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SPECTRUM OF BLOODSTREAM PATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN A TERTIARY CARE SETTING: A RETROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background

With a high mortality rate, bloodstream infections (BSI) are a serious worldwide public health burden. Delay in therapy has a significant impact on Patient's outcomes, and rapid and precise pathogen diagnosis significantly improves patient care. Drug-resistant bacterial pathogens are a result of frequent inappropriate antibiotic usage and undiagnosed bloodstream infections. A lack of investigation on BSIs in Nepal has limited the understanding of their causes, prevention, and treatment.

Objectives

The purpose of this study is to examine the bacteriological profile and antibiotic susceptibility of bloodstream infections in order to assess current trends.

Methods

This is a retrospective analysis of blood cultures from individuals suspected of having a bloodstream infection conducted at a hospital. We performed a retrospective examination of blood culture data from individuals suspected of having a bloodstream infection over a three-year period. Bloodstream infection, blood culture contamination, bacterial profile, and patterns of antibiotic resistance were all determined using data from the laboratory reports.

Results

A total of 544 isolates were isolated with Gram negative organisms being the most dominant. *Acinetobacter* spp. (20.6%), followed by Coagulase negative Staphylococci (CoNS) (17.28%), *S. aureus* (14.5%), and *E. coli* (9.4%) were the most significant bacteria. Excluding CoNS, the MDR rate among the remaining 447 isolates was 40.4%. *K. pneumoniae* had the greatest MDR burden (73.9%), then A. baumannii complex (58.3%) and S. aureus (46.8%). Overall, third-generation cephalosporin resistance was concerning, and glycopeptides and carbapenems are still effective against the majority of pathogens.

Conclusions

The presence of high-level multidrug resistant (MDR) Gram negative bacteria, especially Acinetobacter spp and K. pneumoniae, makes empirical treatment less effective. Our results suggest the need for local susceptibility-based approach and improved antibiotic stewardship in resource limited settings.

Keywords: *Antimicrobial resistance (AMR), Antimicrobial stewardship (AMS), Blood culture, Blood stream infections (BSI), Multi Drug Resistance (MDR)*

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INTRODUCTION

The current known method for identifying suspected cases of bacteremia is blood culture. When bacteremia is suspected in Febrile patients, blood cultures are routinely obtained in the majority of high-income nations. However, due to inadequate or nonexistent laboratory capacity, physicians may decide to treat patients empirically in resource-constrained settings [1,2]. Despite the availability of broad-spectrum antibiotics and significant advancements in supportive care, Blood Stream Infections (BSI) remains a significant cause of morbidity and mortality [3]. According to data released in 2020, there were 11 million sepsis-related fatalities and 48.9 million cases globally, accounting for 20% of all deaths [4].

Every organ in the body is at risk from microorganisms in circulating blood, whether they are present continually, sporadically, or temporarily. Additionally, BSI can develop into potentially fatal complications like organ failure and sepsis. It has been demonstrated that early diagnosis and timely delivery of the proper antibiotic therapy enhance patient outcomes and lower mortality in patients with BSIs [5].

In BSIs, a range of organisms are isolated. Enterobacteriaceae, Enterococci, and Staphylococci are frequently implicated. Members of the Enterobacteriaceae family and other Gram negative bacteria, such as Pseudomonas and Acinetobacter species, are responsible for the current rise in the incidence of bacteremia [6]. Contamination is defined as a microorganism recovered from BC bottle that is indeed absent in the patient blood. Most often, contaminations are bacteria from the skin microbiota (e.g. coagulase negative staphylococci, coryneform). Antibiotic resistance is one of the top 10 health risks, according to the World Health Organization. Research from Nepal has also shown that BSI organisms are becoming more resistant [7,8]. For the purpose of directing appropriate medication and enhancing patient outcomes, precise and up-to-date information on the microbiological profiles and antimicrobial susceptibility profiles linked to BSIs is crucial.

