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ANTIMICROBIAL RESISTANCE IN BLOODSTREAM INFECTION ISOLATES IN A TERTIARY CARE HOSPITAL OF TRIPURA: A ONE-YEAR RETROSPECTIVE ANALYSIS

Medical Microbiology

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ABSTRACT

Background: Blood stream infections (BSI) are a global health issue that requires high clinical suspicion and early detection, identification of etiological agents, and antimicrobial susceptibility testing. Delay in therapy initiation decreases survival by 8% per hour.[1] This study was conducted to determine the bacterial profile and antimicrobial susceptibility pattern of BSI in a tertiary care hospital in India. **Materials & Methods:** Blood samples were collected from suspected BSI patients and processed for bacterial culture. The samples were tested for antimicrobial susceptibility using standard methods. The bacterial profile, percentage of contamination, gender distribution, and prevalence of gram-positive and gram-negative isolates were analyzed. **Results:** Out of 3327 suspected BSI patients, the bacterial culture positivity rate was 3.54% (118/3327). Gram-positive isolates predominated over gram-negative isolates (72% vs. 28%). Among the bacterial isolates, Staphylococcus aureus (61%) was the most prevalent followed by Enterococcus species (8.5%) and coagulase-negative Staphylococcus (2.5%). The most common gram-negative pathogens were Klebsiella species, E. coli, and Acinetobacter species. The rate of isolation was lower than other Indian studies, which could be attributed to differences in methods, patient population, and infection control strategies. The contaminants were isolated in 11% (365/3327) of cases, and there was a female preponderance (51%). All gram-positive isolates showed 100% sensitivity to vancomycin and high sensitivity to other antimicrobials, which is comparable to other Indian studies. **Conclusion:** This study provides insight into the bacterial profile and antimicrobial susceptibility pattern of BSI in a tertiary care hospital in Tripura. The prevalence of gram-positive isolates was higher than gram-negative isolates, and S. aureus was the most common isolate. The results highlight the need for early detection, identification of etiological agents, and antimicrobial susceptibility testing to ensure adequate patient care. Further studies are required to arrive at a definite conclusion.

KEYWORDS

Blood Stream Infection, Sepsis, Antibiotic Resistance, Antimicrobial Stewardship

INTRODUCTION:

Bloodstream infections (BSIs) are a significant and potentially life-threatening condition, resulting from the presence of microorganisms in the bloodstream. According to Harrison's Principles of Internal Medicine, a blood stream infection (BSI) is defined as the presence of viable microorganisms in the bloodstream, which can lead to a wide spectrum of clinical manifestations ranging from asymptomatic bacteremia to sepsis, septic shock, and multiorgan failure.

The prevalence of BSIs varies across different regions of the world, with a higher burden observed in developing countries. According to the World Health Organization (WHO), sepsis accounts for approximately 20% of global deaths each year, with BSIs being a significant contributor.[2] A recent meta-analysis study revealed that the prevalence of community-onset BSI was 14.6% (range, 3.4 to 38.2%) in Africa, 7.3% (range, 2.0 to 48.4%) in Asia, 2.9% (range, 2.1 to 19.2%) in Europe, and 7.3% (range, 2.9 to 15.6%) in the Americas.[3]

In India, the burden of BSIs is substantial, with estimates suggesting that BSIs account for approximately 30% of hospital-acquired infections.[4] A recent body of literature highlights the concerning prevalence of blood stream infections (BSIs) and antimicrobial resistance (AMR) in pediatric and neonatal patients in India. Dhanya et al. reported a high rate of AMR at 29.1%.[5] In another study conducted by Vasudeva et al., blood culture positivity was reported at 31.2% [6]. Chaturvedi et al. found that 22.78% of their study population had blood culture positivity with 14.88%.[7] Kumar et al. conducted a study at a teaching hospital in West Bengal and reported blood culture positivity rates of 52.1%, with neonates at 49.3%, infants at 17.8%, and pediatric patients at 32.9% [8]. Meanwhile, Yangzom et al. reported a BSI rate of 10.2% among BSI-suspected patients attending a tertiary care hospital in East Sikkim[9]. Palewar et al. found a culture positivity of 10.73% in septicemic cases [10].

