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Burden of Antibiotic Resistance in Bloodstream Infections

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ABSTRACT

Bloodstream infections (BSIs) are among the leading causes of morbidity and mortality worldwide. In developing countries, rising cases are driven by changing epidemiology, antibiotic resistance, lack of standardized treatments, and inadequate diagnostics, which contribute to an increased rate of BSI-associated mortality. The main objective of this research was to identify multidrug-resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing bacteria isolated from blood specimens. The cross-sectional study was conducted in Alka Hospital, Lalitpur, from April to October 2023. A total of 737 blood specimens from suspected BSI patients were inoculated on BACTEC 9050 and subcultured on Blood agar and MacConkey agar. Bacteria were identified based on colony morphology and biochemical tests, and then the antibiotic susceptibility pattern was determined using the Kirby-Bauer disk diffusion method following CLSI 2018. ESBL production was detected by screening and phenotypic confirmatory tests, and methicillin-resistant *S. aureus* (MRSA) was detected using a Cefoxitin disc. Out of 737 specimens processed, 52 (7.06%) were culture-positive, with 37 (71.15%) Gram-negative bacteria and 15 (28.85%) Gram-positive bacteria. A total of 70.27% of Gram-negative isolates and 73.33% Gram positive isolates were found to be multidrug resistant (MDR). A total of 29.73% Gram-negative bacteria and 20% Gram-positive bacteria were phenotypically confirmed as ESBL producers. MDR and ESBL-producing strains limit the treatment options. Therefore, strict regulations on antibiotic prescription and sales, and increased public awareness of their proper use must be implemented to prevent ineffective use, misuse and overuse, which drive the emergence of drug-resistant bacteria and exacerbate antimicrobial resistance.

Keywords: Bloodstream infection, multidrug resistance, Extended Spectrum Beta Lactamase, Methicillin Resistant *S. aureus*, BACTEC

Introduction

Bloodstream infections (BSIs) can be defined as a positive blood culture in a patient with clinical signs of infection and is a major public health burden worldwide, with a high mortality and morbidity worldwide and representing an increasing public health concern (Timsit et al., 2020; Lamy et al., 2020; Laupland, 2013). BSIs occur in community and hospital settings from different sources and may affect a diverse group of patients with various microorganisms (Liu et al., 2024). BSIs are one of the major causes of illness and one of the leading causes of death worldwide. BSI has a mortality rate ranging from 20-50% and has the potential to be fatal (Bhandari et al., 2015). The rate of morbidity increases in case of patients with severe comorbidities such as oncologic-hematologic diseases, cirrhosis, or solid-organ transplants (Franco et al., 2021). However, very little information exists regarding the pattern of bacterial bloodstream infection and antibiotic susceptibilities in Nepalese children and adults (Parajuli et al., 2017).

The term bloodstream infection and bacteremia are synonymously used and bacteremia may arise from minor injuries during tooth brushing, tooth extraction, abscesses, wounds, surgery, use of catheter, and existing infections like urinary tract infection (UTI), gastrointestinal tract infection (GTIs), burns, bedsores, or from areas of localized disease as in pneumococcal pneumonia, meningitis, osteomyelitis, etc. (Simkhada et al., 2016). The term septicemia is not similar to bacteremia but closely interacts with it, in which septicemia refers to the invasion and persistence of pathogenic bacteria in the bloodstream (Komori et al., 2020).

Increasing BSIs globally over time with a shift in antimicrobial resistance, particularly the emergence of multidrug-resistant (MDR) organisms and

ESBL-producing organisms, pose a significant therapeutic challenge in BSI management (Liu et al., 2024). Infections caused by antibiotic-resistant pathogens such as MDR strains of *E. coli, S. aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*, Methicillin resistant *S. aureus* (MRSA), Coagulase-negative Staphylococci and Carbapenem-resistant Enterobacteriaceae and ESBL-producing organisms are critically challenging treatment options (Bohra et al., 2017; Timsit et al., 2020; Shaikh et al., 2015; Siwakoti et al., 2024).

The most common cause of antibiotic resistance in bacteria could be the unmonitored and uncontrolled use of antibiotics. Since only a limited number of new antimicrobial agents are in development, antimicrobial resistance (AMR) has become more threatening worldwide. This issue has become a main public health threat because if they are not adequately and promptly treated, these infections are associated with lengthy Intensive Care Unit (ICU) stays, high expenses, and may also increase the mortality rate (Azimi et al., 2019; Franco et al., 2021). Administering the correct antibiotics promptly for bloodstream infections (BSI) is the key factor in improving patient survival and recovery while reducing healthcare costs by preventing complications, prolonged treatments, and hospital stays. In bacteremia, the risk of death doubles when proper antibiotics are not administered within 24 hours (Tabah et al., 2022).