Therefore, examining regional patterns of antibiotic susceptibility among BSI pathogens throughout time will serve as a guide for creating antibiograms and developing antibiotic policies. Furthermore, such data offers valuable insights on the scope and swift increase of antibiotic resistance in this area or similar resource-constrained environments. Antimicrobial resistance (AMR) surveillance at the local and regional levels supports national initiatives, which in turn assist the establishment of coordinated action to address AMR at the international level.

METHODS

The current study was conducted at the Rapti Academy of Health Sciences Department of Microbiology in Ghorahi, Dang, Nepal, from January 2023 to December 2025. The samples originate from inpatients with BSI symptoms, people who visited various OPDs, and hospitalized individuals. All blood culture specimens from people of all ages and newborns with a clinical suspicion of bloodstream infection were considered during the study period. Ethical clearance was obtained from the Institutional Review Committee (IRC) of Rapti Academy of Health Sciences (RAHS), Dang, Nepal (Ref. No. 1599). Given the retrospective nature of the study, informed consent was waived by the IRC as no direct patient interaction was involved. All patient identifiers were removed and replaced with anonymous numerical codes prior to analysis to ensure confidentiality. The study was conducted in full accordance with the Declaration of Helsinki.

Every blood culture that revealed a known bacterial infection was also analyzed. Duplicate organisms that were isolated from several specimens collected simultaneously and had the same antibiotic sensitivity from the same patient (identified by matching hospital ID) were excluded from the analysis.

Procedure: Standard microbiological techniques were used to obtain blood samples, which were then placed in Bactec bottles (TDR Resin Aerobic Culture Vials for Adults and TDR Resin Peds Culture Vials for children) and stored in the Mindray company TDR-X 120 Blood Culture System. If there was growth, the Bactec bottle was subculture on Blood Agar (BA), MacConkey's agar (MA), and Chocolate agar plates (HI Media). All of the BA, CA, and MA were kept in candle jars and incubated at 37°C for 18 to 24 hours. Periodic subculture was performed following an overnight incubation period of up to 72 hours. The acquired growth was identified using colony characteristics, biochemical tests, and Gram staining. The Kirby-Bauer disc diffusion method was used to assess the isolates susceptibility to various antibiotics. The Clinical and Laboratory Standards Institute (CLSI) criteria were followed in the measurement and interpretation of zone sizes [9].

Study definitions

- BSI was described as the presence of live bacteria or fungi in the bloodstream, as evidenced by a positive blood culture in an individual suspected of having an infection.
- Resistance to at least one agent in three or more antimicrobial groups was defined as Multidrug Resistance (MDR) [10]

Statistical Analysis

Demographic data of patients, including age, isolates, and drug susceptibility results obtained were entered in the Microsoft Excel professional plus 2021. Data were analyzed using descriptive statistics. The bacterial growth rate was calculated in percentage base distribution. Organism spectrum is presented

as tabulations. Trend pattern of antibiotic resistance was analyzed in R programming Language Version R 4.5.2. The contaminants included species of Bacillus, micrococcus, and polymicrobial organisms and were excluded from the entry.

RESULTS

Over the course of three years, 8736 blood samples with suspected cases of BSI were collected; 544 of these samples showed growths after culture. Gram negative bacteria accounted for 321/544 (59%) of BSI pathogens and Gram positive bacteria were found in 220/544 (40.44%) and fungal in 3/544(0.55%) of total BSI pathogens. The most common etiological agent was found to be *Acinetobacter* species (20.59% of all the positive cultures) followed by Coagulase negative staphylococci (17.28%). Majority of Gram negative bacteria belonged to *Acinetobacter* spp and Gram positive bacteria belonged to Coagulase negative Staphylococci. The commonest pathogen was *Acinetobacter* species (accounting for over 20.59% of BSIs), followed by Coagulase negative Staphylococci (17.28%), *Staphylococcus aureus* (14.52%), *Escherichia coli* (9.38%) and *Enterobacter* spp. (8.27%). It is shown in **Table 1**.

Male to female ratio is 1.17:1 was seen as displayed in **Table 2**. Mean age of 24.9 years and a median age of 11 years, patient ages ranged from 1 to 90 years, indicating a greater number of pediatric representations in the population, which is displayed in **Table 3**.

