Original article:

Bacteriological profile of blood stream infections at a Rural tertiary care teaching hospital of Western Uttar Pradesh

Vijay Prakash Singh[a] , Abhishek Mehta[b*]

[a,b]Department of Microbiology, K.D. Medical College, Hospital & Research Center , 24 Km. milestone, NH#2, AKBARPUR, Distt. Mathura, Uttar Pradesh, India -281406.

*Corresponding Author : Dr.Abhishek Mehta, Department of Microbiology, K.D. Medical College, Hospital & Research Center, 24 Km.milestone, NH#2, AKBARPUR, Distt.Mathura, Uttar Pradesh, India -281406.

Abstract:

Background: Bloodstream infections (BSIs) are one of the most important infections responsible for morbidity and mortality among hospitalized patients worldwide. Blood culture is the most important procedure to detect systemic bacterial infections. A wide range of organisms have been implicated in the etiology of BSIs. This cross sectional study was conducted to determine the percentage distribution of various bacterial isolates among sepsis patients in a tertiary care teaching hospital of Western Uttar Pradesh, India..

Material and Methods: In this cross sectional study stretched over a period of 1 year, blood culture bottles from 120 patients with clinically suspected cases of blood stream infections were received at the Department of Microbiology for routine culture sensitivity and were processed using standard microbiological techniques so as to determine the percentage distribution of bacterial pathogens causing BSI and their antibiotic susceptibility pattern.

Results: Out of the total 120 patients under study, Blood culture was positive in 37 cases. *Escherichia coli* (10) was the commonest isolate followed by *Klebsiella pneumoniae (8), Salmonella typhi (7), Staphylococcus aureus* (7), *Coagulase negative Staphylococcus*(4) and *Acinetobacter spp*.(1). Gram-negative bacterial isolates exhibited a high degree of sensitivity towards amikacin, imipenem, levofloxacin and linezolid. Gram-positive bacterial isolates were found to be highly susceptibile towards amikacin, gentamycin, linezolid, piperacillin tazobactam and vancomycin. This study has shown that the Blood stream infections in our clinical setting is caused predominantly by Gram-negative organisms and to a lesser extent by Gram-positive organisms with *S. aureus* and *E.coli* being the most common organisms in respective categories.

Conclusion: The knowledge of etiological pattern and their antibiogram pattern can be applied while framing the antibiotic policies for any healthcare institution.

Keywords: Bloodstream infections, Sepsis, Blood culture, Antibiotic susceptibility test, antimicrobial drug resistance

INTRODUCTION

Bloodstream infections (BSIs) are one of the most important infections responsible for morbidity and mortality among hospitalized patients worldwide [1]. Blood culture is the most important procedure to detect systemic infection due to bacteria [2]. A wide range of organisms have been implicated in the etiology of BSIs. These include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Acinetobacter spp.* among

Gram-negative bacteria and *Staphylococcus aureus*, Coagulase-negative Staphylococci (CoNS), Enterococci and alpha-hemolytic Streptococci among Gram-positive bacteria. There is a wide variation of predominant microorganisms in blood cultures among different healthcare facilities [1].

Antimicrobial susceptibility test helps in precise identification of the most appropriate choice of drugs administered to the patients. Emerging antimicrobial drug resistance among bacterial pathogens implicated in the etiology of BSIs can limit therapeutic options and complicate patient management[3]. Blood culture results generally give a low positive yield as a large proportion of patients presenting at a tertiary care hospital are already treated with antibiotics elsewhere previously[4].

BSIs are one of the main causes of death in hospitalized patients with mortality rates ranging from 30% to 70% [5]. Illness associated with BSIs may range from self limiting infection to life threatening sepsis which requires rapid empirical treatment with a proper antibiotic[6]. Hence regular surveillance of the cause of BSIs by blood culture helps in monitoring the spectrum of bacterial pathogens and their antimicrobial sensitivity pattern in a particular area. These data provide a platform to the clinicians on the base of which they can initiate effective empirical therapy, thus preventing the irrational use of antibiotics.

