

Identify the Bacterial Pathogens Causing Hospital Acquired Septicemia and the Antibiotic Sensitivity Profile of the Bacterial Isolates

Ashan Hamidi¹, Ram Naresh Sharma², Ram Shankar Prasad³

¹Assistant Professor, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India

²Assistant Professor, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India

³Associate Professor and HOD, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India

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Corresponding author: Dr. Ram Shankar Prasad

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Abstract

Objective: The objective of this study was to determine the bacterial agents responsible for hospital acquired septicemia and to determine the antibiotic sensitivity profile of the bacterial isolates. **Methods:** This was a prospective observational study conducted in the Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India for 1 year. A total of 550 patients with suspected blood stream infection were included for this study. The sample collected was inoculated immediately into culture bottle containing 70 ml to Brain Heart infusion (BHI) broth. The culture bottle was incubated at 35-37°C aerobically. After 24h incubation, the sample was sub-cultured on to Blood agar, MacConkey agar, Chocolate agar and special media which were suitable for isolation and identification of the species. Antimicrobial susceptibility test antimicrobial sensitivity was determined by Kirby Bauer's disc diffusion method on Mueller Hinton agar (MHA). **Results:** Out of 550, 100(18.18%) were culture positive. Out of 100 positive cultures (65%) were male while (35%) were female. The positive samples belonged to maximum from infants 52(52%) followed by adults 48 (48%). Out of 100 positive cultures, 50 (50%) were gram-positive, 44 (44%) were gram-negative and 6 (6%) were *Candida* spp. Among the 100 isolates, the most predominant organism was *Klebsiella species* 35(35%), followed by *Staphylococcus aureus* 20(20%) and the least was *Escherichia Coli* 2(2%). Among Gram positive sepsis, *Staphylococcus aureus* was found to be most sensitive to vancomycin (95%) & linezolid (95%) followed by amoxyclav (70%), Amoxicillin (70%), tetracycline (55%), ciprofloxacin (55%) & netilmicin (50%). Low sensitivity to clindamycin (35%), cotrimoxazole (35%), gentamycin (45%) & least sensitive to erythromycin (10%), ampicillin (10%) and penicillin (0%). (Table 2). *Klebsiella* spp. showed 88.57% sensitivity for imipenem followed by amikacin (62.86%), tetracycline (45.71%), aztreonam (42.86%), gentamycin (37.14%), netilmicin (37.14%) and ciprofloxacin (37.14%). Imipenem showed 90% efficacy against the *Pseudomonas aeruginosa*. **Conclusion:** 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.

Keywords: blood stream infections, bacterial profile, antimicrobial susceptibility.

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Introduction

Septicemia is defined as the presence of microorganisms or their toxins in the bloodstream[1]. Bacteremia indicates the presence of bacteria in the circulating blood; it may be transient, continuous or intermittent[2]. Septicemia is a leading cause of morbidity and mortality in India. Neonates are particularly vulnerable to infection (septicemia) because of their weak immune system[3]. Septicemia may present with nonspecific signs and symptoms severe febrile episodes with fever, chills, malaise, tachycardia, mental confusion, hyperventilation, hypotension or shock[4]. Various conditions in which bacteria are present in the blood stream include manipulation of infected tissues, instrumentation of contaminated mucosal surfaces, bacterial endocarditis, typhoid fever, undrained abscesses, meningitis, pneumonia etc. causing significant septicemia[5]. Septicemia is caused either by a single type of organism or it may be caused species of bacteria. Recent literature suggests that the incidence of polymicrobial bacteremia is increasing. Blood culture remains the gold standard for the diagnosis of septicemia[6,7]. The vascular compartment is sterile and usually intact. Microbes gain entry from breakages of blood vessels adjacent to skin or mucous surfaces or by phagocytic cells carrying organisms into capillaries or the lymphatic system. Gram-negative lipids (endotoxins) or Gram-positive toxins initiate a cascade of events involving cytokines, interleukin-2, vascular mediators and platelets leading to hypotension. This process becomes irreversible and produces failure of all major organs, so sepsis is life threatening emergency that demands urgent diagnosis and treatment. High rate of antibiotic resistance against bacterial pathogen has worsened the situation.

Septicemia is caused either by a single type of organism or it may be caused by multiple species of bacteria. Recent literature suggests that the incidence of polymicrobial bacteremia is increasing. Blood culture remains the gold standard for the diagnosis of septicemia[8,9]. The vascular compartment is sterile and usually intact. Microbes gain entry from breakages of blood vessels adjacent to skin or mucous surfaces or by phagocytic cells carrying organisms into capillaries or the lymphatic system. Gram-negative lipids (endotoxins) or Gram-positive toxins initiate a cascade of events involving cytokines, interleukin2, vascular mediators and platelets leading to hypotension. This process becomes irreversible and produces failure of all major organs, so sepsis is life threatening emergency that demands urgent diagnosis and treatment. High rate of antibiotic resistance against bacterial pathogen has worsened the situation. Detection of causative organisms and their antibiotic susceptibility is crucial for diagnosis of sepsis in order to initiate the appropriate antibiotic treatment therapy which reduces the adverse effects of antibiotic treatment on patient prognosis; hence we had done study to identify most common organisms and its sensitivity pattern in our hospital. 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

This was a prospective observational study conducted in the Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India for 1 year. after taking the approval of the protocol review committee

and institutional ethics committee. 100 patients with anaemia were included in the study.

