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Microbiological Profile and Antimicrobial Susceptibility Pattern on Blood Culture Isolates Enrolled in Microbiology Department at Rajshahi Medical College

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ABSTRACT: ***Background:*** Blood stream infections (BSI) are a significant global health concern, contributing to both mortality and morbidity. These infections can range from mild to life threatening, often requiring antimicrobial treatment. Due to increasing antimicrobial resistance and changing patterns of antibiotic use, the epidemiology and outcomes of BSI are constantly evolving. Therefore, continuous monitoring of the bacterial causes of BSI and their antibiotic resistance pattern is necessary. ***Objective:*** To find out the bacteriological profile and their antibiotic sensitivity patterns among suspected BSI patients. ***Materials and Methods:*** A cross-sectional study was carried out in the Department of Microbiology, Rajshahi medical college from January 2024 to December 2024. A total of 1228 suspected BSI patients were included in the study. The BacT/Alert automated blood culture method was used to isolate bacterial pathogens and antimicrobial susceptibility test was performed by modified Kirby-Bauer disc diffusion method following CLSI 2024 guidelines. ***Results:*** Out of 1228 cases, 125 (10.17%) were culture positives, where male were 72(57.6%) and female were 53(42.4%). Among 125 culture positive cases, 86 (68.8%) were Gram negative bacteria and 39 (31.2%) were Gram positive bacteria. The most prevalent pathogens were *Coagulase negative staphylococcus* 29(23.2%) and *E. coli* 27(21.6%). Gram negative bacteria are resistant to Amoxyclav, 2nd and 3rd generation Cephalosporin and lower resistance was shown to Meropenem, Piperacilin/tazobactam and Amikacin. ***Conclusion:*** Regular monitoring of sensitivity patterns, creating hospital antibiotic policies based on current data and following treatment guidelines can encourage appropriate antibiotic use and reduce bacterial resistance.

Keywords: Antibiotic-Resistance, Antibiotic Sensitivity, Bacterial Isolates, Blood Culture, Bloodstream Infections.

Article at a glance:
Study Purpose*: To contribute to existing knowledge or propose new ideas.*
Key findings: *Among Gram negative bacteria, E. coli 27 (21.6%) and among Gram positive bacteria CoNS 29(23.2%) were identified as most common isolates.*
Newer findings: *In this study, Gram negative bacteria were showed higher resistance to commonly used antibiotics from BSIs.*

INRODUCTION

Blood stream infections (BSI) are a significant cause of mortality and morbidity globally, ranging from self-limiting to life threatening sepsis requiring antimicrobial treatment.[1] Bacteremia is the presence of viable bacteria in the blood while septicemia indicates systemic manifestations caused by bacteria or their toxins in blood. Hospital-acquired BSIs range from 9% to 11% in developed nations and up to 19% in low and middle countries.[2, 3] Factors contributing to BSIs include medical devices, patient age and pre-existing medical condition such as diabetes mellitus, cancer, renal failure, burns and previous hospitalization.[4] Other risk variables such as the severity, age, sex all affects the death rate from bloodstream infections.[5] Blood culture remains gold standard for diagnosing blood stream infections, despite bacteria are not always being identified.[6] Multidrug resistance especially in Gram negative bacteria causing BSIs, poses a significant therapeutic challenge, leading to fewer treatment options, higher cost, longer hospital

stay and increased morbidity and mortality.[7] Antibiotic-resistance varies geographically, necessitating surveillance and documentation of blood culture isolates to guide the best empirical antibiotic use and reduce resistance.[8] With this above view, to isolate and identify different bacterial causes of BSIs, determining the antibiotic susceptibility patterns of isolated bacteria and suggesting empirical treatment of BSIs.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted in the Rajshahi Medical College's Microbiology department over a period from January to December 2024. The study involved collecting two venous blood samples were taken from two separate locations, 30 minutes apart, from each participant who had a suspected BSIs following strict aseptic procedures. The sample sets consisted of 8-10 ml of adult venous blood, 0.5- 2 ml of neonatal venous blood or 2-5 ml of blood from pediatric patients. These sample were immediately inoculated into aerobic blood culture bottles (adult or pediatric bottles, depending on the situation) and incubated using BacT/ALERT® 3D automated blood culture analyzer. All broths that tested positive for BacT/ALERT were subcultured onto Salmonella Shigella agar, MacConkey's agar and blood agar. Subculturing onto blood agar and MacConkey's agar was carried out on days 2, 5, and 7 days of incubation for those bottles without positive signs. For 18 to 24 hours, the inoculated blood agar, MacConkey's agar, and Salmonella Shigella agar plates were incubated at 37 °C. Using standard laboratory procedures, colony morphology, gram staining, and traditional biochemical testing were used to identify bacterial growth. [9] The modified Kirby-Bauer disc diffusion method assessed the antibiotic susceptibility of isolated bacteria on Mueller-Hinton agar with CLSI guideline, determining if the results were sensitive or resistant. [10]

