MED-PHOENIX: JOURNAL OF NATIONAL MEDICAL COLLEGE

ORIGINAL ARTICLE

BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ISOLATES CAUSING WOUND INFECTIONS

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Date of Submission : May 14, 2025
Date of Acceptance : May 20, 2025
Date of Publication : August 6, 2025

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Citation:
Kayastha U, Kayastha P, Adhikari A, Pokhrel S, Kayastha J, Kayastha K, Ghale D. Bacteriological Profile and Antimicrobial Susceptibility Pattern of Isolates causing Wound Infections. Medphoenix. 2025;10(1):28-33.

DOI:https://doi.org/10.3126/medphoenix.v10i1.82628

Conflict of interest: None, ***Funding:*** None

Publisher: *National Medical College Pvt. Ltd.*
MedPhoenix - Journal of National Medical College (JNMC); 2025,10(1), available at www.jnmc.com.np

ISSN:2631-1992 (Online); ISSN:2392-425X (Print)

ABSTRACT

Introduction: *Wound infections are a major cause of morbidity, often involving a wide range of bacterial pathogens, including multidrug-resistant organisms. Among them, Methicillin-Resistant Staphylococcus aureus (MRSA) has emerged as a significant challenge due to its resistance to commonly used antibiotics and its association with poor clinical outcomes. The rising incidence of MRSA and other resistant pathogens highlights the need for continuous surveillance. This study aims to determine the bacteriological profile and antimicrobial susceptibility patterns of wound infection isolates, with a focus on the prevalence of MRSA, to guide effective empirical therapy and inform antibiotic stewardship practices.*

Materials and methods: *A descriptive cross-sectional study was conducted from April 14, 2022 to August 14, 2023 in the Department of Microbiology at Shree Birendra Hospital. A total of 120 pus swab specimens were collected and processed using standard microbiological procedures. Bacterial species were identified through conventional methods, and antimicrobial susceptibility testing was conducted using the disk diffusion technique, following the Clinical and Laboratory Standards Institute (CLSI) guidelines to ensure accuracy and reliability of results.*

Results: *Of the total bacterial isolates recovered, Gram-negative organisms accounted for 51%, while Gram-positive organisms comprised 49%, indicating a nearly equal distribution. A total of six distinct bacterial species were identified. Staphylococcus aureus emerged as the predominant isolate, representing 35% (n=42) of the total, followed by Klebsiella pneumoniae (17.5%, n=21), Escherichia coli (12.5%, n=15), and Enterobacter spp. (8.33%, n=10). Among the less frequently isolated organisms were coagulase-negative Staphylococci (5.83%, n=7) and Pseudomonas aeruginosa (4.16%, n=5). The most effective antibiotic for Gram positive organism was found to be Linezolid and the less effective was Ciprofloxacin. However, for Gram negative bacteria the most effective antibiotic was Amikacin and the less effective was Cefotaxime. Hence, the etiological agents of wound infection along with their antibiotic susceptibility pattern were determined.*

Conclusion: *The study highlights a diverse bacteriological profile in wound infections, with a nearly equal distribution of Gram-negative and Gram-positive bacteria, and Staphylococcus aureus as the most prevalent isolate. The presence of multidrug-resistant organisms, including MRSA, underscores the urgent need for regular surveillance and adherence to antibiotic stewardship programs. Continuous monitoring of local antimicrobial susceptibility patterns is essential to guide effective empirical therapy and improve patient outcomes.*

Keywords: *Antimicrobial Resistance, Klebsiella pneumoniae, Shree Birendra Hospital, Staphylococcus aureus*

INTRODUCTION

The skin is the largest organ in the human body, playing a vital role in regulating water and electrolyte balance, maintaining body temperature, and serving as a primary barrier against environmental hazards such as microbes. When the epithelial integrity of the skin is compromised, a wound is formed.[1] A wound is defined as a disruption in the continuity of the epithelial layer that can extend deeper into the dermis, subcutaneous fat, fascia, muscle, or even bone.[2] Infection is characterized by the invasion and multiplication of microorganisms in body tissues,

which may or may not produce visible clinical symptoms. These infections can result from competitive microbial metabolism, toxin production, intracellular replication, or immune responses.[3] They may be caused by a single microorganism (mono-microbial) or multiple organisms (poly-microbial).[4] Wound infections are among the most frequent complications encountered in both outpatient and inpatient healthcare settings. They are particularly common in the postoperative period, often delaying recovery and increasing the risk of complications.[5] Timely identification of causative organisms and their antimicrobial susceptibility patterns is essential for effective treatment, prevention of chronic infection, and containment of antibiotic resistance.