Therefore, the aim of the present study was to determine the prevalence of various bacterial isolates causing sepsis in a tertiary care hospital and their antibiograms and help physicians in guiding the choice of the empirical antibiotics.

MATERIAL AND METHODS

This study was a prospective study conducted at the Department of Microbiology of a Rural tertiary care teaching hospital of Western Uttar Pradesh, India over a period of 1 year from January 2016 to December 2016 after getting clearance from institutional ethical committee.

Blood culture bottles from 120 patients with clinically suspected cases of Blood stream infections were received at the department of Microbiology and were studied regarding the pattern of bacteriological isolates in culture of sepsis patients.

Blood samples were collected from clinically suspected cases of Blood stream infections admitted in various inpatient departments of the hospital for routine blood culture sensitivity before the beginning of empirical antibiotic therapy. In case of adult patients, 5 ml of blood was collected using strict aseptic precautions , and inoculated immediately into 50 ml of " Brain heart infusion" (BHI) broth with 0.025% of sodium polyanethol sulphonate as anticoagulant. In paediatric cases, 1-2 ml of blood was inoculated in 5-10 ml of BHI broth. Then the blood culture bottles were labelled with the patient details (name, identification number, date and time of collection). The bottles containing specimens were transported within half an hour to the Bacteriology laboratory. Blood culture bottles were incubated aerobically at 37°C for 24 hrs. After incubation, primary subculture from the BHI broth was done on blood agar & MacConkey agar after regular intervals at 2nd day, 5th day and 7th day. The culture was reported negative if all subcultures showed no growth by the end of 1 week.

Bacterial growth on the subcultures was identified by their colony morphology, gram stain of the isolated

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colonies and other conventional biochemical reactions as per the standard protocol followed in our laboratory[7].

The antibiotic susceptibility of bacterial isolates was determined by the Kirby Bauer disc diffusion method on Mueller-Hinton agar plates as per CLSI (Clinical and Laboratory Standards Institute) guidelines[8]. The antibiotics tested on Gram-positive cocci included amoxyclav, amikacin, cefoxitin, clindamycin, erythromycin, gentamycin, levofloxacin, linezolid, piperacillin tazobactam and vancomycin. The antibiotics tested on Gram-negative bacilli included amikacin, ampicillin, ampicillin sulbactam, amoxyclav, ciprofloxacin, ceftriaxone, cefixime, cefuroxime, colistin, gentamycin, imipenem, levofloxacin and piperacillin tazobactam. The screened strains were further processed for detection of ESBL production and methicillin resistance according to CLSI guidelines[8] .

Statistical analysis

All the results collected were subjected to descriptive statistics. Microsoft Excel 2007 was used for making tables and bar-charts.

RESULTS

Out of the 120 blood samples that were processed in our study, 88 samples showed no growth while 35 showed significant growth on aerobic culture. *Escherichia coli* (10) was the commonest isolate followed by *Klebsiella pneumoniae (8),Staphylococcus aureus* (7)*, Salmonella typhi* (5) *Coagulase negative Staphylococcus*(4) and *Acinetobacter spp*.(1). (Table-1).

The antibiotic susceptibility test (AST) was performed separately for all the isolated bacterial pathogens. For AST, a panel of 10-12 antibiotic discs were used on MHA plates depending on the isolated micro-organism.

In case of *E.coli*, Colistin (100%), imipenem (90%) and Amikacin (90%) showed the highest sensitivity. Other antibiotics showing modest sensitivity were Levofloxacin (80%) , Linezolid (80%) and Amoxyclav (70%). Ampicillin showed the highest resistance (60%). High degree of resistance was also seen in case of Ampicillin sulbactam, second and third generation cephalosporins. (Table-2)

Other Gram negative bacilli isolated were *Klebsiella spp*., *Salmonella typhi* and *Acinetobacter spp.* In case of *Klebsiella spp.* , antibiotics with high sensitivity were Colistin (100%), Imipenem, Amikacin and Levofloxacin (88% each). High degree of resistance was seen in Ampicillin(75%), Ampicillin sulbactam, second and third generation cephalosporins.(Table-2)

In the present study, 30% of E.coli and 37.5% of Klebsiella were found to be ESBL producers (Fig.-1).