This study highlights the burden of antimicrobial resistance in BSIs, therefore, continuous improvement of the whole BSI diagnostic process, including sampling quality, proper diagnostic process and time to get result, proper identification of BSI pathogens and perform antibiotic susceptibility test to guide empiric antibiotic treatments and enabling precision medicine should be a priority to improve patient outcome and avoid unnecessary antibiotic treatment (Liu et al., 2024; Lamy et al., 2020; Siwakoti et al., 2024). Similarly, a systematic study is needed to investigate the etiology and trend of bacterial pathogens and the role of drug-resistant isolates in these infections. By providing insights into the prevalence of antimicrobial resistant (AMR) organisms in causing blood stream infections in clinical settings in Nepal and identifying patients with risk factors that make them more susceptible to BSIs, this research will support policymakers, healthcare professionals, and microbiologists in developing evidence-based measures to reduce the spread of antibiotic-resistant bacteria.

Materials and methods

Research design

The study was a hospital-based cross-sectional study.

Study site and Study duration

Samples were collected and processed at Alka Hospital Pvt. Ltd, Lalitpur, Nepal. The study was conducted over the period of 7 months (from April to October 2023).

Sample size

A total of 737 blood specimens were collected from patients suspected of bloodstream infections.

Sample Collection and Transport

Using a sterile syringe and needle, a total of 10 ml of blood sample was collected from the adults and 2 ml of blood sample was collected in case of children (Cheesbrough, 2017) and inoculated on BACTECH 9050.

Sample processing

Collected blood was cultured using BACTECH 9050 and incubated up to 7 days at 37 °C. The bottle was removed from the system for subculture when the indicator signaled for positive growth and the subculture was done onto blood agar and MacConkey agar and was incubated at 37 °C for 24 hours. For a negative result after 72 hours, it was discarded and reported as no growth (Cheesbrough, 2017).

Identification of the isolates

Isolates grown on Blood agar and MacConkey agar were identified based on Colony morphology, Gram's staining, and biochemical tests. sulfide indole motility test (SIM), methyl red (MR) test, Voges Proskaeur (VP) test, citrate utilization test, urease test, oxidase test and triple sugar iron agar (TSIA) tests were performed for Gram negative bacteria and coagulase test, catalase test and oxidation-fermentation (OF) tests were performed for Gram positive bacteria (Cheesbrough, 2017).

Detection of antibiotic resistance and ESBL production

Antibiotic susceptibility pattern was tested by the Modified Kirby Bauer disc diffusion method using the antibiotics of Hi Media following the CLSI guideline 2018. When three different sets of first-line medications were analyzed, an isolate that showed resistance to at least one antibiotic from each of the three groups was classified as multidrug resistant (MDR) (CDC, 2024). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were detected using a 30 mg Cefoxitin disk on a Muller-Hinton agar plate. Zone size was interpreted using CLSI (2018) Criteria: sensitive (>22mm) and resistant (<21mm).

Similarly, to identify ESBL-producing bacterial isolates, Ceftazidime and Cefotaxime discs were used with cut-off zones of inhibition ≤22 mm and ≤27 mm, respectively, following CLSI (2018) standards. To validate ESBL production, a combination disc method was performed by using a disc containing Ceftazidime and Cefotaxime alone and in combination with Clavulanate. ESBL was confirmed with an increase in inhibition zone of ≥5 mm when combined with clavulanate, compared to the zone of inhibition of the antibiotic alone (CLSI 2018).

Results

Growth Profile of Bacteria

Out of the total 737 samples processed, 52 specimens (7.06%) were found culture positive, and out of culture-positive specimens, Gram-negative bacteria were isolated from 37 (71.15%) specimens, while Gram-positive isolates were isolated from 15 (28.85%) specimens.

