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Bacteriological profile and antimicrobial susceptibility of blood culture in a tertiary care hospital Ajmer

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Abstract
Background: Bloodstream infection (BSI) is a significant cause of morbidity and mortality throughout the world. Illness associated with BSI ranges from self limiting infection to life threatening sepsis that requires rapid and aggressive antimicrobial treatment. The emergence of resistant bacteria makes it a requisite to know the prevailing antibiotic susceptibility pattern of the pathogens causing bloodstream infection.
Objective: To identify the bacteriological profile and antibiotic susceptibility patterns of blood culture isolates in a tertiary care hospital
Material and Methods: A total of 400 patients with suspected blood stream infection from various wards in the hospital were included for this study. Blood samples were processed in microbiology laboratory and isolates were identified by standard laboratory methods and then antibiotic susceptibility test was performed by using CLSI guidelines.
Results: Out of 400 blood samples, 75 (62.7%) were culture positive. Out of 75 positive cultures, 39 (52%) were gram-positive, 32 (42.7%) were gram-negative and 2 (5.3%) were Candida spp. The most predominant organism was Klebsiella species 20(26.8%), followed by *Staphylococcus aureus* 14 (18.67%) Most of the Gram-positive cocci (GPC) were susceptible to vancomycin and linezolid. Most of the Gram-negative bacilli (GNB) showed sensitivity to imipenem followed by amikacin.
Conclusion: Increase in antibiotic resistance for BSI causing pathogens has necessitated continuous monitoring of the susceptibility of organisms towards antibiotics to prevent and spread of drug resistance.

Keywords: blood stream infections, bacterial profile, antimicrobial susceptibility

Introduction
Blood stream infections (BSI) are the leading causes of morbidity and mortality throughout the world and are amongst the most common health care associated infections [1]. Illness associated with BSI ranges from self limiting infection to life threatening sepsis that requires rapid and aggressive antimicrobial treatment [2].
Bacteremia refers to presence of viable bacteria in blood. Septicemia indicates systemic symptoms caused by bacteria or their toxins in blood. BSI on the basis of time or setting of acquisition, can be community-acquired or nosocomial BSI. Usually community - acquired BSI is defined as a BSI that is detected (onset of symptoms) within the first 48 hrs after hospitalization while BSI is referred to as nosocomial; if detected more than 48 hour after hospitalization [3].
The incidence of nosocomial BSI has been reported to correlate with increasing use of central venous catheters, patient illness (eg. Carcinoma, trauma and high risk nursery) and other predisposing factor like intensive care unit stay, hand washing practice of medical staff [4]. Common portal of entry for extra vascular blood stream infection are genitourinary tract (25%), respiratory tract (20%), abscess (10%), surgical wound infections (5%) and miscellaneous and uncertain sites (25%) [5].
Common organism isolated from blood culture are *Staphylococcus aureus, Escherichia coli,* Coagulase Negative Staphylococci (CoNS), *Enterococcus species, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Proteus* species and β-hemolytic streptococci. CoNS have long been considered mainly as non pathogenic blood culture contaminant, however as a result of combination of increased use of intravascular devices and an increase in the number of hospitalized immune compromised patients, CoNS has emerged as a major cause of nosocomial blood stream infection [6-8].
The emergence of resistant bacteria makes it a requisite to know the prevailing antibiotic susceptibility pattern of the pathogens causing bloodstream infection. There is increase in antimicrobial resistance rates and a shift in organism distribution among important blood stream pathogen both in hospital and community settings. In hospital setting there has been a shift from a predominance of gram negative organism in the late 70's to the present day primacy of gram positive organism [9]. This study was conducted to identify the bacteriological profile and their antibiotic susceptibility patterns from blood culture in a tertiary care hospital to guide clinicians to initiate empiric antibiotic therapy and to formulate antibiotic policy.