In case of the *S.typhi* isolates, highest sensitivity was shown towards Ceftriaxone and Piperacillin tazobactum (100%) followed by Cefixime,Levofloxacin,Imipenem,Amikacin(80% each) and comparatively lesser activity towards Ampicillin sulbactam, Amoxycillin Clavulanate, Cefuroxime and Ciprofloxacin (60%). (Table-2)

In case of *Acinetobacter spp.*, Imipenem, Piperacillin tazobactam and Amikacin showed the highest sensitivity (100% each). Highest resistance rate was seen in Ampicillin (100%). (Table-2)

Among the gram positive pathogens isolated, *S.aureus* was the commonest isolate (21.9%). Overall, It was the fourth most common isolate after *E.coli*, *Klebsiella spp.* and *S.typhi*. In case of *S.aureus*, Linezolid (100%) and Vancomycin (100%)

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showed the highest sensitivity while Erythromycin showed the highest resistance. Among Gram positive bacteria, 28.6% (2) of *S.aureus* isolates were found to be MRSA and 50%(2) of CONS isolates were MR-CONS (Fig.- 2).

Table-1: Distribution of Bacterial isolates in blood culture positive cases

S.No.	Organism isolated	Number of isolates (%)
1.	*E.coli*	10 (28.57 %)
2.	*Klebsiella pneumoniae.*	8 (22.86 %)
3.	*Salmonella typhi*	5 (14.28 %)
4.	*Acinetobacter spp.*	1 (2.8 %)
5.	*Staphylococcus aureus*	7 (20 %)
6.	*Coagulase negative staphylococcus spp.*	4 (11.43 %)
	Total	35(100 %)

Table-2 :Antibiotic susceptibility pattern of Blood stream infections

Antibiotic drug	***E.coli*** **n=10**	***Klebsiella spp.*** **n=8**	***S.typhi*** **n=5**	***Acinetobacter spp.*** **n=1**	***S.aureus*** **n= 7**	***CoNS*** **N=4**
Ampicillin	40	25		00	00	00
Ampicillin sulbactam	50	38	60			
Cefixime	60	50	80			
Cefuroxime	50	38	60	50		
Ceftriaxone	60	62	100	50		
Levofloxacin	80	88	80	50	86	80

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Linezolid	80	75			100	100
Imipenem	90	88	80	100		
Amoxyclav	70	75	60	50	71	60
Erythromycin					30	40
Piperacillin tazobactam			100	100	86	80
Vancomycin					100	100
Ciprofloxacin	60	75	60	50		
Amikacin	90	88	80	100	86	100
Gentamycin				50	86	80
Cefoxitin					71	60
Clindamycin					71	80
Colistin	100	100				

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Fig.-1 : Distribution of ESBL producers