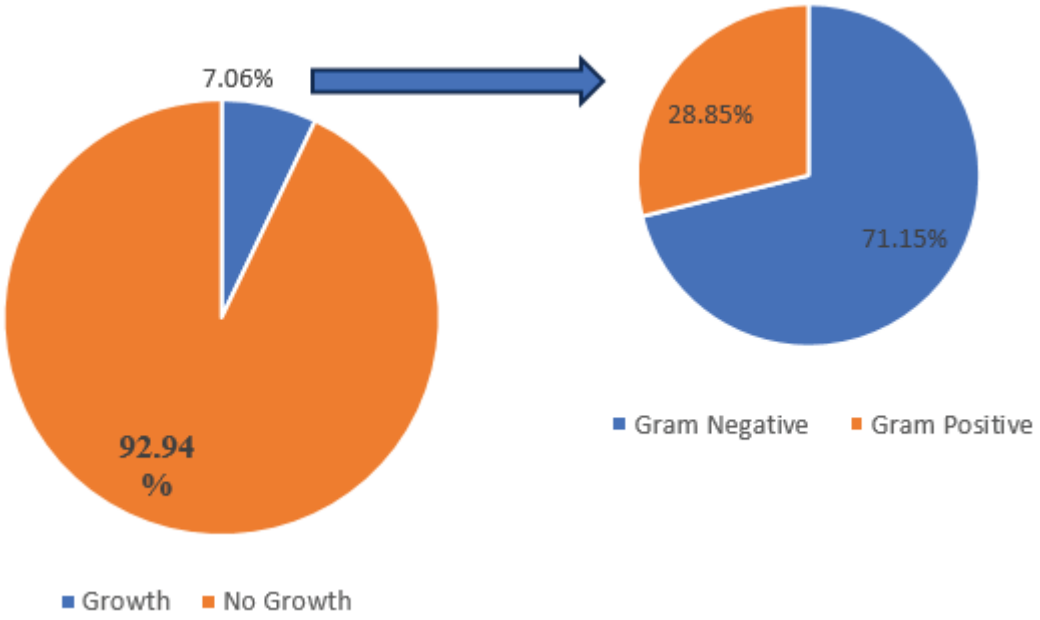


Figure 1: Growth profile and distribution of Gram-positive and Gram-negative bacterial isolates

Distribution of isolates based on Sex and Age of the patients

Among 37 Gram-negative isolates, 22 (59.5%) were isolated from the specimens collected from male patients, and the remaining 15 (40.5%) were isolated from female patients. Similarly, in the case of a total of 15 Gram-positive isolates, 8 (53.3%) isolates were from male patients and 7 (46.7%) were from female patients. The maximum number of Gram-negative isolates (i.e., 40.5%) was from the age group of 61-80, while that in the case of Gram-positive isolates, the maximum growth (i.e., 53.3%) was obtained from the age group of more than 80 Years.

Table 1: Distribution of the isolates according to sex and age of the patients

Bacteria	Male	Female	<1-20	21-40	41-60	61-80	>80	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
Salmonella Typhi	5 (62.5)	3 (37.5)	1(12.5)	1(12.5)	3(37.5)	3 (37.5)	0	8
Salmonella Paratyphi A	8 (72.5)	3 (27.5)	1 (9.2)	3(27.3)	4 (36.4)	3 (27.3)	0	11
P. aeruginosa	2 (100)	0 (0)	0	0	2 (100)	0	0	2
E.coli	5 (62.5)	3 (37.5)	0	0	2 (25)	4 (50)	2 (25)	8
K.pneumoniae	2 (33.3)	4 (66.7)	0	0	2 (33.3)	4 (66.7)	0	6
Acinetobacter spp.	0 (0)	2 (100)	1 (50)	0	0	1 (50)	0	2
Total (Gram Negative)	**22(59.5)**	**15(40.5)**	**3 (8.1)**	**4(10.8)**	**13(35.1)**	15(40.5)	**2 (5.5)**	**37**
MSSA	4 (57.1)	3 (42.9)	1(14.2)	0	0	3 (42.9)	3(42.9)	7
CONS	3 (75.0)	1 (25.0)	0	1 (25)	0	0	3 (75)	4
MRSA	1 (25.0)	3 (75.0)	0	0	0	2 (50)	2 (50)	4
Total (Gram Positive)	**8 (53.3)**	**7 (46.7)**	**1 (6.7)**	**1 (6.7)**	**0**	5 (33.3)	**8(53.3)**	**15**

Month-wise distribution of bacterial isolates

A maximum number of Gram-negative isolates were obtained in the month of June, followed by August. *Salmonella* Typhi, *Salmonella* Paratyphi A and *E. coli* were obtained mostly in the month of June. Gram positive bacteria were mostly isolated in the month of April.