Current Scenario

Globally, wound infections remain a significant public health concern, with varying prevalence across different regions. Studies have reported a prevalence of 36% in Ethiopia,[6] while soft tissue infections account for 16–19% of cases in the United States.[7] In South Asia, the burden is also notable, with wound infection rates reported at 11% in India and 23% in Nepal.[8,9], the morbidity and mortality associated with these infections stem from prolonged hospital stays, impaired healing, risk of systemic infections, and increased healthcare costs.[10]

Among the diverse range of pathogens involved in wound infections, Staphylococcus aureus is a major causative agent in both community and healthcare settings.[11] Over the years, the emergence and rapid spread of Methicillin-Resistant Staphylococcus aureus (MRSA) have become a global challenge. Initially, Methicillin was introduced as an effective treatment for S. aureus infections; however, resistance soon developed, leading to widespread MRSA infections. Surveillance studies across continents have shown a rising trend in MRSA prevalence, contributing to higher mortality rates and necessitating the use of expensive, last-resort antibiotics. The ability of S. aureus to acquire resistance mechanisms rapidly has made MRSA a key pathogen in the context of multidrug resistance.[12]

These developments underscore the importance of continued surveillance of the bacteriological profile and antimicrobial susceptibility patterns of wound pathogens, particularly in resource-limited settings, to inform empirical therapy and improve clinical outcomes.

Current options:

Effective management of wound infections relies on accurate identification of causative pathogens and their antimicrobial susceptibility profiles. Current options include culture-based diagnostics, guided antibiotic therapy, and implementation of infection control practices. Empirical treatment is often initiated based on local antibiogram data, with MRSA coverage considered in high-risk settings. Advanced methods like molecular diagnostics and antimicrobial stewardship programs are increasingly being adopted to improve treatment outcomes and combat rising resistance. Regular surveillance and updated treatment guidelines are essential for effective infection management.

MATERIALS AND METHODS

Study Design and Ethical approval

A descriptive cross-sectional study was carried out in the Department of Microbiology at Shree Birendra Hospital, Kathmandu, Nepal. The study spanned April 14, 2022 to August 14, 2023, following ethical clearance from the Institutional Review Committee (Ref: 245 dated August 2022)

Sample size determination

During this study period, 120 specimens were processed from patients. The studies consider 95% confidence interval and 80% power to calculate the sample size. Using the formula sample size becomes

$$n = [z^2 p (1-p)]/d^2$$

Where z = Z score for 95% confidence interval, p = prevalence, d = tolerable error Using z = 1.96, p = 49.28% [13] (, d = (10 %), the sample size will be

$$n= (1.96)^2 x 0.49 x 0.51/ (0.098)^2$$

$$=96$$

In determining the sample size for this study, initial calculation based on 95% confidence level and using corrected sample size formula for finite population suggested a sample size of 96 participants. However, to ensure a more balanced allocation between groups and to minimize the bias, the statistical robustness a slightly larger size of sample 100 was chosen.

Sample collection

Samples including pus, wound swab, high vaginal swab were obtained from both inpatient and outpatient departments. Patients of all age groups were included. Patients of both sexes (female and male) and all age groups were included. Samples inadequately labeled,

cracked, or transported in damaged containers, were excluded from the study.

Culture and Identification

Each sample underwent Gram staining, followed by culture on Blood agar, MacConkey agar, Coagulase test, Oxidase test and other relevant biochemical properties. Plates were incubated at 37°C for 24 to 48 hours. Colonies indicative of identified by their Gram-positive cocci appearance (in clusters), catalase positivity, and colony morphology.

Biochemical Tests for Species Differentiation

Various biochemical tests were conducted to identify the isolated bacteria. Initially, a pure culture was obtained from the primary culture, and subsequently, it underwent biochemical testing as outlined in Appendix IV, VI, and VII. The employed biochemical media included Triple Sugar Iron (TSI) media, Sulphide Indole Motility (SIM) media, Simmons citrate media, Christensen's Urea media, Decarboxylase test media, Hugh and Leif'son's (OF) media, MR/VP media, Phenylalanine agar, and other tests as needed.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was employed for antibiotic susceptibility testing in line with Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included:

- Amoxicillin (10µg)
- Amikacin (30 µg)
- Amoxicillin-Clavulanic acid (20 µg/10 µg)
- Azithromycin (15 µg)
- Ciprofloxacin (5 µg)
- Co-trimoxazole (1.25/23.75 µg)
- Cefotaxime (30 µg)
- Ceftazidime (30 µg)
- Chloramphenicol (30 µg)
- Cefepime (30 µg)
- Cefoxitin (30 µg)
- Clindamycin (2 µg)
- Doxycycline (30 µg)
- Erythromycin (15 µg)
- Gentamicin (10 µg)
- Imipenem (10 µg)
- Meropenem (10 µg)

A 0.5 McFarland standard was used to prepare bacterial suspensions, followed by lawn culture on Mueller-Hinton agar. Antibiotic discs (HiMedia, India) were then applied, and plates were incubated at 37°C for 48 hours. Zones of inhibition were measured using a Vernier caliper. Based on CLSI guidelines, isolates were categorized as sensitive or resistant.

