

Bacteriology of blood stream infections in patients on hemodialysis and antibiotics susceptibility pattern

Abstract

Background: Bacteriological blood stream infection is the most common cause of mortality in hemodialytic patients as they are immunocompromised. In such patients blood stream infections is mainly due to gram positive cocci. Patients under the course of haemodialysis are particularly vulnerable if it is due to *Staphylococcus aureus* that accounts more than 8% mortality rate.

Methods: A cross-sectional study was carried out at National Kidney Center of Nepal for duration of six month. Blood sample collected from the patients undergoing hemodialysis course was inoculated in brain heart infusion biphasic media in blood culture bottle and incubated aerobically at 37°C for 18-24 hours (up to 1 week if necessary). It was then subcultured on blood agar plate where colony morphology and haemolytic reactions was observed. A set of conventional biochemical tests was performed to identify the isolated organisms. Antimicrobial susceptibility test was carried out by modified Kirby Bauer technique. All obtained data were recorded and analyzed using SPSS software.

Results: Gram-positive bacteria were isolated with the highest frequency (88.5%). Among them *Staphylococcus aureus* (69.6%) was found predominant. The prevalence of gram negative bacteria was just 11.5% they were *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*. During antimicrobial susceptibility testing, most of the *Staphylococcus aureus* were found resistant to amoxicillin and sensitive to cephoxitin. Minimum inhibitory concentration of this antibiotic against *Staphylococcus aureus* was determined to be 64 and 128mg/L. All coagulase negative staphylococci were found resistant to β-lactam antibiotics and most of them were sensitive to ciprofloxacin. Out of total 16 *Staphylococcus aureus* isolates 37.5% were found to be methicillin resistant.

Conclusion: Among the gram positive isolates *Staphylococcus aureus* was found to be predominant organisms in hemodialytic patients. And most of these *S. aureus* were found resistant to amoxicillin and sensitive to cephoxitin. Out of total 16 *S. aureus* isolates 37.5% were found to be methicillin resistant. Amoxicillin was found to be least effective drug and ciprofloxacin was found most effective drug for most gram positive bacteria from HD patient.

Keywords: AST, MRSA, CoNS, HD

Background

Patients requiring hemodialysis are at high risk for blood stream infection (BSI) mainly due to Gram- positive bacteria.. Within gram positive bacteria *Staphylococcus aureus* (*S. aureus*) and coagulase negative staphylococci (CoNS) are most common.¹ BIS due to methicillin resistant *S. aureus* (MRSA) has been found to have a higher attributable mortality than methicillin susceptible *S. aureus* (MSSA).² Although the incidence of MRSA is emerging threat, there is no study of BSI in persons under the course of HD in Nepal. Besides, the etiology of BSI is poorly characterized in this country, mainly due to limited laboratory resources, a poor recording system and an inadequate number of trained personnel. This study was thus designed to investigate the antibiotic resistance patterns of bacterial isolates during blood culture from HD patients.

Methods

Ethical considerations

This study was conducted with the approval of the committee of National College and National Kidney Center and declaration of Helsinki was strictly followed.

Design, period and location of the study

A cross-sectional study was conducted at National Kidney Center, Kathmandu, Nepal from June 2013 to December 2013. All the patients who had attended this kidney center for hemodialysis and agreed to provide the blood sample after informed consent were included in this research. However, HD patients with HIV or Hepatitis C were not included.

After obtaining informed consent, 5ml of blood sample was collected aseptically and inoculated in 45ml sterile brain heart infusion (BHI) biphasic media. The BHI biphasic media culture bottles were labeled and incubated at 37°C. Incubation was continued for up to 7 days unless the visible growth was observed. After getting a visible sign of microbial growth, it was subcultured on blood agar and MacConkey agar plate medium for isolation and identification of the organisms. Isolates were identified using standard microbiological techniques as describe by Forbes et al.,³ using cultural, morphological and biochemical tests.

The antibiotic susceptibility pattern of all the isolates was determined by modified Kirby Bauer disc diffusion method. The antibiotics were selected and the results were interpreted according to CLSI 2012 guidelines. Strains of *S. aureus* resistant to cefoxitin (CX/30mcg) and oxacillin (OX/1mcg) were considered as MRSA strain.

The statistical analysis was formed using SPSS version 17. The Chi-square test was used to calculate probabilities and determine significance. P value <0.05 was considered to be statistically significant.

Results

Out of 100 blood samples subjected for culture 26% showed culture positive result. Among these positive cases, 43.3% were male. The prevalence of BSI was found to be highest in the age group 45-59 years. And there is no significant association between the age group of patients and culture positive result (Table 1).