With the aim to provide an overview of the bacteraemia and antibiotic susceptibility profile of the isolates we conducted a retrospective analysis of one year- from 1st January 2020- 31st December 2020. The paper also discussed the causative agents of BSIs and their antibiotic resistance patterns, highlighting the need for appropriate antibiotic stewardship and infection control measures to combat the spread of AMR.

MATERIALS AND METHODS:

Sample Collection:

Blood samples were collected aseptically from patients suspected to have BSI. Approximately 10ml-20ml of blood was collected in 2 sets using sterile needles and syringes from each patient. The samples were then transported immediately to the laboratory and inoculated into aerobic and anaerobic culture bottles containing brain heart infusion broth.

Isolation and Identification:

Blood culture bottles were incubated at 37°C for a maximum of 7 days. Subculturing was done on to appropriate culture media and colonies were identified based on their morphology, Gram staining, and biochemical tests. Confirmation of the isolates was done using automated identification systems such as VITEK.

Antimicrobial Susceptibility Testing:

AST of the isolates was performed using the Kirby-Bauer disk diffusion method. Antibiotic disks (HiMedia, Mumbai, India) were placed on to Mueller Hinton agar plates using a sterile wire loop, and the plates were incubated for 24 hours at 37°C. The zone of inhibition around the disk was measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Data Analysis:

The data obtained were entered into a Microsoft Excel spreadsheet, and the percentage of susceptibility and resistance to antibiotics was calculated. Descriptive statistics, such as mean, median, and standard deviation, were calculated, and the data were presented in tables and graphs.

Quality Control:

To ensure the accuracy and reliability of the results, appropriate quality control measures were taken at every stage of the analysis. These included the use of standard reference strains for identification and antimicrobial susceptibility testing, daily monitoring of culture media, and regular calibration of the equipment used for the analysis.

RESULTS:

The study included a total of 3327 blood samples from patients suspected of blood stream infections (BSI). The overall proportion of BSI in the study population was found to be 3.54%, with 118 cases identified. Of these cases, there was a nearly equal distribution between males and females, with 57 (49%) male and 61 (51%) female patients.

Table 01: Socio-demographic and sociodemographic characteristics

Variables	Category	Frequency	Percentage (%)
Sex	Male	57	49%
	Female	61	51%
Age	≤18 years	46	39%
	19 to 40 years	15	12.7%
	40 to 60 years	32	27.1%
	> 61 years	25	21.1%

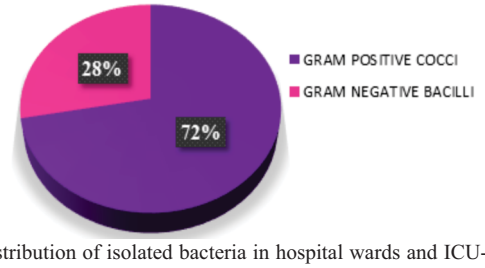
Majority of the culture-positive cases were observed among patients admitted in the departments of medicine, NICU, ICU, PICU, and Paediatric ward. The age groups 19–40 and 41–59 had the highest number of positive cases, accounting for 47 (40%) of the total cases with quite alarmingly high positivity among the age group ≤18 years. These findings suggest that bloodstream infections are a significant health concern in the study population, particularly in patients admitted to certain departments of the hospital.

The results of the bacteriological profiles revealed that out of the 118 bacterial pathogens isolated from the 3327 blood culture sets, the majority of the isolates (72%) were Gram-positive, which included S. aureus, CoNS, and Enterococci species. Among the Gram-positive bacteria, S. aureus was the predominant organism accounting for 61% of the cases, followed by Enterococci species (8.5%) and CoNS (2.5%). The remaining 28% of the isolates were Gram-negative bacteria, with Klebsiella species being the most frequently obtained (12.7%), followed by E. coli (9.3%), Acinetobacter species (5.1%), and Salmonella typhi (0.9%). It is noteworthy that all cases only showed monobacterial infection. These findings highlight the importance of identifying the causative organisms in bloodstream infections, particularly Gram-positive bacteria, and their antimicrobial susceptibility patterns for the appropriate management of patients.