RESULTS

A total of 1228 blood culture samples were collected from clinically suspected patients with BSI during the study period. Only 125 (10.17%) of the 1228 samples tested positive for culture, whereas 1103 (89.83%) tested negative (Figure 1). Among the culture 125 positive cases, higher in males (72; 57.6%) as compared to females (53; 42.4%) (Figure 2). Of the 125 bacterial isolates, 86 (68.8%) were Gram-negative bacteria, while the remaining 39(31.2%) were Gram-positive bacteria (Table 1). Amongst the Gram-negative bacteria, most isolated organism was *E. coli* (21.6%) followed by *Klebsiella spp.* (19.2%), *Pseudomonas spp.* (14.4%), *Salmonella spp* (12%) and *Acinetobacter spp.* (1.2%) respectively. Antimicrobial susceptibility pattern of Gram-negative bacteria showed highest susceptibility to Cefepime, Meropenem, Piperacillin/tazobactam and lowest susceptibility to Amoxyclav, Cefuroxime, Cefixime and Ceftriaxone (Table 2). The susceptibility pattern of Gram-positive isolates showed highest susceptibility to Vancomycin, Linezolid, Meropenem and lowest susceptibility to Azithromycin, Ciprofloxacin (Table 3).

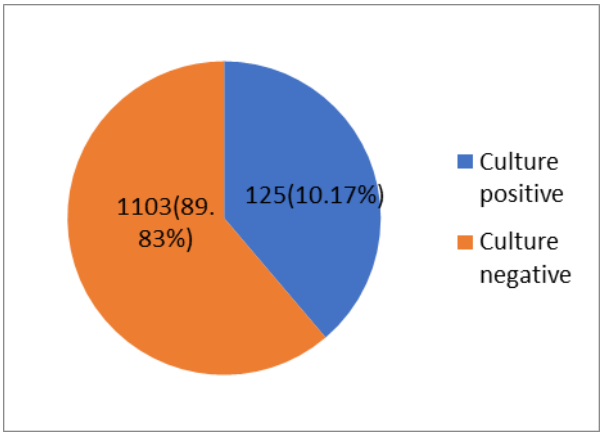

Figure 1: Frequency of culture positive and culture negative cases (N=1228)

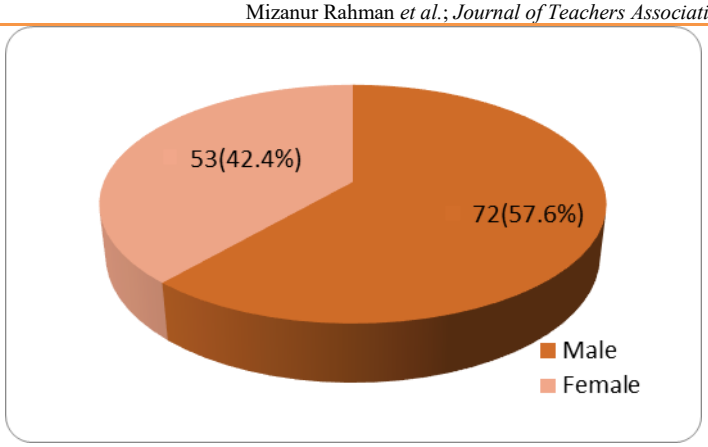

Figure 2: Gender variation regarding growth positive cases: (n=125)

Table 1: Gram positive and Gram-negative bacteria among bacterial growth (n=125)