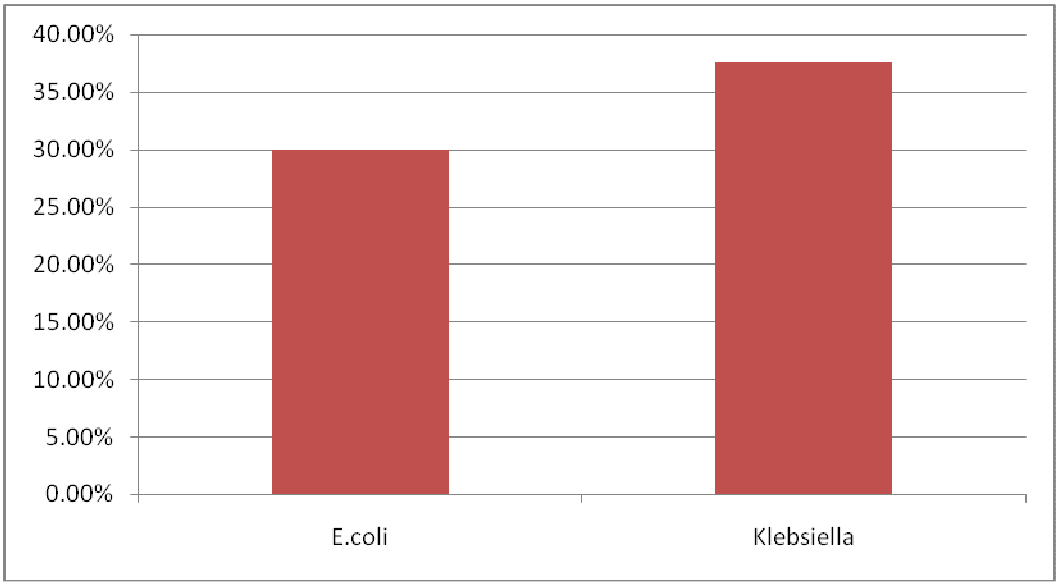
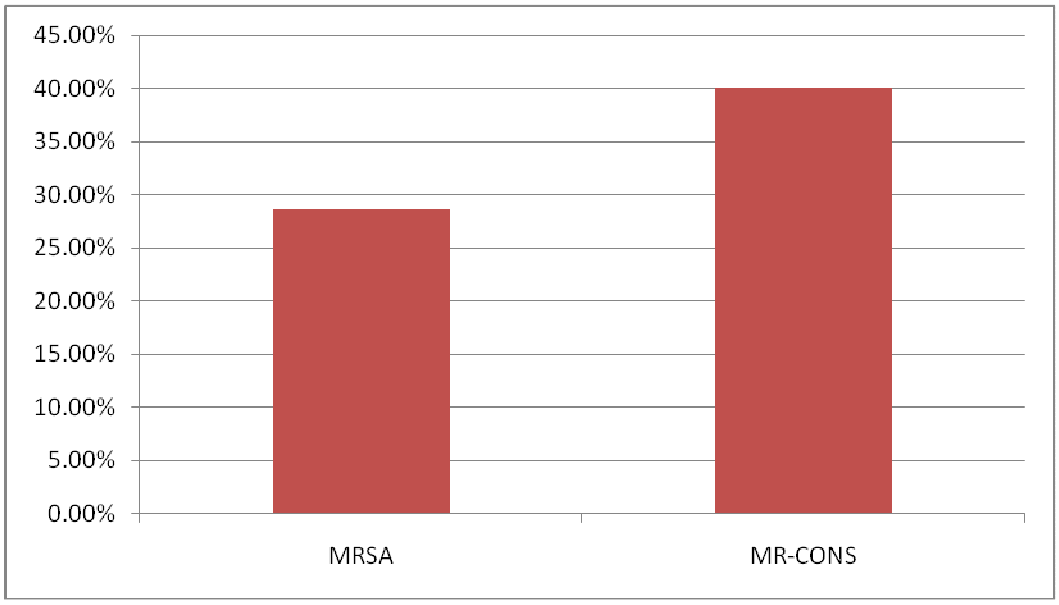


Fig.-2 : Distribution of MRSA and MR-CoNS



DISCUSSION

Septicemia presents as a serious challenge for the clinicians as it is associated with considerable morbidity and mortality. Hence timely detection and treatment of BSI plays a very important role on the final clinical outcome. The gold standard of diagnosis of BSIs is blood culture for the isolation of the etiological agents responsible for sepsis.

This study found that 37 out of 120 total blood sample screened from suspected sepsis cases were positive for the presence of bacteria [Table/Fig.-I]. Thus, the overall prevalence of bacteraemia in the whole population was 30.83%. The isolation rate reported from other indian studies where routine blood culture was performed show a wide variation like Alam et al. (20.9%)[9], Arora et al. (20.02%)[10], Sharma et al (33.9%)[11], Roy et al. (16.4%)[12] and Gohel K et al (9.2%)[13]. In India, the variation in isolation rate may be due to the reason that practice of prescribing antibiotics is very common among the local health practitioners before the patients reach the tertiary care hospital.