Quality control

Staphylococcus aureus ATCC 25923, E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were concurrently tested in every experiment set as a part of quality control.

Data Analysis

The gathered data were examined using the Statistical Package for the Social Sciences (SPSS) version 20 for Windows.

RESULTS

Gender Distribution of Patients

Out of 120 samples 67 were male (55.8%) were received from male patients whereas (53) were female (44.2%) were received from female patients.

Table 1: Gender Distribution of Patients

Gender	Number (n)	Percentage (%)
Male	67	55.8
Female	53	44.2
Total	120	100

The specimens were collected from age group 0-90 years old patients. Among them, highest number of specimens was from age group 31-40 years (25.8%) followed by 21-30 years (18.3%) as shown in table 2.

Table 2: Age- wise Distribution

Age group	Number(n)	Percentage (%)
0-10	2	1.7
11-20	10	8.3
21-30	22	18.3
31-40	31	25.8
41-50	17	14.2
51-60	11	9.2
61-70	18	15
71-80	3	2.5
81-90	6	5

Staphylococcus aureus was found to be most sensitive to Linezolid (78.5%) followed by Doxycycline (66.66%) and Aminoglycosides (Gentamicin (59.5%) and Amikacin (59.5%). Erythromycin, Clindamycin and Ciprofloxacin, Cloxacillin and Cefoxitin have shown least susceptibility. Coagulase negative Staphylococcus species were sensitive to Amikacin and Doxycycline (71.42%) followed by Gentamicin and Linezolid (57.14%). Azithromycin, Erythromycin, Clindamycin, Cotrimoxazole, Cloxacillin and Cefoxitin have shown least susceptibility

Table 3. Antibiotic susceptibility pattern of Gram-positive bacteria

Antibiotic	Staphylococcus aureus (n=42)		Coagulase negative Staphylococcus species (n=7)	
	(n)	%	(n)	%
Gentamicin	25	59.5	4	57.14
Amikacin	25	59.5	5	71.42
Azithromycin	8	19.04	1	14.28
Erythromycin	5	1.90	1	14.28
Doxycycline	28	66.66	5	71.42
Ciprofloxacin	5	11.90	0	0
Ofloxacin	8	19.04	0	0
Clindamycin	5	11.90	1	14.28
Cotrimoxazole	10	23.80	1	14.28
Linezolid	33	78.5	4	57.14
Cloxacillin	12	28.5	1	14.28
Cefoxitin	12	28.5	1	14.28

Klebsiella pneumoniae was found to be most sensitive to Amikacin (61.9%) followed by Meropenem (57.1%), Gentamicin (47.6%), Imipenem, Piperacillin Tazobactam (42.8%), Doxycycline have shown least susceptibility. Escherichia coli was found to be most sensitive to Meropenem (93.33%), followed by Imipenem (86.66%) and Amino glycosides (Gentamicin and Amikacin) (73.33%), Ampicillin, Cefotaxime, Ciprofloxacin and Ofloxacin have shown least susceptibility. Enterobacter species was found to be most sensitive to Meropenem (80%), followed by Imipenem and Amikacin (60%), Gentamicin (50%), Cotrimoxazole (40%). Third generation cephalosporins have shown least susceptibility as shown in table no.4

Table 4: Antibiotic susceptibility pattern of Enterobacterales

Antibiotics	Klebsiella pneumonia (n=21)		Escherichia coli (n=15)		Enterobacter spp (n=10)	
	(number)	%	(n)	%	(n)	%
Ampicillin	0	0	1	6.66	0	0
Amoxyclav	5	23.80	6	40	0	0
Piperacillin	4	19.04	8	53.33	3	30.0
Piperacillin Tazobactam	9	42.8	8	53.33	3	30.0
Cefotaxime	3	14.2	1	6.66	1	10.0
Cefoperazone	2	9.52	0	0	0	0
Ceftriaxone	2	9.52	2	13.33	1	10.0
Ceftazidime	1	4.76	1	6.66	0	0
Cefixime	2	9.52	0	0	0	0
Imipenem	9	42.8	13	86.66	6	60.0
Meropenem	12	57.1	14	93.33	8	80.0
Gentamicin	10	47.6	11	73.33	5	50.0
Amikacin	13	61.9	11	73.33	6	60.0
Doxycycline	8	38.0	7	46.66	2	20.0
Ciprofloxacin	3	14.28	1	6.66	3	30.0
Ofloxacin	6	28.57	1	6.66	2	20.0
Cotrimoxazole	6	28.57	6	40.0	4	40.0

Methicillin resistant Staphylococcus aureus was found to be most sensitive to Doxycycline (40.0%) followed by Gentamicin, Linezolid (36.66%), Amikacin (33.33%), Clindamycin, Erythromycin and Ciprofloxacin have shown least susceptibility.