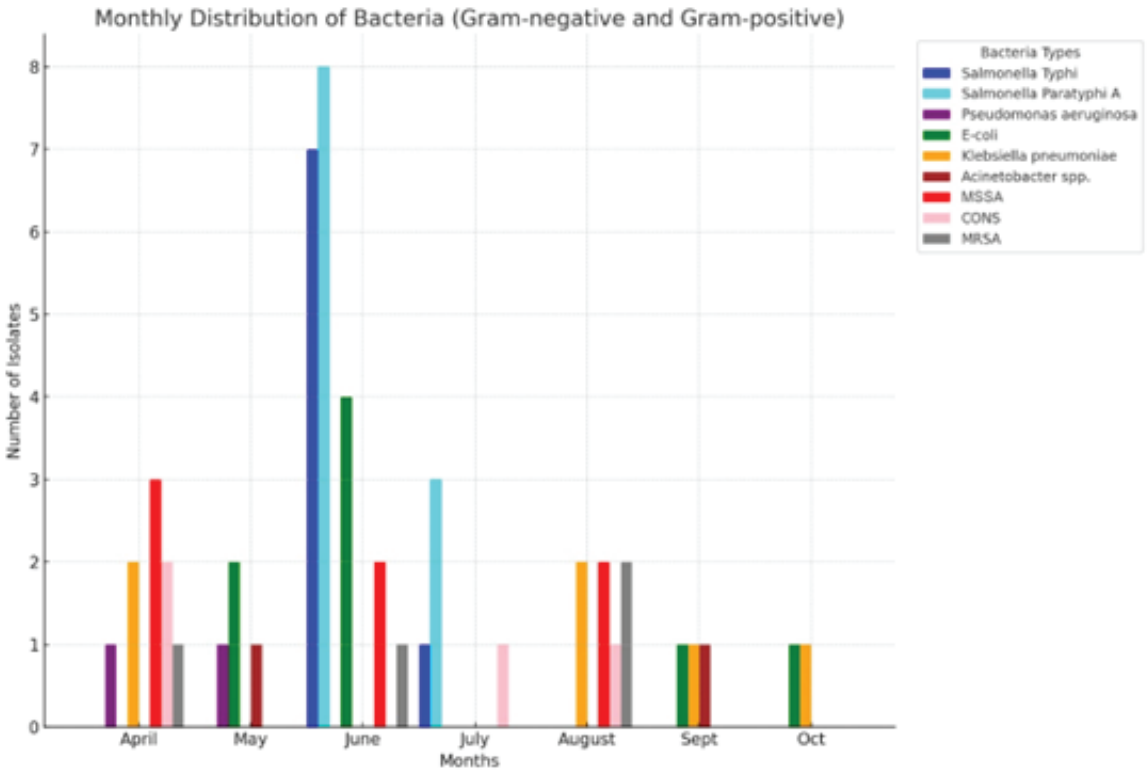


Figure 2: Month-wise distribution of bacterial isolates

***Antibiotic Susceptibility Pattern of Salmonella* Paratyphi *A and Salmonella* Typhi**

In case of *Salmonella* Paratyphi A, Chloramphenicol was found to be the most effective antibiotic with a 100% susceptibility rate, whereas 63.6% of isolates were resistant towards Nalidixic acid. Similarly, *Salmonella* Typhi was found to be 100% susceptible to Chloramphenicol, and the least effective antibiotic was Amoxyclav with 62.5% resistance.

Table 2: Antibiotic susceptibility pattern of *Salmonella* Paratyphi A and *Salmonella* Typhi

Antibiotics	Salmonella Paratyphi A (N=11) n (%)			Salmonella Typhi (N=8) n (%)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ciprofloxacin	7 (63.6)	2 (18.2)	2 (18.2)	7(87.5)	1(12.5)	0
Nalidixic Acid	4 (36.4)	0	7 (63.6)	5(62.5)	0	3(37.5)
Amoxicillin	8 (72.7)	0	3 (27.3)	5(62.5)	0	3(37.5)
Cefixime	9 (81.8)	0	2 (18.2)	6(75)	0	2(25)
Cefotaxime	9 (81.8)	0	2 (18.2)	6(75)	0	2(25)
Ceftriaxone	9 (81.8)	0	2 (18.2)	6(75)	0	2(25)
Amoxyclav	4 (36.4)	1 (9.1)	6 (54.5)	3(37.5)	0	5(62.5)
Azithromycin	6 (54.5)	1 (9.1)	4 (54.5)	5(62.5)	0	3(37.5)
Chloramphenicol	11 (100)	0	0	8(100)	0	0
Cotrimoxazole	10 (90.9)	0	1 (9.1)	7(87.5)	0	1(12.5)

Antibiotic susceptibility pattern of E. coli

E. coli was found to be 100% susceptible to Gentamicin, Amikacin, Chloramphenicol, and Imipenem while 100% resistance was seen towards ceftazidime.