Methodology

A total of 550 patients with suspected blood stream infection from various OPDs, IPDs and ICUs in the hospital regardless to their age, sex, occupation, were included for this study. 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 35-37°C aerobically. After 24h incubation, the sample was sub-cultured on to Blood agar, MacConkey agar, Chocolate agar and special media which were suitable for isolation and identification of the species. The plates incubated for 18-24 hours at 35-37°C 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, Amoxicillin + Clavulanic acid, Aztreonam, Cefepime, Cefoperazone, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Cotrimoxazole, Erythromycin, Gentamicin, Imipenem, Linezolid, Netilmicin, Ofloxacin, Penicillin-G, Piperacillin, Tetracycline, Vancomycin.

Results

A total of 550 blood samples were collected for blood culture in present study among that 100(18.18%) were culture positive. Out of 100 positive cultures (65%) were male while (35%) were female. The positive samples belonged to maximum from infants 52(52%) followed by adults 48 (48%). Out of 100 positive cultures, 50 (50%) were gram-positive, 44 (44%) were gram-negative and 6 (6%) were *Candida* spp. Among the 100 isolates, the most predominant organism was *Klebsiella* species 35(35%), followed by *Staphylococcus aureus* 20(20%) and the least was *Escherichia Coli* 2(2%) (Table 1).

Among Gram positive sepsis, *Staphylococcus aureus* was found to be most sensitive to vancomycin (95%) & linezolid (95%) followed by amoxycylav (70%), Amoxacillin (70%), tetracycline (55%), ciprofloxacin (55%) & netilmycin (50%). Low sensitivity to clindamycin (35%), cotrimoxazole (35%), gentamycin (45%) & least sensitive to erythromycin (10%), ampicillin (10%) and penicillin (0%). (Table 2). *Klebsiella* spp. showed 88.57% sensitivity for imipenem followed by amikacin (62.86%), tetracycline (45.71%), aztreonam (42.86%), gentamycin (37.14%), netilmicin (37.14%) and ciprofloxacin (37.14%) table 3. Imipenem showed 90% efficacy against the *Pseudomonas aeruginosa*. Among the antibiotics used for susceptibility testing for gram positive isolates, vancomycin (95%) & linezolid (95%) showed highest activity. 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

Organism	Number	Percentage
<i>Klebsiella species</i>	35	35
<i>Staphylococcus aureus</i>	20	20
CoNS	10	10
<i>Enterococcus species</i>	10	10
<i>Pseudomonas aeruginosa</i>	10	10
<i>Citrobacter freundii</i>	5	5
<i>Candida species</i>	5	5
<i>Streptococcus viridians</i>	3	3
<i>Escherichia Coli</i>	2	2
Total	100	100%

Table 2: Antibiotic sensitively pattern of Gram-positive organism

Organism		AMP	PAMC	CIP	TE	GEN	NET	E	LZ	OX	VAC	COT	CX	CD	
<i>Staphylococcus aureus</i> (n=20)	No	2	0	14	11	11	9	10	2	19	14	19	7	13	7
	%	10	0	70	55	55	45	50	10	95	70	95	35	65	35
CoNS (n=10)	No	2	0	8	6	8	7	7	5	10	8	10	3	7	5
	%	20	0	80	60	80	70	70	50	100	80	100	30	70	50
<i>Enterococcus species</i> (n=10)	No	3	0	6	3	5	5	6	3	10	4	9	3	4	3
	%	30	0	60	30	50	50	60	30	100	40	90	30	40	30

Table 3: Antibiotic sensitive pattern of Gram-negative organisms

Organism	No	AM	AM C	CIP	CT X	CA	CF M	CT	CPZ	CP M	AT	IMP	TE	GE N	NET	AK	PI
<i>Klebsiella spices</i> (n=35)	No	0	11	13	9	7	9	7	0	12	15	31	16	13	13	22	Na
	%	0	31.43	37.14	25.71	20	25.71	20	0	34.28	42.86	88.57	45.71	37.14	37.14	62.86	-
<i>Pseudomonas aeruginosa</i> (n=10)	No	3	3	7	3	5	Na	3	Na	4	5	9	3	5	Na	6	8
	%	30	30	70	30	50	-	30	-	40	50	90	30	50	-	60	80
<i>Citrobacter freundii</i> (n=5)	No	0	3	4	2	2	2	2	0	3	3	5	5	4	5	5	Na
	%	0	60	80	40	40	40	40	0	60	60	100	100	80	100	100	-
<i>E. coli</i> (n=2)	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Na
	%	0	0	0	0	0	0	0	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 18.18% 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 (65% male and 35 % 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 52(52%) than other age groups were 48(48%). 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 (50%) than Gram negative bacteria (45%) which are consistent with the studies conducted by various authors[24-27] we found that most common organism isolated in our study was *Klebsiella species* (35%). 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* (17.78%), 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 10%. 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 (95%) & linezolid (95%) 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 (95%) & linezolid (95%) followed by amoxycylav (70%), Amoxicillin (70%), tetracycline

(55%), ciprofloxacin (55%) & netilmycin (50%). Low sensitivity to clindamycin (35%), cotrimoxazole (35%), gentamycin (45%) & least sensitive to erythromycin (10%), ampicillin (10%) and penicillin (0%). 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 isolate's imipenem showed the highest sensitivity (85%) 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.

Conclusion

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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