Isolated Organism	Number (%)
Gram negative	86(68.80%)
Gram positive	39(31.20%)

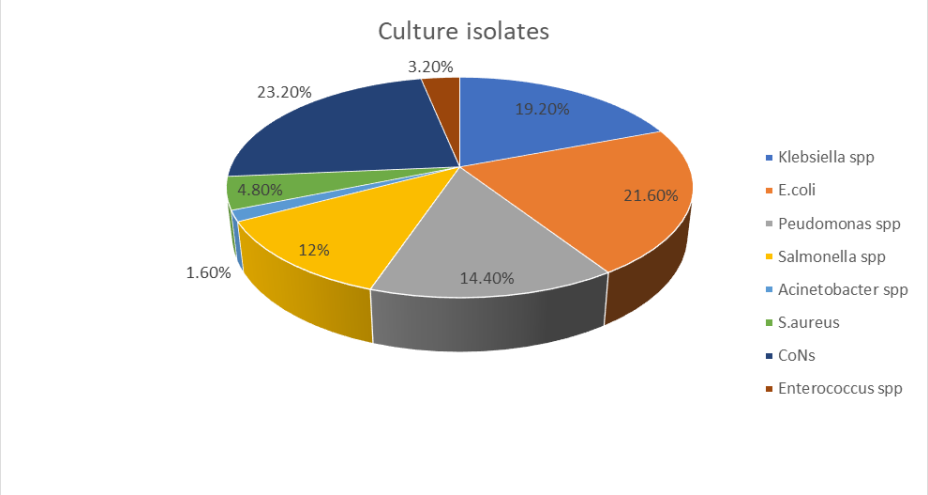

Figure 3: Distribution of culture positive isolates. (n=125)

Table 2: Antimicrobial susceptibility pattern of Gram-negative organism. (n=86)

Antimicrobial Agent		*E.coli* (27)	*Klebsiella spp.* (24)	*Pseudomonas spp.* (18)	*Salmonella spp.* (15)	*Acinetobacter spp.* (02)
Amikacin	S	70.83%	81.49%	66.67%	66.67%	100%
	R	29.17%	18.51%	33.33%	33.33%	0%
Amoxyclav	S	62.50%	88.89%	61.11%	80%	-
	R	37.5%	11.11%	38.89%	20%	-
Aztreonam	S	70.83%	48.14%	72.22%	-	-
	R	29.17%	51.86%	27.78%	-	-
Azithromycin	S	-	-	61.11%	26.67%	-
	R	-	-	38.89%	73.33%	-
Ampicillin	S	-	-	-	86.67%	-
	R	-	-	-	13.33%	-
	S	37.5%	74.08%	-	66.67%	-

Cefixime	R	62.5%	25.92%	-	33.33%	-
Cefepime	S	70.83%	70.38%	-	60%	50%
	R	29.17%	29.62%	-	40%	50%
	S	33.33%	81.49%	-	80%	0%
Ceftriaxone	R	66.67%	18.51%	-	20%	100%
	S	37.5%	25.92%	-	-	-
Cefuroxime	R	62.5%	74.08%	-	-	-
Cotrimoxazole	S	-	-	-	80%	0%
	R	-	-	-	20%	100%
Ciprofloxacin	S	66.67%	29.62%	66.67%	73.33%	100%
	R	33.33%	70.38%	33.33%	26.67%	0%
Ceftazidime	S	66.67%	44.44%	72.22%	-	50%
	R	33.33%	55.56%	27.78%	-	50%
Colistin	S	-	-	-	-	100%
	R	-	-	-	-	0%
Meropenem	S	75%	66.67%	83.33%	100%	100%
	R	25%	33.33%	16.67%	0%	0%
Netilmicin	S	-	-	66.67%	-	-
	R	-	-	33.33%	-	-
Piperacillin/	S	83.33%	48.14%	83.33%	-	100%
tazobactam		16.67%	51.86%	16.67%	-	0%
	R					
Levofloxacin	S	-	-	-	100%	-
	R	-	-	-	0%	-

Table 3: Antimicrobial susceptibility pattern of Gram-positive bacteria (n=39)