Table 3: Antibiotic susceptibility pattern of ***E. coli***

	E. coli (N=8) n (%)		
Antibiotics	**Sensitive**	**Intermediate**	**Resistant**
Gentamicin	8 (100)	0	0
Amikacin	8 (100)	0	0
Cotrimoxazole	3 (37.5)	1 (12.5)	4 (50)
Aztreonam	3 (37.5)	1 (12.5)	4 (50)
Chloramphenicol	8 (100)	0	0
Piperacillin-tazobactam	7 (87.5)	0	1 (12.5)
Ciprofloxacin	5 (62.5)	0	3 (37.5)
Ceftriaxone	3 (37.5)	0	5 (62.5)
Ceftazidime	0	0	8 (100)
Imipenem	8 (100)	0	0

Antibiotic susceptibility pattern of K. pneumoniae

The most effective antibiotic for *K. pneumoniae* was found to be Chloramphenicol with 100% sensitivity while the least effective drug was Ceftazidime with 83.3% resistance.

Table 4: Antibiotic susceptibility pattern of *K. pneumoniae*

Antibiotics	*K.pneumoniae* (N=6) n (%)		
	Sensitive	Intermediate	Resistant
Gentamicin	5 (83.3)	0	1(16.7)
Amikacin	4 (66.6)	1 (16.7)	1 (16.7)
Cotrimoxazole	3 (50)	0	3 (50)
Aztreonam	3 (50)	0	3 (50)
Chloramphenicol	6 (100)	0	0
Piperacillin-tazobactam	2 (33.3)	2 (33.3)	2(33.3)
Ciprofloxacin	1 (16.7)	3 (50)	2 (33.3)
Ceftriaxone	2 (33.3)	1 (16.7)	3 (50)
Ceftazidime	1 (16.7)	0	5 (83.3)
Imipenem	4 (66.7)	0	2 (33.3)

***Antibiotic susceptibility pattern of Acinetobacter* spp.**

Among the isolated *Acinetobacter* spp, it was found that Gentamicin, Amikacin, Piperacillin-Tazobactam, Imipenem, Cotrimoxazole and Cefixime was 100% effective drug, followed by Ceftazidime to which one isolate was found to be sensitive (50%) and one was found to be resistant (50%). Two isolates were found to be resistant towards Doxycycline and Ciprofloxacin.

***Antibiotic susceptibility pattern of Pseudomonas* spp.**

In case of *Pseudomonas* spp, Amikacin was found to be a 100% effective drug, followed by Imipenem, to which one isolate was found to be sensitive and one was found to be resistant. Both isolates were resistant to Ceftazidime and Ciprofloxacin.

Antibiotic susceptibility pattern of Methicillin-sensitive Staphylococcus aureus (MSSA)

S. aureus was found to be 100% sensitive to Vancomycin. S. *aureus* was found to be sensitive to Amikacin (85.71%), followed by Gentamicin, Meropenem, Erythromycin, and Chloramphenicol (71.44%). *S. aureus* was found to be resistant to Cotrimoxazole and Amoxicillin (71.44%), followed by Ciprofloxacin and Tetracycline (57.14%).

Table 5: Antibiotic susceptibility pattern of Methicillin sensitive *S. aureus (MSSA)*

Antibiotics	*S. aureus* (N=7) n (%)		
	Sensitive	Intermediate	Resistant
Gentamicin	5 (71.44)	0	2 (28.56)
Amikacin	6 (85.71)	0	1 (14.28)
Amoxycillin	1 (14.28)	1 (14.28)	5 (71.44)
Cefoxitin	3 (42.86)	0	4 (57.14)
Meropenem	5 (71.44)	0	2 (28.56)
Vancomycin	7 (100.00)	0	0

Erythromycin	5 (71.44)	0	2 (28.56)
Chloramphenicol	5 (71.44)	0	2 (28.56)
Ciprofloxacin	3 (42.86)	0	4 (57.14)
Cotrimoxazole	2 (28.56)	0	5 (71.44)
Tetracycline	3 (42.86)	0	4 (57.14)

Antibiotic susceptibility pattern of MRSA

MRSA was found to be 100% resistant to Amoxicillin and Cefoxitin. Vancomycin was found to be most effective for MRSA, i.e., 100% sensitive. MRSA was found to be sensitive towards Amikacin (75%), followed by Gentamicin (50%). Only one isolate was found resistant to Tetracycline.

Antibiotic susceptibility pattern of CONS

CONS was found to be 100% sensitive to Vancomycin and it was found to be 60% resistant to Gentamicin, followed by Cotrimoxazole (50%).

Multidrug Resistant (MDR) Gram-positive and Gram-negative bacterial isolates

Out of 37 Gram-negative isolates, 26 (70.27%) strains were found to be multidrug resistant. Eight (100%) *E. coli* was found to be MDR. Four (66.6%) *K. pneumoniae* was found to be multidrug resistant, which was followed by 6 (54.5%) *Salmonella* Paratyphi A, followed by *Salmonella* Typhi 4 (50%).