Table 1: Frequency of blood culture isolates

Organism	Numbers	%
Gram Positive organisms		
Coagulase Negative Staphylococci (CoNS)	94	17.28
Staphylococcus aureus	79	14.52
Enterococcus spp.	35	6.43
Streptococcus spp.	11	2.02
Viridans Streptococci	1	0.18
Gram Negative organisms		
Acinetobacter spp.	112	20.59
Escherichia coli	51	9.38
Enterobacter spp.	45	8.27
Pseudomonas aeruginosa	43	7.9
Klebsiella pneumoniae	23	4.23
Acinetobacter baumannii complex	12	2.21
Salmonella Typhi	10	1.84
Pseudomonas spp.	8	1.47
Proteus vulgaris	5	0.92
Citrobacter freundii	3	0.55
Salmonella spp.	2	0.37
Klebsiella spp.	2	0.37
Klebsiella oxytoca	2	0.37
Citrobacter koseri	2	0.37
Proteus mirabilis	1	0.18
Fungus		
Candida	3	0.55

Table 2: Distribution of isolates among gender

Sex	Frequency	%
Male	293	53.86
Female	251	46.14
Total	544	100

Table 3: Distribution of isolates among different age-groups

Age groups	Frequency	Percent
0-15	297	54.6
16-30	68	12.5
31-45	37	6.8
46-60	51	9.4
>61	91	16.7
Total	544	100.0

The highest percentage of isolates was from the Emergency Department (302; 55.5%), followed by the Pediatrics Department (173; 31.8%) and the Medicine ward (36; 6.6%). A total of 120 isolates were recorded in 2023, which increased to 255 in 2024, before declining to 169 in 2025.

Table 4: Antibiotic Susceptibility Profile of Clinically Significant Bacterial Isolates % Resistant (R%)

Organism	N	CIP	IMP	AMK	CRO	CFX	CTX	LVFX	MER	PIT	COT	PEN	VAN	GEN
		R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%
Acinetobacter spp.	n=112	24.1	18.3	15.5	77.0	82.65	81.1	7.5	23.0	27.5	45.0	--	--	30.7
S. aureus	n=79	37.8	--	--	--	--	--	31.2	--	--	56.0	87.0	0	27.5
E. coli	n=51	50.0	0	24.4	62.0	70.8	57.4	28.2	3.7	19.5	42.4	--	--	42.3
Enterobacter spp.	n=45	24.3	36.3	35.7	48.5	60.6	63.8	10.7	3.1	21.9	43.3	--	--	50
P. aeruginosa	n=43	14.3	0	10.2	---	--	---	0	6.2	10.5	--	--	--	9.0
Enterococcus spp.	n=35	13.3	--	--	--	--	--	10.7	---	--	--	77.0	0	--
K. pneumoniae	n=23	40.0	9.0	66.6	82.3	83.3	85.7	46.6	24.0	27.2	53.0	--	--	81.2
A. baumannii complex	n=12	22.2	22.2	54.0	83.3	91.67	90.9	25	33.3	36.6	75.0	--	--	50
S. typhi	n=10	16.6	0	0	0	30	11.1	50.0	0	11.1	22.2	--	--	0

(CIP=Ciprofloxacin, IMP=Imipenem, AMK=Amikacin, LVFX=Levofloxacin, MER=Meropenem, PIT=Piperacillin Tazobactam, COT=Cotrimoxazole, PEN=Penicillin, VAN=Vancomycin, GEN=Gentamicin, CFX-Cefixime, CTX-cefotaxime, -- = Not tested

The antibiotic susceptibility profile showed varying levels of resistance among clinically significant bacterial isolates. **Table 4** Higher resistance rates were observed against commonly used antibiotics, particularly among organisms such as *Acinetobacter* species, *Acinetobacter baumannii complex, Escherichia coli, Klebsiella pneumoniae*. Most of the gram-negative organism has low resistance percentage against Imipenem and Meropenem

Table 5: Multi-Drug Resistance (MDR) Prevalence by Organism (MDR = resistance to ≥3 antibiotic classes).