Antimicrobial agent		***Enterococcus spp.* (04)**	***CoNS* (29)**	***Staphylococcus aureus* (06)**
Amikacin	S	75%	89.65%	66.67%
	R	25%	10.34%	33.33%
Amoxyclav	S	50%	62.06%	33.33%
	R	50%	37.94%	66.67%
Azithromycin	S	50%	27.58%	33.33%
	R	50%	72.41%	66.67%
Cotrimoxazole	S	50%	55.17%	50%
	R	50%	44.82%	50%
	S	75%	37.93%	66.67%
Ciprofloxacin	R	25%	62.07%	33.33%
Linezolid	S	100%	100%	83.33%
	R	0%	0%	16.67%
Meropenem	S	75%	81.10%	66.67%
	R	25%	18.90%	33.33%
Vancomycin	S	100%	100%	100%
	R	0%	0%	0%

DISCUSSION

Blood culture is a well-established procedure of the standard diagnostic workup for many infectious diseases. In Bangladesh, all kinds of drugs including the antibiotics are sold over the counter, misuse of antibiotics has been found to be responsible for developing pool of resistant bacteria as well as negative results of blood culture.[11] A total of 1228 blood culture samples were collected from clinically suspected patients with BSI during the study period. Only 125 (10.17%) of the 1228 samples tested for culture positive, whereas 1103 (89.83%) tested negative (Figure 1). This study were nearly similar

with Laxmi Kant Khanal *et al.,* in Nepal; Nasrin *et al*., in Bangladesh with 10.3%, 13% were cultures positive and 89%, 87% were found culture negative respectively.[12,13] The current findings were dissimilar with Shewta *et al*., in India; Daniel Ningthoujam *et al*., in India with culture positive 25.8%, 31.2% and 74.2%, 68.8% were culture negative.[14,15] The variation in positivity rates among different studies may be attributed to differences in the methodology used for blood culture, the volume or number of blood culture samples taken, study design, geographical differences, nature of the patient population, differences in the epidemiological agents and variations in infection control policies.[15] Among the 125 culture positive cases, higher in males (72; 57.6%) as compared to females (53; 42.4%) (Figure 2). The finding corresponded with the study of Belew *et al*., in Ethiopia (50.9% male and 49.1% female) and Shewta *et al*., in India (male 59% and female 41%).[14,20] Dissimilar with R.S. Parihar *et al*., in India (male 68.75% and female 31.25%).[1] This may be explained as men are involved in more physical activities for livelihood and less frequent hand hygiene practice which could potentially provide environment for large reservoirs of common pathogens responsible for causing blood stream infections.[1] Of the 125 bacterial isolates, 86 (68.8%) were Gram-negative bacteria, while the remaining isolates 39(31.2%) were Gram-positive bacteria (Table 1). In contrast to this study, similar findings were found with 68% Gram negative and 32% Gram positive bacteria of Habyarimana *et al*., in Africa and 60% Gram negative and 40% Gram positive were found of Laxmi Kant Khanal *et al.,* in Nepal.[12, 18] Dissimilarity was found with Daniel Ningthoujam *et al*., in India and Cheema K H *et al*., in Pakistan which were found 24.7% and 96.9% were Gram negative bacteria and 75.3% and 3.1% as Gram positive bacteria respectively.[15,19]

