similarly, in another study from our hospital, 17% of the *S. Paratyphi A* was resistant to ampicillin and 50% were resistant to chloramphenicol [4], indicating that the antibiotic susceptibility pattern of isolates greatly varies even in same area. Therefore regular monitoring and update of resistance pattern of pathogens is of great importance in the management of BSIs. Resistance data obtained from surveillance programs can be used as important information for understanding the pattern of antibiotic resistance and encourage the physician to reduce misuse of antibiotics, which would be a key point in controlling the spread of drug resistance among pathogens.

Increasing antimicrobial resistance among blood pathogens is a matter of great concern to start empirical antibiotic treatment especially among gram negative bacilli as majority of cases of BSIs are caused by them. Among gram negative bacilli, the members of Enterobacteriaceae such as *K. pneumoniae, Enterobacter spp.* and *E. coli* were frequently resistant to the first-line antibiotics such as ampicillin and cefazolin. About 24.5% of the *S. aureus* isolated were MRSA comparable with other studies[3, 14]. All isolates of Staphylococci were

Table 1: Demographic details of the patients with Blood-Stream Infections
Characteristics:

Sex:	
Male: 140 (57.7%)	Female: 111 (42.3%)
Location In-patient	
In-patient (wards): 190 (75.7%)	Out-patient Department (OPD): 61 (24.3%)
Clinical diagnosis of patients at presentation	
Pyrexia of unknown origin (PUO): 51	Enteric fever: 40
Neonatal sepsis: 35	Pneumonia: 30
Urinary tract infection: 25	Cellulitis and sepsis: 20
Infective Endocarditis: 10	Upper respiratory tract infection: 08
Other lower respiratory tract infection: 07	Acute gastroenteritis (AGE): 06
Burns: 06	Meningitis / encephalitis: 04
Malignancy: 04	Puerperal sepsis: 03

Table 2: Etiological agents of Blood-Stream Infections

Gram negative bacteria

Bacteria	Number	Percentage (%)
Escherichia coli	30	11.9
Klebsiella species	27	10.7
Salmonella typhi	09	3.6
Salmonella paratyphi A	14	5.6
Salmonella species	07	2.8
Enterobacter species	15	6.0
Citrobacter species	07	2.8
Pseudomonas aeruginosa	10	4.0
Pseudomonas species	26	10.3
Acinetobacter species	10	4.0
Other non fermentative GNB	07	2.8
Serratia marcescens	02	0.8

Gram positive bacteria

Bacteria	Number	Percentage (%)
Staphylococcus aureus	49	19.5
CoNS	30	11.9
Enterococcus species	06	2.3
Viridans streptococci	02	0.8

sensitive to Vancomycin. Majority of the non-fermenters such as *Pseudomonas spp.* and *Acinetobacter spp.* were sensitive to the routinely used antibiotics such as ciprofloxacin, gentamicin and amikacin. Moreover, all the non-fermenters were sensitive to imipenem.

Isolation of higher numbers of *Pseudomonas species* and Coagulase negative *Staphylococci* may be associated with contamination during blood collection. Therefore strict aseptic condition should be maintained during blood collection and injecting it into blood culture bottles in order to avoid unnecessary antibiotic therapy.

Table 3(A): Resistance pattern of the Gram negative bacteria (Enterobacteriaceae) causing BSI (Percentage of antibiotic resistance pattern)

	AMP	CFZ	GEN	CIP	CRO	NT	AK	CXM	CHL
Escherichia coli (30)	80	50	23	30	47	03	03	-	-
Klebsiella species (27)	89	70	56	40	85	18	15	-	-
Enterobacter species (15)	73	87	40	20	53	13	13	-	-
Citrobacter species (07)	43	43	57	14	57	29	29	-	-
Salmonella typhi (09)	44	11	11	11	11	-	-	11	11
Salmonella paratyphi A (14)	57	29	43	00	14	-	-	50	07
Other Salmonella (07)	71	57	71	14	29	-	-	43	14

Table 3(B). Antibiotic resistance pattern of Non-fermenters (%):

	CB	PC	GEN	AK	CAZ	CIP	IPM
Pseudomonas aeruginosa (10)	70	40	20	10	60	10	00
Pseudomonas species (26)	42	19	08	04	23	04	00
Acinetobacter species (10)	60	60	20	10	60	10	00
Other non-fermentative GNB (07)	43	29	14	14	57	14	00

AMP – ampicillin, GEN – gentamicin AMK – amikacin, NT – Netilmicin, CFZ – cefazolin, CRO – Ceftriaxone, CIP – ciprofloxacin, CHL- Chloramphenicol, CXM – cefuroxime CRB – carbenicillin, PIP – piperacillin, TOB – tobramycin, CAZ – ceftazidime, IPM – Imipenem, ERT-erythromycin.

CONCLUSION

Gram-negative bacteria were the predominant causes of BSIs. *Salmonella spp., Pseudomonas Spp, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus* were the common etiological agents of BSIs. The members of Enterobacteriaceae were frequently resistant to the first-line antibiotics such as ampicillin and cefazolin. Based on results of our study, Amikacin was found most effective against various members of Enterobacteriaceae (excluding Salmonella) and Ciprofloxacin provided the best coverage for the treatment for enteric fever.

However, the non-fermenters were unusually sensitive to most antibiotics. Isolation of higher numbers of *Pseudomonas species* and Coagulase negative *staphylococci* may be associated with contamination during blood collection.

Limitations of the study:

Only one blood sample was cultured in majority of cases and isolation of doubtful pathogens such as CoNS, Pseudomonas species were not confirmed by repeated blood culture. Species identification of genus Salmonella by using specific antiserum was done for *S. typhi* and *S. paratyphi A* only where as other Salmonella species were identified by using genus specific Salmonella polyvalent antiserum.

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