Organism	Total Isolates (n)	MDR Cases (n)	MDR Rate (%)
K. pneumoniae	23	17	73.9%
A. baumannii complex	12	7	58.3%
S. aureus	79	37	46.8%
Enterobacter spp.	45	19	42.2%
Acinetobacter spp.	112	46	41.1%
P. vulgaris	5	2	40.0%
P. aeruginosa	43	16	37.2%
Streptococcus spp.	11	4	36.4%
E. coli	51	17	33.3%
Enterococcus spp.	35	9	25.7%
S. Typhi	10	1	10.0%

Klebsiella pneumoniae recorded the highest MDR prevalence at 73.9% (17/23 isolates), followed by A. baumannii complex at 58.3% (7/12). S. aureus showed an MDR rate of 46.8% (37/79), while Enterobacter spp. (42.2%, 19/45) and Acinetobacter spp. (41.1%, 46/112) were similarly burdened. E. coli and Enterococcus spp. showed lower but clinically relevant MDR rates of 33.3% and 25.7%, respectively. Salmonella Typhi demonstrated the lowest MDR prevalence at 10.0%. **Table 5**.

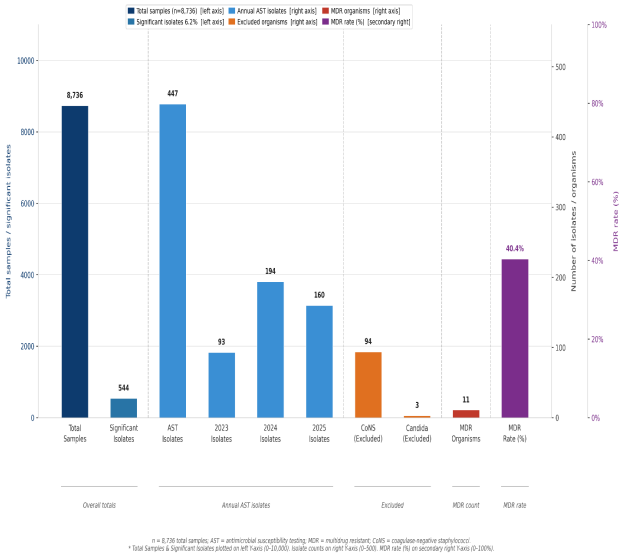

Figure 1: Bar diagram including total sample, Number of Isolate with year wise distribution of isolate include Multi Drug Resistance Percentage

Figure 1: Shown total number of samples were 8736 in which 544 were significant isolates.

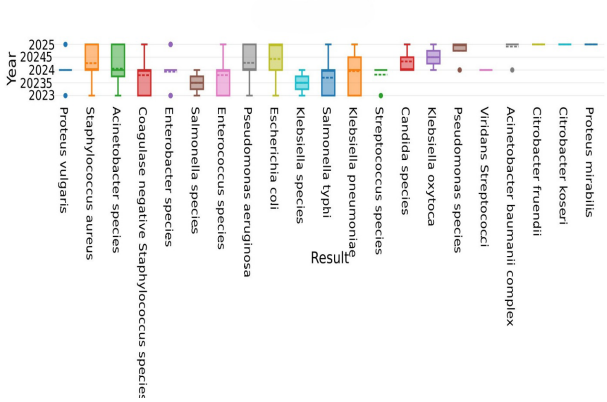

Figure 2: Yearly Distribution of Bacterial Isolate

Figure 2: Shows that a variety of bacterial and fungal species were isolated between 2023 and 2025, with Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Acinetobacter species appearing most frequently. Other organisms were present in smaller or more variable numbers. Overall, the pattern suggests a diverse group of pathogens with some year-to-year variation in their occurrence.