Table 02: Distribution of Bacterial isolates based on Gram's Stain Reaction

	Frequency	Percent (%)
Gram Positive Cocci (GPC)	85	72.0
Gram Negative Bacilli (GNB)	33	28.0
Total	118	100.0

Chart 01: Gram's Stain Result of isolates



The distribution of isolated bacteria in hospital wards and ICU-wise revealed that the maximum number of culture-positive cases were observed among patients admitted in Medicine wards (27.1%), followed by NICU (16.1%), ICU (14.4%), and PICU (11.9%). The Paediatrics ward had 8.5% cases while Cabin had 5.1% of positive cultures. Obs & Gynae wards and Casualty block had an equal distribution of 4.2% each. Orthopaedics wards, ENT, cardio and Neuro wards together had 2.5% each, and only 0.8% of positive cultures were observed from RCC. This data reflects the varying degree of positivity rates of bloodstream infections in different hospital wards, which may help in identifying the high-risk areas for infection control measures.

Table 03: Ward Wise Distribution of Blood Culture Positivity	Frequency	Percent (%)
NICU	19	16.1
PICU	14	11.9
Paediatric Ward	10	8.5
NNW	3	2.5
Medicine Ward	32	27.1
ICU	17	14.4
Cabin	6	5.1
Obstetrics & Gynaecology Wards	5	4.2
Orthopaedics Wards	3	2.5
Casualty Block	5	4.2
ENT, CARDIO, NEURO wards	3	2.5
RCC (Regional Cancer Centre)	1	.8
Total	118	100.0

Table 04: Ward Wise Distribution Of The Isolates

Name of the Ward/ ICU	Name of the Isolate	Frequency	Percentage (%)
NICU	Staphylococcus aureus	12	10.2%
	Enterococcus species	4	3.4%
	Klebsiella pneumonia	3	2.5%
PICU	Enterococcus species	3	2.5%
	Staphylococcus aureus	6	5%
	Klebsiella pneumoniae	4	3.4%
	Staphylococcus epidermidis	1	0.8%
Paediatrics	Staphylococcus aureus	10	8.5%
NNW	Staphylococcus aureus	3	2.5%
Medicine	Escherichia coli	3	2.5%
	Acinetobacter species	3	2.5%
	Staphylococcus aureus	15	12.7%
	Salmonella typhi	1	0.8%
	Escherichia coli	4	3.4%
	Klebsiella pneumoniae	3	2.5%
	Klebsiella oxytoca	2	1.7%
	Staphylococcus haemolyticus	2	1.7%
ICU	Acinetobacter species	2	1.7%
	Klebsiella pneumoniae	2	1.7%
	Staphylococcus aureus	12	10.1%
	Enterococcus species	1	0.8%
Cabins	Staphylococcus aureus	5	4.2%
	Enterococcus species	1	0.8%
Obs& Gynae	Staphylococcus aureus	2	1.7%
	Escherichia coli	2	1.7%
	Enterococcus species	1	0.8%
Orthopaedics	Escherichia coli	1	0.8%
	Staphylococcus aureus	2	1.7%
Casualty block	Escherichia coli	1	0.8%
	Staphylococcus aureus	2	1.7%
	Klebsiella pneumoniae	1	0.8%
ENT, CARDIO, NEURO	Staphylococcus aureus	3	2.5%
RCC	Acinetobacter species	1	0.8%