Table 5: Antibiotic susceptibility pattern of Methicillin Resistant

Antibiotics	MRSA isolates (n)=30	
	(n)	Percentage (%)
Erythromycin	2	6.66
Ciprofloxacin	2	6.66
Ofloxacin	4	13.33
Azithromycin	3	10.0
Cotrimoxazole	4	13.33
Amikacin	10	33.33
Gentamicin	11	36.66
Clindamycin	2	6.66
Doxycycline	12	40.0
Linezolid	11	36.66

DISCUSSION

This study was carried out with an objective to find out the causative pathogens of different types of wound infection and their antibiotic susceptibility patterns in this study out of 120 patients 67(55.8%) were males and 53(44.2%) were females. The growth was found to be

higher in male patients 56 (56%) than in female patients 44(44%). A similar study carried out by Yakha et al (2012) at B & B Hospital showed that pus samples were collected more from male patients (46.95%) than female (37.4%).[14] The children with the age group 0-10 and patients with age above 70 were found to be less affected. In Nepal, Tuladhar et al (1999) also found that patients with age group 21-30 were more susceptible to wound infection.[15] Oslon & Lee (1990) reported that 90% were aged 50 years or older but in our study lesser number of patients belonged to this age group.[16] In this study the prevalent microorganism associated with wound infection was found to be Staphylococcus aureus (42%) followed by Klebsiella pneumoniae (21%). Additional isolates included Escherichia coli (15%), Enterobacter species (10%), Coagulase negative Staphylococcus species (7%) and Pseudomonas aeruginosa (5%). This study was very much similar to study conducted by Valarmathi S et al. and Pant MR et al. which showed Staphylococcus aureus (54.1%) and (56.8%) respectively.[17,18] Mumtaz et al (2002) in aerobic pyogenic isolates from wounds and abscesses. S. aureus was the most common pathogen (49%)[19] which is higher than this study, the reason for the higher incidence might be due to contaminated environment, poor wound hygiene, delay in the treatment, pre-existing health conditions, foreign body or debris in the wound. Antibiotic susceptibility tests were performed for all the 100 bacterial isolates. The antibiotics used were Amoxycillin Clavulanic acid, Piperacillin, Piperacillin Tazobactam, Cefixime, Ceftriaxone, Cefepime, Ciprofloxacin, Ofloxacin, Cotrimoxazole, Imipenem and Meropenem. Out of 42 isolates of Staphylococcus aureus, 33(78.5%) isolates were sensitive to linezolid, 28(66.66%) were sensitive to Doxycycline, followed by Amikacin and Gentamicin (25(59.5%). Similar result was observed in the study conducted by Raut S et al. (43.6%) were sensitive to Linezolid, Amikacin (39.0%) followed by Cotrimoxazole (30.8%), Gentamicin (33.3%) and Ciprofloxacin (31.5%)[20] 30(71.4%) were found to be Methicillin Resistant Staphylococcus aureus (MRSA). Dibah S et al in India showed (68.4%) resistance of S.aureus to methicillin.[21] in the study by Belbase A et al. Staphylococcus aureus isolates which was alarmingly high prevalence of MRSA which was (47.4%)[22] A study conducted by Shrestha LB et al. MRSA accounts for 41.7 %[23] In contrast in the study done by Kayastha BB 8.92 % of Staphylococcus isolates was identified as MRSA.[24] the reason for this contrast might be due to proper hand washing, public awareness and education, infection control measures, antimicrobial stewardship.

This study has some limitations. It was conducted in a single hospital, which may not reflect the situation across Nepal. Also, it relied on phenotypic and biochemical methods, not molecular testing. The antibiotic panel used was also limited.

This study has several limitations. First, it was confined to a single tertiary hospital, which may limit the applicability of results to other regions of Nepal. Second, the study relied solely on phenotypic and biochemical methods without molecular confirmation, which may affect species- level accuracy.

CONCLUSION

In conclusion, Staphylococcus aureus remains the dominant in clinical samples in Shree Birendra Hospital. These findings highlight the importance of local antimicrobial surveillance and targeted stewardship interventions. Future research should incorporate molecular typing and multicentric approaches to obtain a comprehensive understanding of the epidemiology and resistance mechanisms of Staphylococcus species in Nepal

ACKNOWLEDGEMENT

We express our sincere gratitude to all the faculty members, laboratory staffs of the Department of Microbiology, as well as the Institutional Review Committee (IRC) of Nepal Army Institute of Health Sciences, for their valuable support and assistance in conducting this research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

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