Similarly in the case of Gram-positive bacteria, 93.33% was found to be MDR. CONS and MRSA were 100% multidrug resistant. Six (85.7%) of *S. aureus* was found to be MDR.

Table 6: Multidrug resistant Bacterial isolates

Organisms	**Total Isolates**	**MDR**	**MDR Percentage**
Gram Negative Bacteria			
Salmonella Typhi	8	4	50
Salmonella Paratyphi A	11	6	54.5
P. aeruginosa	2	2	100
E. coli.	8	8	100
K. pneumoniae	6	4	66.6
Acinetobacter spp	2	2	100
Total	**37**	**26**	**70.27**
Gram positive Bacteria			
MSSA	7	6	85.7
CONS	4	2	50
MRSA	4	3	75
Total	**15**	**11**	**73.33**

Distribution of ESBL producers

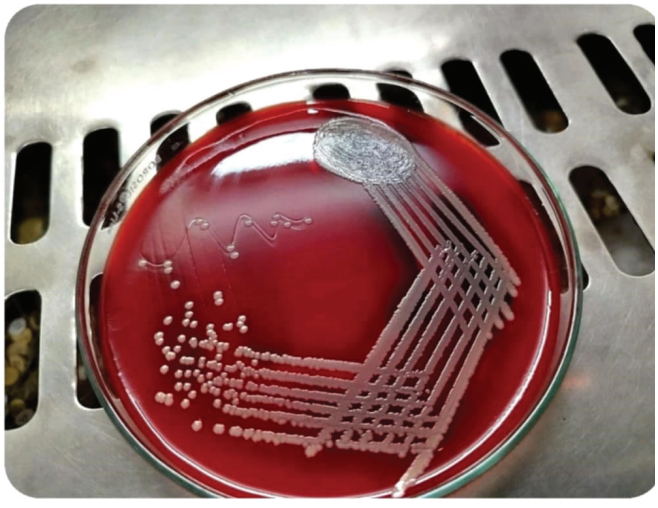
Among Gram-negative bacteria, *E. coli* had the highest screening positivity rate (100%), while *Salmonella* Paratyphi A had the highest phenotypic confirmation rate (85.7%). The overall screening positivity for Gram-negative bacteria was 67.6%,

with a phenotypic confirmation rate of 44%. In contrast, Gram-positive bacteria showed lower screening positivity and confirmation rates, with *Staphylococcus aureus* being the most common, showing 57.1% ESBL screening positivity, and 50% were ESBL confirmed. Overall, Gram-negative bacteria had higher positivity and confirmation rates compared to Gram-positive bacteria.

Table 7: Distribution of ESBL Producers

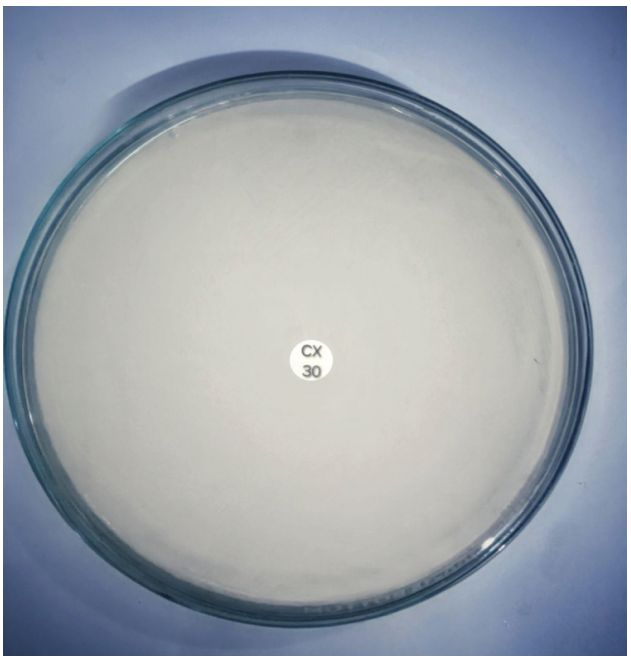
Bacteria	Total sample	Screening positive	Phenotypic confirmation
Gram Negative Bacteria			
Salmonella Typhi	8	5 (62.5%)	3 (60%)
Salmonella Paratyphi A	11	7 (63.6%)	6(85.7%)
Pseudomonas aeruginosa	2	1 (50%)	0
E-coli.	8	8 (100%)	6 (75%)
Klebsiella pneumoniae	6	4 (66.7%)	2 (50%)
Acinetobacter spp	2	0	0
Total	**37**	**25(67.57%)**	**11(29.73%)**
Gram Positive Bacteria			
MSSA	7	4 (57.1%)	2 (50%)
CONS	4	1 (25%)	0
MRSA	4	2 (50%)	1 (50%)
Total	**15**	**7(46.67%)**	**3(20%)**