The susceptibility patterns of Gram-positive bacteria (n = 85) isolated from the blood cultures of patients suspected with BSI against eleven antibiotics were evaluated. The majority of the isolates were resistant to ceftriaxone (89%) and ofloxacin (81%), followed by cefoxitin (28%), co-trimoxazole (28%), clindamycin (28%), ciprofloxacin (23%), gentamicin (20%), linezolid (6%), doxycycline (5%), erythromycin (5%), and vancomycin (0%). All isolates were found to be susceptible to vancomycin. The least resistance was observed to antibiotics such as doxycycline, erythromycin, and linezolid. S. aureus isolates showed a lower resistance against gentamicin (19%), ciprofloxacin (19%), erythromycin (1%), doxycycline (1%), linezolid (1%), co-trimoxazole (28%), and clindamycin (28%). The percentage of MRSA among S. aureus was 28%. Among Enterococci species, 50% of the isolates were resistant to ciprofloxacin and 40% were resistant to linezolid. All isolates were susceptible to vancomycin. Overall susceptibility patterns of Gram-positive isolates showed that 100% were susceptible to vancomycin, 95% to doxycycline and erythromycin, 94% to linezolid, and 80% to gentamicin.

Table 05: Susceptibility pattern of commonly used antimicrobial agents in the treatment of Gram positive isolates.

Antibiotic	Organism		Organism		Organism		Total isolates	
	Staphy. aureus (n=72)		Enterococcus spp. (n=10)		CoNS (n=3)		n = 85	
	S(%)	R (%)	S (%)	R (%)	S(%)	R(%)	S (%)	R(%)
Ceftriaxone	11 (8)	89 (64)	NT	NT	NT	NT	11 (8/72)	89(64/72)

Cefoxitin	72 (52)	28% (20)	NT	NT	67 (2/3)	33% (1/3)	72 (54/75)	28 (21/75)
Gentamicin	81 (58)	19 (14)	70 (7)	30 (3)	100 (3/3)	0%	80 (68/85)	20 (17/85)
Ciprofloxacin	81 (58)	19 (14)	50 (5)	50 (5)	67 (2/3)	33% (1/3)	77% (65/85)	23 (20/85)
Ofloxacin	17 (12)	83 (60)	NT	NT	67 (2/3)	33% (1/3)	19 (14/75)	81 (61/75)
Trimetho/sul	72 (52)	28 (20)	NT	NT	NT	NT	72 (52/72)	28 (20/72)
Clindamycin	72 (52)	28 (20)	NT	NT	NT	NT	72 (52/72)	28 (20/72)
Erithromycin	99 (71)	1 (1)	70 (7)	30 (3)	NT	NT	95 (78/82)	5 (4/82)
Linezolid	99 (71)	1 (1)	60 (6)	40 (4)	NT	NT	94 (77/82)	6 (5/82)
Vancomycin	100 (72)	0	100 (10)	0	100 (3)	0	100 (85/85)	0
Doxycycline	99 (71)	1 (1)	70 (7)	30 (3)	100 (3)	0	95 (81/85)	5 (4/85)
NT: not tested								

The findings of Table 06 demonstrate the extent of antimicrobial resistance in Gram-negative isolates. High levels of resistance were observed against ampicillin (73%, 8/11), cefotaxime (73%, 8/11), cefepime (47%, 15/32), ciprofloxacin (44%, 14/32), piperacillin-tazobactam (24%, 5/21), and amikacin (14%, 3/21). However, the resistance against ciprofloxacin, gentamicin, tetracycline, and cefepime were comparatively lower, i.e., 22.2% (2/9), 33.3% (3/9), 42.8% (3/7), and 33.3% (3/9), respectively. The susceptibility rates of the Gram-negative isolates were high for meropenem (91%, 29/32), imipenem (88%, 28/32), amikacin (86%, 18/21), and piperacillin-tazobactam (76%, 16/21). These findings highlight the importance of choosing appropriate antimicrobial agents to manage infections caused by Gram-negative organisms, taking into account their resistance profiles.

Table 06: Susceptibility pattern of commonly used antimicrobial agents in the treatment of Gram Negative isolates.