Among the Gram-negative bacteria (Figure 3), most isolated organism was *E.coli* (21.6%) followed by *Klebsiella spp* (19.2%), *Pseudomonas spp* (14.4%), *Salmonella spp (*12%*)* and *Acinetobacter spp* (1.6%) respectively. Among Gram positive 39 cases, highest organism *CoNS* (23.2%) followed *S. aureus* (4.8%) and *Enterococcus spp* (3.2%) (Figure 3). Among the Gram-negative bacteria, the similar study were found *E. coli* (29.4%), *Klebsiella spp* (20.6%), *Pseudomonas spp* (8.8%) with Oluwalana T *et al*., in Nigeria and *E.coli* (14%), *Klebsiella spp*. (13%), *Acinetobacter spp* (7%), *Pseudomonas spp.* (7%) with Kaur C and Sharma S, in India.[16,20] Dissimilar were found *E.coli* (5.7%), *Klebsiella spp*. (5.7%), *Acinetobacter spp.* (7%), *Pseudomonas* spp. (1.4%) *Salmonella spp.* (59.5%) with Cheema K H *et al*., in Pakistan and *Pseudomonas spp* (11.3%), *Acinetobacter spp*. (6%), *Klebsiella spp* (6%), *E. coli* (0.7%), *Salmonella spp* 1 (0.7%) with Daniel Ningthoujam *et al*., in India.[19,15] Figure 3 showed that among 39 Gram positive cases the highest organism was *CoNS* (23.2%) followed *S. aureus* (4.8%) and *Enterococcus spp.* (3.2%). Similar with Kaur C and Sharma S., in India were found *CoNS* (31.2%) *S. aureus* (7%) followed by *Enterococcus spp* (6%) and Laxmi Kant Khanal, in Nepal were found *CoNS* (26.8%) and *Enterococcus spp* (6.52%).[20,12] Different dissimilar were found with R.S. Parihar *et al*., in India , Oluwalana T *et al*., in Nigeria , where *CoNS* were (41.3%, 8.8%) and *S. aureus* was (23.8%) respectively.[16, 17]

Antimicrobial susceptibility pattern of Gram-negative bacteria showed highest susceptibility to Cefepime, Meropenem, Piperacillin/tazobactam and lowest susceptibility to Amoxyclav, Cefuroxime, Cefixime and Ceftriaxone (Table 2). *E. coli* was the most susceptible to Meropenem (75%), Amikacin (70.83%) and Piperacillin-tazobactam (83.33%) whereas Cefixime (62.5%) and Ceftriaxone (66.66%) were the least susceptible drug. This study similar to Shewta *et al*., in India for Piperacillin-tazobactam (81.81%), Meropenem (81.81%) and Amikacin (72.72%) & Zerin T *et al*., in Bangladesh Meropenem (84.57%), Amikacin (60.64%) and Cefixime (60%), Ceftriaxone (44.68%), respectively had the lowest susceptibilities.[14, 21]

Among the Gram-negative isolates, *Klesiella spp* showed highest susceptibility towards Meropenem (66.67%), Amikacin (81.49%) and Cefepime (70.38%) and lowest susceptibility to Amoxyclav (11.11%), Ceftriaxone (18.51%), Cefixime (25.92%) respectively. Similar study was found Belew *et al.,* in Ethiopia in highest susceptibility to Meropenem (62.5%), Amikacin (78.5%) and lowest susceptibility Amoxyclav (0%) and Ceftriaxone (18.51%) respectively.[21] Dissimilar with Cheema K H *et al*., in Pakistan was found to Meropenem (40%), Amikacin (40%) and lowest susceptibility Amoxyclav (0%) and Ceftriaxone (30%).[19] and R.S. Parihar *et al*., in India was found Meropenem (100%), Amikacin (57.1%) respectively.[1]

Table 3 *CoNS* had the lowest susceptibility to Azithromycin (72.41%) and Ciprofloxacin (62.06%) among Gram-positive isolates, whereas the highest susceptibility to Vancomycin (100%), Linezolid (100%) and Meropenem (81.1%) was observed. Similar studies were conducted in India by R.S. Parihar *et al.*, in with 100% susceptible to Vancomycin and Linezolid and Shewta *et al.*, in with 100% Vancomycin and 92.85% Linezolid.[1, 14] The three antibiotics that *S aureus* was most susceptible to were Vancomycin (100%), Linezolid (83.33%), and Meropenem (66.67%). Similar studies were conducted in India by Shewta *et al.*, in with Vancomycin (100%) and Linezolid (80%) and R.S. Parihar *et al.*, in with Vancomycin (100%) and Linezolid (94.73%).[14, 1]

CONCLUSION

The resistance pattern of Gram-negative bacilli to commonly used drugs has raised concerns among clinicians and hospital groups to treat blood stream infections. So, detection of bloodstream infection-causing pathogens and their antibiogram which can help choose the best empirical treatment.

Ethical Approval

Ethical clearance for the study was taken from the Instutional Review Board and concerned authority, Rajshahi Medical College & Hospital.

Conflict of Interest: None declared.

Funding: No funding sources.

Consent: Informed written consent was taken from each patient's attendant.

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