Material and Methods
Study Design:
This study was carried out in the Department of Microbiology, JLN Medical College Ajmer from August 2015 to November 2016 to study bacteriological profile and antibiotic susceptibility pattern of blood culture isolates. A total of 400 patients with suspected blood stream infection from various OPDs, IPDs and ICUs in the hospital regardless to their age, sex, occupation, religion were included for this study.
Sample Collection and Methods
10-20 ml of blood was drawn in case of adult and 2 to 5ml in

children in aseptic condition. The sample collected was inoculated immediately into culture bottle containing 70 ml to Brain Heart infusion (BHI) broth with 0.05% Sodium Polyanethol Sulfonate (SPS) as anticoagulant in adult and 20ml of BHI broth with 0.05% SPS in children with utmost precaution and aseptic procedure.

The culture bottle was incubated at 35-37ºC aerobically. After overnight incubation, the sample was sub cultured on to Blood agar, Mac- Conkey agar, Chocolate agar and special media which were suitable for isolation and identification of the species. The plates incubated for 18-24 hours at 35-37ºC in the incubator. If no growth observed on plate by next day subculture were repeated on day 3, day4 and finally on day 7.

Isolation and identification of organisms was done by using standard microbial procedure by colony character, gram staining, motility testing and standard biochemical test.

Antimicrobial susceptibility test

Antimicrobial sensitivity was determined by Kirby Bauer's disc diffusion method on Mueller Hinton agar (MHA) as per CLSI guidelines [10]. Antibiotic discs used for sensitivity testing were amikacin, ampicillin, Amoxycillin + Clavulinic acid, Aztreonam, Cefepime, Cefoperazone, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Cotrimoxazole, Erythromycin, Gentamicin, Imipenem, Linezolid, Netilmicin, Ofloxacin, Penicillin – G, Piperacillin, Tetracycline, Vancomycin. *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used as quality control throughout the study for culture and antimicrobial susceptibility testing.

Results

A total of 400 blood samples were collected for blood culture in present study among that 75(18.75%) were culture positive. Out of 75 positive cultures (62.7%) were male while (37.3%) were female. The positive samples belonged to maximum from infants 43(57.33%) followed by adults 19 (25.33%). Out of 75 positive cultures, 39 (52%) were gram-positive, 32 (42.7%) were gram-negative and 2 (5.3%) were Candida spp. Among the 75 isolates, the most predominant organism was Klebsiella species 20(26.8%), followed by *Staphylococcus aureus* 14 (18.67%) and the least was Escherichia Coli 1(1.3%) (Table 1)

Klebsiella species show 90% sensitivity to imipenem followed by amikacin (65%). Imipenem showed 85.7% efficacy against the *Pseudomonas aeruginosa*. Vancomycin and Linezolid remained the most active drug in infections caused by Gram positive organisms. The antibiotic sensitivity patterns of GPC are shown in Table 2 and those of GNB are shown in Table 3.

Table 1: Distribution of Isolates

S. No.	Organism	Number	Percentage
1	Klebsiella species	20	26.8
2	Staphylococcus aureus	14	18.67
3	CoNS	12	16
4	Enterococcus species	12	16
5	Pseudomonas aeruginosa	7	9.33
6	Citrobacter freundii	4	5.33
7	Candida species	4	5.33
8	Streptococcus viridians	1	1.33
9	Escherichia Coli	1	1.33
	Total	75	100%

Table 2: Antibiotic sensitively pattern of Gram positive organism

Organism		AMP	P	AMC	CIP	TE	GEN	NET	E	LZ	OX	VA	COT	CX	CD
Staphylococcus aureus (n=14)	No	1	0	10	8	8	6	7	1	14	10	14	5	9	5
	%	7.14	0	71.4	57.14	57.14	42.8	50	7.14	100	71.4	100	35.71	64.28	35.71
CoNS (n=12)	No	2	0	9	7	9	8	8	5	12	9	12	3	8	5
	%	16.66	0	75	58.33	75	66.7	66.7	41.67	100	75	100	25	66.67	41.67
Enterococcus species (n=12)	No	2	0	7	2	5	5	6	3	12	4	10	3	4	3
	%	16.66	0	58.33	16.66	41.67	41.67	50	25	100	33.33	83.3	25	33.33	25