Table 1 Blood culture pattern of patients, according to age

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Status	Age of patients				Total	P value
	15-29	30-44	45-59	≥ 60		
Culture Positive (%)	26.9	19.2	38.5	15.4	100	
Culture Negative (%)	21.6	28.4	20.3	29.7	100	0.177
Total	48.5	47.6	58.8	45.1	200	

Among the total 88.5% Gram positive isolates, 62.5% *S. aureus* isolates were found resistant to amoxicillin and cotrimoxazole. On the other hand, the most effective drug for the isolates was found to be cephoxitin (62.5%). But all CoNS isolates (30.5%) were found to be resistant to amoxicillin and oxacillin. Among Gram negative isolates 66.7% were sensitive to ofloxacin but all were resistant to cotrimoxazole (Table 2).

Table 2 Antibiotics susceptibility pattern of Gram positive and Gram negative organisms in percentage

Organisms	AMX	C	CIP	CN	COT	CTR	CTX	GEN	OF	OX
For Sensitive:										
Gram positive <i>S. aureus</i> (n=16)	25	31.2	50	62.5	37.5	37.5	56.3	43.7	-	12.5
CoNS (n=7)	0	42.8	71.4	42.8	28.6	28.6	57.1	57.1	-	0
Gram negative	33.33	0	33.33	-	0	33.33	0	33.33	66.66	-
For Resistance:										
Gram positive <i>S. aureus</i> (n=16)	62.5	31.2	31.2	37.5	62.5	43.7	37.5	50	-	81.2
CoNS (n=7)	100	28.6	0	42.8	57.1	71.4	28.6	14.3	-	100
Gram negative	33.33	66.66	0	-	100	33.33	66.66	0	0	-

AMX, amoxicillin; C, chloramphenicol; CIP, ciprofloxacin; CN, Cephoxitin; COT, cotrimoxazole; CTR, ceftriaxone; GEN, gentamycin; OF, ofloxacin; OX, oxacillin

Discussion

In this study from 26% sample processed and these samples were considered culture positive cases. No organism were detected from 74% blood samples processed until 1 week of incubation at 37°C and these cases were considered as culture negative.

Difference in the prevalence of BSI in HD patients of different age group was found statistically insignificant.

Gram positive bacteria were found highly prevalent than gram negative bacteria. It can also be revealed that more than half (61.5%) of the bacteriological blood stream infection episodes is due to *S. aureus*. Similar finding was also reported by Fysarakis et al.,⁴ in hemodialytic patients. According to Jaber⁵ and Taylor et al.,⁶ the majority cases of the BSIs are due to gram positive microorganisms especially skin-derived *S. aureus* and *S. epidermidis*. Similar finding was also reported by Chaudhry et al.,⁷ and Lee et al.⁸ According to Thiene,⁹ the gram positive organisms are comparatively resistant to bactericidal activity of serum than gram negative and this could be the reason behind gram positive being the most common etiological agent of bacterial BSIs.

Diekema et al.,¹⁰ reported that, gram positive bacteria specially *S. aureus* cause majority of both nosocomial and community acquired BSIs however, the rank of pathogen is different for community and nosocomial infections.

Gram negative isolates though less frequently isolated were found more sensitive to ofloxacin and all of them were found resistant to cotrimoxazole. The gram positive isolates were tested for different groups of antibiotics generally used for the treatment purpose. Cephoxitin, ciprofloxacin and gentamycin were found to be most effective for gram positive isolates and oxacillin was found to least effective antibiotic for those gram positive organisms. Similar finding had been reported by Manandhar.¹¹ Strains of *S. aureus* have developed resistance to antibiotics including penicillin, cephalosporins, methicillin, aminoglycosides and quinolones. *S. aureus* abrogates the effects of penicillin by producing β-lactamase and MRS strains have acquired the *mec* gene, which encodes PBP 2a, and the *fem* gene, which confer resistant to methicillin, penicillinase-resistant penicillins, and cephalosporins.¹²

The incidence of *S. aureus* bactemia (SAB), particularly bacteremia caused by MRSA strains, has increased dramatically in recent years in the United States and in some European countries.^{13,14}

Determination of minimum inhibitory concentration (MIC) of antibiotics is an important aid to determine antibiotic resistant to bacteria.¹⁵ The MIC value of ceftriaxone was detected as 64mg/L for 3 strains of *S. aureus* and 128mg/L for 1 strain of *S. aureus*.

Conclusion

The most prevalent microorganisms in BSIs were gram positive bacteria such as *S. aureus* 69.5% and CoNS 26.9% among asymptomatic patients under course of hemodialysis. In the study, *S. aureus* was found to be resistant towards oxacillin and cephoxitin (37.5%), and they are termed as MRSA. The finding of this study, however, gives an indication of which major pathogens causing BSI in patients and their drug susceptibilities. Further research could evaluate the impact of surveillance culture to prevent microorganisms' distribution in this population.

Acknowledgments

We would like to express my special thanks to National Kidney Center and the HD patients who were co-operative to give blood sample and their history. We are also thankful to National College for administrative support.

Conflicts of interest

Authors declare that there is no conflict of interest.

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