Photographs

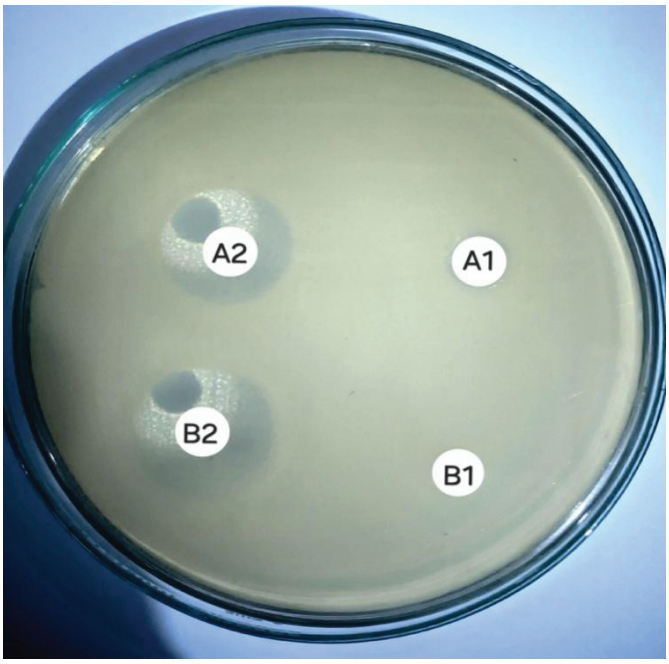


Photograph 1: *S. aureus* on Blood Agar: Golden yellow color, smooth, circular, and convex colonies

Photograph 2: Biochemical test of *E. coli.* From left to right: Indole +ve, MR +ve, VP -ve, Citrate -ve, TSI A/A, Gas +ve, Urease -ve.



Photograph 3: *Staphylococcus aureus* in MHA showing resistance to Cefoxitin (No zone of inhibition).



Photograph 4: Phenotypic confirmation of ESBL (*E coli*)
A2 showing increase zone of inhibition (≥ 5mm) than A1
B2 showing an increased zone of inhibition (≥ 5mm) than B1
A1= ceftazidime, A2= ceftazidime + clavulanate
B1= cefotaxime, B2= cefotaxime + clavulanate

Discussion

Bloodstream infections (BSIs) remain a significant global health concern, contributing to high morbidity and mortality rates. These infections often result in prolonged hospital stays, increased healthcare costs, and severe complications, including septic shock and multi-organ failure. Identifying the causative microbial pathogens and their antibiotic susceptibility patterns is crucial for effective management and treatment.

In this study, a total of 737 blood samples were analyzed, of which 52 (7.06%) were found to be culture positive. This positivity rate was consistent with studies conducted in Nepal by Simkhada et al. (2016) and Parajuli et al. (2017), which reported positivity rates of 7.28% and 7.48%, respectively. However, studies by Chaturvedi et al. (2021), Bhandari et al. (2015), and Habyarimana et al. (2021) observed higher positivity rates, ranging from 11.71% to 40%. The variation in positivity rates could be due to several factors, including sample collection techniques, variations in blood culture systems, geographical variations, months of the year when the blood is collected, types of patients included, and the prior antibiotic use, which can suppress bacterial growth and reduce culture yields (Chaturvedi et al., 2021).

Among the 52 culture-positive cases, Gram negative bacteria accounted for 71.15%, with the predominance of *Salmonella* Paratyphi A (21.2%). A similar finding of predominance of *Salmonella* Paratyphi A was reported in various other studies conducted in Nepal (Simkhada et al., 2016).

A total of 28.85% of the isolates were identified as Gram-positive bacteria, with the predominance of Methicillin-sensitive *Staphylococcus aureus* (MSSA) accounting for 13.4% of the total isolates and 46.7% of the total Gram-positive isolates, followed by CONS, which was in line with findings from Simkhada et al. (2016), Parajuli et al. (2017), and Habyarimana et al. (2021). The study by Shrestha et al. (2012) in Nepal also identified methicillin-sensitive *S. aureus* (MSSA) as the predominant isolated bacteria. The high frequency of *S. aureus* in both this research and comparable studies could be attributed to hospital environments, where

colonization from patient flora, staff hands, air, surgical treatments, inanimate objects, and prolonged hospital stays could play a significant role (Shrestha et al., 2012). The predominance of Gram-negative bacteria aligns with findings from studies conducted by Habyarimana et al. (2021) and Chaturvedi et al. (2021). Gram-negative bacteria are known for their ability to survive in diverse environmental conditions and their intrinsic and acquired resistance mechanisms, which pose significant challenges for treatment.