Table 3: Antibiotic sensitive pattern of Gram negative organisms

Organism	No	AMP	AMC	CIP	CTX	CAZ	CFM	CTR	CPZ	CPM	AT	IMP	TE	GEN	NET	AK	PI
Klebsiella spices (n=20)	No	0	7	8	5	3	5	3	0	7	9	18	10	8	8	13	Na
	%	0	35	40	25	15	25	15	0	35	45	90	50	40	40	65	-
Pseudomonas aeruginosa (n=7)	No	1	1	5	2	3	Na	1	Na	2	3	6	1	3	Na	4	4
	%	14.2	14.2	71.4	28.57	42.85	-	14.2	-	28.57	42.85	85.7	14.2	42.85	-	52.14	52.14
Citrobacter freundii (n=4)	No	0	2	3	1	1	1	1	0	2	2	4	4	3	4	4	Na
	%	0	50	75	25	25	25	25	0	50	50	100	100	75	100	100	-
E. coli (n=1)	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Na
	%	0	0	0	0	0	0	O	0	0	0	0	0	0	0	100	-

Discussion

Blood stream infections range from transient bacteremia to

septic shock. Blood culture is a gold standard for accurate detection of etiological agents of infectious diseases and can assist in choice of appropriate antimicrobial therapy. Furthermore, early detection of bloodstream infections could prevent implantation of microorganisms into vital organs such as brain, heart or kidneys.

In this study the isolation rate of blood culture positive cases was 18.75% which is similar to studies conducted by Mehta MP *et al*. [11], Qureshi M *et al*. [12] and A. Vijaya Devi *et al*. [13] who reported a culture positive rate of 16.4% and 16.6%and 16.8% respectively. The low rate of isolation may be explained by the fact that many of the patients probably received antibiotic therapy before they came to the tertiary care hospital. However Khanal *et al*. [14] and Sharma PP *et al*. [15] reported high frequency of positive blood cultures accounting for 44%, 33.9% and 20.2% respectively whereas studies by Anbumani *et al*. [16] and Arora U *et al*. [17] reported lower frequency of positive blood cultures accounting for 7.89% and 9.94%, respectively.

In this study, men had high culture positivity as compared with women i.e (62.7% male and 37.3 % were female) The result was consistent with the study done by Vanitha Rani *et al*. [18] who reported high culture positivity 60.2% in male & 36.7% in female. A similar study was done by Kaur and Singh [19] who reported high culture positivity in 65.22% men. However, Zenebe *et al*. [20] reported more high culture positivity in women, 59.2% than men, 40.8%, in their study. The reason for this difference is because of gender bias. Secondly, it may be due to more male newborns being admitted in NICU as they are more prone to neonatal septicemia as compared to female newborns [21].

In this study we found that most of the blood culture positive cases were from infant (57.3%) than other age groups. This is in accordance with study conducted by Ayobola *et al*. [22] & Bichitrananda S *et al*. [23] who reported culture positivity in infants up to 58.3% and 50% respectively. The high rate of isolation from infants may be due to their weak immune system as compared to adults & most infants take medication by means of intravascular devices that may easily introduce bacteria into their blood stream.

In this study we found that the rate of isolation of Gram positive bacteria was higher (52.7%) than Gram negative bacteria (42%) which is consistent with the studies conducted by various authors [24-27] However in some studies, rate of isolation was different as reported by Mehta M *et al*. [10] (80.96%) was Gram negative bacteria and 18% were Gram positive bacteria, Anumani *et al*. [16] 46% Gram positive and 56% Gram negative bacteria.