In the present study, 70.27 % isolates were found to be Gram-negative bacteria , whereas 29.73 % isolates were Gram-positive bacteria causing BSIs. This finding is similar to other previous studies where Gram-negative bacilli have taken over the Gram-positive organisms, especially in hospital settings[14,15]. Among the Gram negative bacteria, *E.coli* was the commonest isolate (37.04%) followed by *Klebsiella pneumonia* (259.63%), *S.typhi* (25.92%) and *Acinetobacter spp.*(3.7%) respectively. In other previous studies also, *E.coli* and *Klebsiella* spp. have been found to be predominant isolates among the Enterobacteriaceae family[13,16,17,18,19]. Among the Gram positive bacteria, *S.aureus* was the most common isolate (21.9%) followed by CoNS (15.6%). Other previous surveillance studies have found CONS as the most common Gram positive bacteria isolated from blood culture specimens of suspected cases of septicemia[15,19]. As only a single blood culture specimen was collected from each patient, hence it was not possible to determine if the patients with CoNS isolation had true bacteremia or the finding was due to skin contamination. Though the CoNS isolation from blood culture specimens have often found to be contaminants in majority of cases[13], repeated isolation of CoNS from same patient suggests bacteremia. CoNS is a well described pathogen in cases of neonatal septicemia and in patients with prolonged use of invasive intravascular devices. Hence , clinicians are suggested to rule out the possible risk factors and to advise for repeat blood culture in case of CoNS isolation.

The rate of antimicrobial resistance was generally higher in Gram-negative microorganisms as compared to Gram-positive microorganisms . This scenario may be due to the injudicious use of antibiotics and lack of appropriate antibiotic policy in the hospital. Among the Gram-negative bacterial isolates, high degree of sensitivity was seen to imipenem, linezolid, amikacin and levofloxacin. The drugs showing high resistance to Enterobacteriaceae isolates were ampicillin and ampicillin sulbactam,. E.coli and Klebsiella spp. also showed a very high resistance towards Cephalosporins. In the present study, 30% of E.coli and 37.5% of Klebsiella were ESBL producers which is in accordance with other previous indian studies[10,15]. As second and third generation cephalosporins are one of the most commonly used drug as empirical therapy for

inpatients in developing countries, it could be the reason behind its high level of resistance.

Among all Gram positive bacteria, 28.6% were MRSA and 50% were MR-CONS which is almost similar to the findings in other indian studies[15,20,21]. Apart from vancomycin and linezolid, that were 100% sensitive in case of Gram-positive bacterial infections, increased susceptibility was seen with piperacillin tazobactam, amikacin and gentamycin. It is important for clinicians to update themselves with current data regarding the etiological agents of common bacterial infections in a particular geographical area as well as its antimicrobial susceptibility pattern.

CONCLUSION:

Septicemia is an important nosocomial infection responsible for morbidity and mortality in the patients attributed to both Gram-negative and Gram-positive organisms. *S. aureus* and *E.coli* were among the most common Gram-positive and Gram-negative organisms identified causing sepsis, respectively. The knowledge of etiological pattern and their antibiogram pattern can be applied while framing the antibiotic policies for any healthcare institution. Majority of Enterobacteriaceae isolates were found to be multi-drug resistant (MDR). The major threat with MDR bacteria is that infections are usually untreatable due to limited options of available antibiotics. Antimicrobial susceptibility test report provided by microbiology laboratory is necessary for early diagnosis and treatment of such cases of sepsis.

The information of predominant organisms and their sensitivity among sepsis patients is essential for making the right choice of antibiotics in the management of sepsis. Hence, blood cultures must be obtained from all suspected cases of bacteraemia or sepsis before prescribing antibiotics. Strict infection control measures along with judicious antibiotic policy for antibiotic therapy should be implemented in the hospitals as control measures against blood stream infections.

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