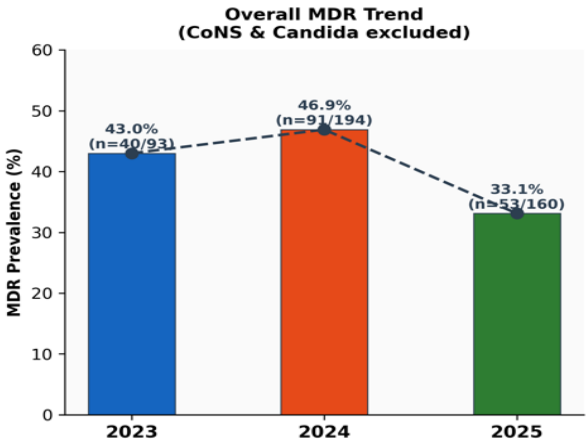

Figure 3: Annual trend in Multi Drug Resistance (Multi-Drug Resistance (MDR) Analysis of Clinical Bacterial Isolates (2023–2025), with CoNS excluded. (A) MDR prevalence by organism; organisms with fewer than five isolates excluded. (B) Annual MDR trend; n denotes the number of non-CoNS isolates per year (2023: n=93/120 total; 2024: n=194/255 total; 2025: n=160/169 total)

Examining the annual trend MDR prevalence was 43.0% in 2023 and higher to 46.9% in 2024, and declined to 33.1% in 2025. **Fig: 3**.

DISCUSSION

Effective management of BSIs requires an understanding of local isolate patterns and their antimicrobial susceptibility. To comprehend the evolving bacterial etiology and the emergence of antibiotic resistance over time, surveillance studies

on bacterial trends are essential.

Overall culture positive was 6.2% (544/8736) and a 3-year prevalence of BSI of 5.1% (447/8736), The prevalence of BSI which is consistent with previous studies from western Nepal reporting rates was 6% [11] also, some studies in Nepal show higher prevalence comparison to our studies i.e. 10.3% [12] and 12.6% [13]. BSIs are caused by many factors like the host's immune system, environmental conditions, and patient characteristics like age, stress, and a chronic physiological strain. Studies show that due to age-related immunological decline, sepsis has more severe effects in the extremes ages like infants and the elderly [14]. Also, the sampling volume of the blood culture, the medium formulation, and the kind of patients included in the study could all be the factors contributing to the difference in the BSI rates throughout different studies. Additionally, patients and medical personnel's improper use of antibiotics prior to referrals to tertiary care facilities may be responsible for lower rates of BSI [15].

In our study, the prevalence of BSI varied across age groups, with higher rates observed in pediatric patients n=297(54.6%). Similar study in Nepal and Ghana shows higher prevalence in pediatric patient [16,17]. It may be due to their developing immune systems and weak skin integrity; pediatric age group often engage in activities that put them at risk for illness [15].

Among the isolated organisms Gram Negative bacteria was predominant (59.02%) in comparison to Gram positive organism which is comparable to other studies [11,18,19]. Acinetobacter species were the most common organism in Blood stream infection which is in accordance with the results of earlier research conducted in Nepal Mediciti hospital and Philadelphia hospital [20,21]. Seasonal variation, endemicity of the etiological agents, patient population characteristics, and geographic distribution could all contribute to the variation.

The antimicrobial susceptibility patterns in this study had shown several important resistance patterns among clinically important bacteria. Multidrug resistant of *Acinetobacter baumannii complex* has been seen high-level resistance to Ceftriaxone (83.3%) and Amikacin (54.0%), also with considerable carbapenem resistance (33.3%). *Klebsiella* spp. had highest level of resistance against Gentamicin (81.2%) and Ceftriaxone (82.3%), while *E. coli* and *Enterobacter* spp. have shown increased resistance to Ciprofloxacin and third generation cephalosporin, indicating the existence of multiple ESBL producing organisms. Vancomycin still shows it is one of the reliable treatments against Gram positive bacteria because *S. aureus* had 87.0% resistance rate against penicillin but no evidence of vancomycin resistance. With low resistance rates against Gentamicin (9.0%) and Meropenem (6.2%), *P. aeruginosa* was found to be less resistant. These results show a rising prevalence of resistance, underscoring the importance of locally customized empirical treatment and antimicrobial stewardship measures in order to achieve the highest potential clinical outcomes.