We found that most common organism isolated in our study was Klebsiella species (26.84%). It is predominant in all age groups being highest in infants. This observation is in concordance with other studies conducted by Sanjay D. Rathod, [28] Mustafa *et al*. [29] and S. Oza Sweta *et al*. [30] The second most common isolate overall and most common Gram Positive Cocci was *Staphylococcus aureus* (18.67%), again similar results were reported from studies done by Ghanshyam D. Kumar *et al*. (24%), [31] Mehta *et al*. (13.86%), [11] Mustafa *et al*. (24%) [29] and S. Oza Sweta *et al*. [30].

In our study, Coagulase Negative Staphylococci (CoNS) were recovered at a frequency of 16%. This is in agreement with studies conducted by Gandham Pavani *et al*., [32] (23%) R. Sharma *et al*. (13.3%), [33] S. Oza sweta *et al*. (20.2%). [30] However Meenakshi Kante *et al*. [34] had reported very low occurrence of CoNS 7.1% & 5.6% respectively. This variation in occurrence of CoNS as blood pathogen is due to fact that they are considered as most common skin commensal and their presence in blood may be result of contamination due to non follow of proper aseptic technique of blood collection. However, there are many studies suggesting that there is increase in occurrence of CoNS as true blood pathogen due to increase use of intravascular devices [35, 36].

Antibiotic sensitivity pattern of micro-organisms is always changing. Earlier it was observed gentamycin sensitivity in 80% cases of Klebsiella sepsis but today it is no cases due to development of resistance to gentamycin. In past, penicillin was effective for gram positive organisms but now days they are usually not effective for such micro organisms.

In this study, among the antibiotics used for susceptibility testing for gram positive isolates, vancomycin (94.8%) & linezolid (100%) showed highest activity.

This correlates with other studies conducted by Mehta M. *et al*. [11], Sharma M *et al*., [37] Atul G *et al*., [38] Mustafa M *et al*. [29] Among Gram positive sepsis, Staphylococcus aureus was found to be most sensitive to vancomycin (100%) & linezolid (100%) followed by amoxyclav (71.4%), oxacillin (71.4%), tetracycline (57.14%), ciprofloxacin (57.14%) & netilmycin (50%). Low sensitivity to clindamycin (35.7%), cotrimoxazole (35.7%), gentamycin (42.8%) & least sensitive to erythromycin (7.14%), ampicillin (7.14%) and penicillin. This high level of resistance to commonly used antibiotics is comparable with R. Sharma *et al*., [33] Vanitha Rani *et al*., [18] S. Rathod *et al*. [28].

In this study we found that among the Gram negative isolates imipenem showed the highest sensitivity (91%) which is consistent with the studies conducted by Sanjay D Rathod *et al*. [28] and Mustafa M *et al*. [29] who also showed imipenem as most effective drug for Gram negative bacilli. Klebsiella spp. showed 90% sensitivity for imipenem. Sensitivity to commonly used drugs were like amikacin (65%), tetracycline (50%), aztreonam (45%), gentamycin (40%), netilmicin (40%) and ciprofloxacin (40%). High level of resistance was found for 3rd generation cephalosporins. Resistance to ampicillin was 100%. This high level of resistance to commonly used drugs is comparable with study conducted by Anubumani *et al*., [16] Karki S *et al*., [39] Mustafa M *et al*. [29] and S. Oza. Sweta *et al*. [30] Similarly Komal take *et al*. show a significantly high percentage of resistance among gram negative bacilli to aminoglycosides, ciprofloxacin, carbenicillin and cephalosporin, but in comparison imipenem was found to be effective.

Conclusion

Blood stream infection is one of the main agent causing morbidity and mortality worldwide. The most predominant organism was Klebsiella species followed by *Staphylococcus aureus*. Imipenem was the most effective drug for gram negative isolates while Vancomycin & Linezolid were most effective against gram positive isolates. As the resistant rate of an antibiotic for bloodstream pathogens is increasing, continuous monitoring of the susceptibility of the organism

towards antibiotics has become mandatory, in order to avoid the inappropriate use of the antibiotics.

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