Antibiotic	Organism		Organism		Organism		Total isolates	
	Klebsiella spp. (n= 15)		Escherichia coli (n=11)		Acinetobacter species (n=6)		n =32 (100%)	
	S (%)	R (%)	S (%)	R(%)	S(%)	R(%)	S (%)	R (%)
AMP	NT	NT	27 (3)	73 (8)	NT	NT	27 (3/11)	73 (8/11)
IPM	100 (15)	0	64 (7)	36 (4)	100 (6)	0	88 (28/32)	12 (4/32)
CFX	NT	NT	27 (3)	73 (8)	NT	NT	27 (3/11)	73 (8/11)
CPM	53 (8)	47 (7)	27 (3)	73 (8)	100 (6)	0	53 (17/32)	47 (15/32)
CIP	53 (8)	47 (7)	36 (4)	64 (7)	100 (6)	0	56 (18/32)	44 (14/32)
COT	73 (11)	27 (4)	45 (5)	55 (6)	83 (5)	17 (1)	66 (21/32)	34 (11/32)
PIT	67 (10)	33 (5)	NT	NT	100 (6)	0	76 (16/21)	24 (5/21)
MRP	80 (12)	20 (3)	100 (11)	0	100 (6)	0	91 (29/32)	9 (3/32)
AK	80 (12)	20 (3)	NT	NT	100 (6)	0	86 (18/21)	14 (3/21)

Note: AMP: ampicillin, IPM: imipenem, CFX: cefuroxime, CPM: cefepime, CIP:ciprofloxacin, COT: trimethoprim-sulfamethoxazole, PIT: piperacillin-tazobactam, MRP: Meropenem, AK: amikacin.
NT: not tested.

DISCUSSION:

Bloodstream infections (BSI) are a challenging and life-threatening health issue globally. Early detection and identification of etiological agents are necessary for adequate patient care. In the present study, bacterial culture positivity among suspected BSI patients was 3.54%, and contaminant isolation was 11%. Gram-positive isolates predominated over Gram-negative isolates (GNI), with Staphylococcus aureus being the most common pathogen. The low rate of isolation in the present study could be due to the fact that most patients had already received antibiotic treatment before being referred to the tertiary care hospital. Variation in culture positivity rates could be attributed to differences in methods employed for blood culture, volume of blood used, number of blood cultures taken, and the lack of clinical indications, eventually increasing the proportion of negative results.

Among the bacterial isolates, 72% were Gram-positive bacteria out of which Staphylococcus aureus alone accounted for 61% of the BSI followed by Enterococcus species (8.5%) and CoNS (2.5%). Predominance of S. aureus as a bloodstream pathogen has been documented by other studies.[11] The prevalence of MRSA ranged from 70% in ICU setup and 62% in wards, complicating management of BSIs. Coagulase-negative Staphylococcus (CoNS) is increasingly being considered bloodstream pathogens in select settings. Improper methods of blood collection and the presence of longstanding intravascular catheters are recognized as possible modes of spread of BSI by CoNS.

Among Gram-negative pathogens, predominance of Klebsiella species, E. coli, and Acinetobacter species was similar to findings reported in other studies.[12] In present study, S. typhi was isolated in 0.9% cases, a finding similar to that in other studies.[13] All gram-positive isolates showed 100% sensitivity to vancomycin and high sensitivity to linezolid, doxycycline, gentamicin, erythromycin, clindamycin, Cotrimoxazole, and cefoxitin, which is comparable to other Indian studies.[14] Staphylococcus aureus showed low resistance to vancomycin and linezolid, making them effective treatment options.

CONCLUSION:

This study revealed that the overall proportion of bloodstream infections (BSI) in AGMC & GBP Hospital is 3.54%, which is comparatively lower than previous findings from various studies in India. Gram-positive bacteria, particularly S. aureus, were found to be the most common cause of BSI. Based on the results of antimicrobial susceptibility tests, it can be inferred that antibiotics such as meropenem and erythromycin/doxycycline are effective against Gram-negative and Gram-positive bacteria, respectively. However, there was notable growth in antibiotic resistance against several clinically relevant antimicrobials (such as penicillin, ampicillin, cefuroxime, ceftriaxone, cefepime, ciprofloxacin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, and ofloxacin) in the study setting. Thus, the present study provides information on the proportion of bacterial pathogens causing BSI and their antibiotic sensitivity patterns in our tertiary care center in Tripura, India. Based on these findings, surveillance of local BSI etiology, judicious use of antibiotics, and robust infection control practices are recommended.

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