The annual MDR prevalence in this study increased from 43.0% (n=93) in 2023 to a peak of 46.9% (n=194) in 2024, declined to 33.1% (n=160) in 2025 (Figure 2). The early increase in MDR prevalence could reasonably be explained by the steady build-up of resistance over time, largely driven by the pressure exerted by antibiotic use and the persistent problem of inappropriate prescribing habits among clinicians, a pattern that mirrors what has been widely reported in global surveillance studies documenting a worrying rise in MDR organisms across various healthcare facilities worldwide [22,23]. The following reduction in 2025 can indicate the beneficial impact of antimicrobial stewardship initiatives or infection control practices applied throughout the study time, as also observed in other similar institutional contexts [24,25]. Nevertheless, the constant prevalence of MDR over 30% in the three years is still a serious clinical issue, in line with data of other low- and middle-income country (LMIC) hospitals [26,27]. and demonstrates the necessity of ongoing resistance surveillance and rational policies of antibiotic use.

The overall antibiotic susceptibility pattern of Gram negative isolates indicate a significant percentage of MDR organisms in our hospital. The synthesis of hydrolytic enzymes, specifically extended spectrum β-lactamases (ESBLs), class C cephalosporinase (AmpC), and carbapenemases (including Metallo-beta-lactamases), is frequently linked to beta-lactam antibiotic resistance in Gram negative bacteria [28]. At 73.9%, *Klebsiella pneumoniae* has the greatest MDR prevalence which is similar to several investigations of a similar nature [7,29]. followed by *Acinetobacter* spp. And *Staphylococcus aureus*. The least MDR is shown by *Salmonella* Typhi (10%) and *Enterococcus* Species (25.7%).

In Nepalese hospitals, broad spectrum antibiotics—particularly cephalosporins and quinolones—are the standard treatment for undifferentiated febrile sickness due to their greater efficacy and low toxicity [13]. A developing issue is the irrational and increased use of these antibiotics, which has led to an increase in multiple drug resistance in bacteria.

The biggest risk associated with MDR, ESBL, and carbapenemase-producing Gram negative bacteria is that bacterial illnesses, especially bacteremia, are becoming incurable due to the restricted therapeutic options of the existing antibiotics, increasing mortality and health care expenses.

The increase in bloodstream infections (BSIs) and antimicrobial resistance (AMR) in Nepal highlights the critical need for efficient infection control procedures and antimicrobial stewardship programs (ASP). To counter this expanding threat, effective diagnostics, infection control, and responsible antibiotic usage are essential. The results of the study provide light on the current antimicrobial profile, which will improve the antimicrobial stewardship program and serve as a guide for creating an efficient antibiogram in this tertiary hospital environment.

CONCLUSIONS

This three-year retrospective analysis shows that clinical isolates of bacteria in this tertiary care setting have a concerning prevalence of multidrug and antibiotic resistance. Gram-negative bacteria, particularly *Acinetobacter* spp. and *K. pneumoniae*, provide the biggest therapeutic challenges because of their high rates of resistance, which almost eliminate the use of traditional empirical therapy methods. The most reliable treatments for the majority of pathogens are carbapenems and glycopeptides, but their application is not always common, particularly in *A. baumannii complex* and *K. pneumoniae*. The sensitivity of some important infections to Aminoglycosides, Fluoroquinolones, and Third-generation cephalosporins has been substantially lowered, and local susceptibility testing should be used to support the use of the empirically selected medication. The growing difficulty of properly managing BSIs in a resource-constrained environment like ours is highlighted by the rising incidence of resistant organisms. These results demonstrate the critical need for improved antibiotic stewardship and local susceptibility-guided treatment in situations with limited resources.

LIMITATIONS

To begin with, this study was only done in one hospital it would have been more insightful if it had also looked at other settings, the data on clinical outcomes was not available as the investigation was a retrospective analysis, therefore, it was not possible to evaluate the effect of resistance on patient mortality or length of stay. Also, not every antibiotic was tested on every organism as if they were outside the standard institutional testing protocol for that species and, therefore, some organism-antibiotic combinations lacked data.

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