The highest rate of infections due to Gram-negative isolates was observed in June. The possible reason behind isolating the highest number of isolates in the month of June might be because of the warm and humid season of the year, and the possibility of rain and transmission of disease by contaminated water (Rijal et al., 2021).

A maximum number of isolates were isolated from male patients (57.69%) and Maximum number of Gram-negative isolates (i.e. 40.5%) were isolated from the age group of 61-80 while that in case of Gram-positive isolates, maximum growth (i.e.53.3%) were obtained from the age group of more than 80. Increased rate of infection observed in extreme ages of life might be due to poor immune competency as well as the presence of comorbidity conditions (Parajuli et al., 2017).

The rise of antimicrobial resistance (AMR) is a significant public health challenge, complicating the treatment of bloodstream infections. Both Gram-positive and Gram-negative bacteria are developing multidrug resistance, rendering some infections difficult or impossible to treat with conventional antibiotics. In this study, out of 37 Gram-negative isolates, 26 (70.27%) isolates were found to be multidrug resistant (MDR), while in the case of Gram-Positive isolates, 73.33 % isolates were MDR. These findings were comparable with other independent studies conducted by Silpakar et al. (2021), Bhandari et al. (2015), and Adhikari et al. (2018). The rise of antimicrobial resistance (AMR) is a significant public health challenge, complicating the treatment of bloodstream infections.

Among Gram-negative isolates, ESBL was screened among 25 out of 37 (67.6%) isolates, which opposes the finding of Nepal et al. (2017), where 34.5% of the total isolates were screened as ESBL producers. The screened isolates were further processed for phenotypic confirmation, where 29.73% of the total Gram-negative isolates were confirmed as ESBL producers which opposes the findings of Abrar et al. (2019). The difference could be because of the difference in study time, number and type of bacterial isolates, types of patients, sample size and the antibiotics selected might also be different. Similarly, in the case of Gram-positive isolates, out of 15 isolates, 46.67% were screened for ESBL production and 20% were confirmed as ESBL producers.

BSIs caused by extended-spectrum beta-lactamase (ESBL) producing bacteria are spreading massively worldwide. Detection of a large number of MDR and ESBL producer organisms highlights the urgent need for antimicrobial-related programs, strict antibiotic prescription guidelines, routine surveillance of resistance patterns, public health interventions such as community awareness programs, improved sanitation, and hygiene practices are required to mitigate the spread of MDR and ESBL-producing pathogens. Routine practice of antibiotic susceptibility testing is crucial for guiding empiric antibiotic treatments and enabling precision medicine (Franco et al., 2021; Liu et al, 2024).

The increase in BSIs due to MDR and ESBL pathogens may stress the need for innovative diagnostic tools that can improve fast and accurate identification of resistance markers and finding alternative ways to treat infections, such as phage therapy (Pirnay et al., 2012).

Conclusion

Gram-negative bacteria were the predominant cause of bloodstream infections (BSIs) in which *Salmonella* spp. and *E. coli* were most commonly isolated. The findings of this study provide valuable data on the rising prevalence of ESBL producing bacteria and multidrug-resistant (MDR) bacteria, indicating that these pathogens have developed resistance to multiple groups of antibiotics and even to the antibiotics of higher generation, such as 3rd generation Cephalosporin, making the infections

caused by them more challenging to treat. This resistance complicates treatment protocols and demands newer and stronger antibiotics, increases the risk of poor clinical outcomes, and poses significant challenges to healthcare systems.

Limitations and recommendations

The highest number of Gram-negative bacterial isolates recorded in this study was in June. However, due to the limited study duration, a comprehensive analysis of the annual and seasonal distribution of isolates could not be conducted. Therefore, a prolonged study covering all seasons is recommended to facilitate comparisons between the prevalence of isolates during rainy and non-rainy seasons.

Various related factors, such as prior use of antibiotics, existence of other diseases, and duration of stay at the hospital in case of inpatients, were not recorded in this study, which might impact the result of the growth percentage and antimicrobial resistance. A high level of resistance to antimicrobial agents was observed, emphasizing the urgent need for regulatory measures. Government agencies should enforce strict regulations on antibiotic sales, mandating valid prescriptions to mitigate the emergence and spread of resistant bacterial strains.

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Conflict of interest

The authors declare no conflict of interest regarding the publication of this research article.

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