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Profile of Aerobic Bacteria and Their Antimicrobial Susceptibility Patterns in Pus and Wound Swab Samples at a Tertiary Care Hospital in Punjab, India

Charanjeev Kaur[1*], Poonam Sharma[1], Sarbjeet Sharma[1]

[1]Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India

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***Correspondence**
Email: drcharanjeev@gmail.com
Tel: +919878890027

ABSTRACT

Introduction: Skin disruptions exposing subcutaneous tissue provide a moist, warm, and an ideal environment for microbial colonisation and proliferation. Wound infections can lead to sepsis, limb loss, prolonged hospital stays, increased healthcare costs, and significant morbidity and mortality worldwide. Therefore, this study aimed to identify the aerobic bacterial pathogens responsible for wound infections and determine their antimicrobial susceptibility patterns, thereby providing valuable guidance for clinicians to inform the selection of empirical antimicrobial therapy. **Methods:** This retrospective study included 1388 wound samples analyzed at the Department of Microbiology, Sri Guru Ram Das Institute, Amritsar, Punjab, India, from July 2021 to June 2022. All specimens underwent bacterial culture and antimicrobial susceptibility testing using standard microbiological techniques, including Gram staining. Identification and susceptibility testing were performed using the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile, France). Minimum inhibitory concentration (MIC) results were interpreted as susceptible, intermediate, or resistant, based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Chi-square tests were applied for comparing categorical variables (gender and culture positivity). A P-value ≤ 0.05 was considered statistically significant. **Results:** Out of 1388 wound samples cultured, 707 (50.9%) showed no bacterial growth, and 681 (49.1%) were culture-positive, of which 618 were monomicrobial and 63 were polymicrobial. A total of 744 bacterial isolates were obtained: 602 (81%) were Gram-negative and 142 (19%) were Gram-positive. The most common isolates were *Escherichia coli* (29.4%, n=219), followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* (17.2%, n=128 each). *Enterobacterales* isolates were most susceptible to amikacin (67%), followed by tigecycline (61%) and meropenem (57%), but showed low susceptibility to amoxicillin-clavulanate and ampicillin. *Pseudomonas aeruginosa* exhibited its highest susceptibility to carbapenems, whereas *Acinetobacter baumannii* was largely resistant, with the highest susceptibility noted for tigecycline (25%). Gram-positive isolates were highly susceptible to linezolid (91%) and tigecycline (91%). **Conclusion:** This study details the local pathogen profile in pus and wound swab samples and their antimicrobial susceptibility patterns, providing critical guidance for empirical therapy and antimicrobial stewardship efforts at our institution.

INTRODUCTION

The skin serves as a protective barrier between internal organs and the external environment but is frequently subjected to trauma, such as cuts, abrasions, or surgical incisions. Consequently, a breach in skin integrity exposes subcutaneous tissues, creating a moist, warm, and nutrient-rich environment conducive to microbial colonization [1]. Wound infections occur when pathogenic microorganisms proliferate, eliciting localized inflammation and pus formation characterized by necrotic tissue, damaged cells, and leukocytes [2].

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Globally, wound infections remain a significant clinical concern. In India, recent regional studies (Northern India and Kashmir) have reported wound infection prevalence rates of 40–52% [3, 4]. Separately, chronic wound prevalence in India has been estimated to range from 1.9 to 4.5 per 1,000 population [5].

Aerobic and anaerobic bacteria both cause wound infections, with their roles varying based on wound characteristics. Aerobic or facultatively anaerobic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, can grow in the presence of oxygen and thus typically dominate superficial, acute**,** and exposed wounds [6, 7]. Under favorable conditions, these bacteria can rapidly proliferate and produce toxins, which exacerbates tissue damage. Conversely, anaerobic bacteria, which thrive in low-oxygen environments, are commonly implicated in deep, chronic, or necrotic wounds, including abscesses, gangrene, and diabetic foot ulcers. Anaerobic pathogens, such as *Clostridium perfringens* and *Bacteroides* spp., can cause severe infections by flourishing in necrotic or poorly perfused tissue. In chronic or advanced wounds, aerobes and anaerobes often form complex polymicrobial communities. Aerobes can deplete local oxygen, creating an environment where anaerobes can thrive, a synergy that increases infection severity and complicates treatment [7]. Understanding these distinctions helps clinicians develop targeted diagnostic and treatment strategies for specific types of wound infections, ultimately improving patient outcomes.

Wound infections, if inadequately managed, can lead to severe outcomes, including sepsis, limb loss, and significant morbidity and mortality, as well as prolonged hospital stays and increased healthcare costs globally. While wound infections cannot be entirely prevented, timely and targeted antimicrobial therapy based on pathogen identification can reduce their incidence. In recent years, shifts in the bacterial profiles of wound infections have been observed across countries, regions, and hospitals, driven by factors such as evolving antibiotic resistance trends, changes in healthcare practices, and environmental conditions [2, 6, 8]. Additionally, significant variations in antimicrobial susceptibility exist among bacterial isolates from pyogenic (pus-forming) infections [9-11]. These trends highlight the importance of continuous regional surveillance to guide effective management strategies.

This study aimed to identify aerobic bacterial pathogens causing wound infections in our hospital and determine their antibiotic susceptibility patterns to guide clinicians in selecting empirical antimicrobial therapy.

MATERIAL AND METHODS

Study design and setting. This retrospective study was conducted at the Sri Guru Ram Das Institute of Medical Sciences and Research, a tertiary care center in Amritsar, Punjab, India, from July 2021 to June 2022, and included a total of 1,388 wound specimens received by the Department of Microbiology for bacterial identification and antimicrobial susceptibility testing. All consecutive specimens meeting the inclusion criteria were analyzed.

Inclusion and exclusion criteria. All consecutive wound specimens received by the laboratory were eligible for inclusion. In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, duplicate isolates from the same patient (defined as the same species isolated within a 30-day period, regardless of body site or antimicrobial susceptibility pattern) were excluded from the final analysis [12].

Sample collection. Wound specimens were collected either by aspirating pus from deep or superficial wounds into a sterile syringe or container, or by using sterile swabs for less accessible wounds. Specimens were transported aseptically to the laboratory and processed within one hour of collection. In cases of delayed transport, samples were stored at 4 °C before processing.

Laboratory examination

Direct microscopy: Gram-stained smears were examined to preliminarily assess the Gram reaction (Gram-positive or Gram-negative), morphology (*e.g*., cocci or bacilli), and arrangement of any organisms present.

Culture and isolation: Specimens were inoculated onto Blood agar, an enriched medium to support fastidious organisms like *Streptococcus pyogenes* [7], and MacConkey agar, to differentiate lactose-fermenting from non-lactose-fermenting Gram-negative organisms, then incubated at 37 °C aerobically for 24 hours.

Identification of isolates: Bacterial isolates were identified to the species level using the VITEK 2 automated system (v9.02; bioMérieux, Marcy-l'Étoile, France). Appropriate identification (ID) and antimicrobial susceptibility testing (AST) cards were selected based on the isolate's Gram-staining properties.

Inoculum preparation and VITEK 2 analysis. For identification and AST, a suspension from a pure culture was prepared in sterile 0.45% (w/v) sodium chloride and standardized to a turbidity equivalent to a 0.5 McFarland standard using a densitometer. Bacterial identification was performed using the VITEK 2 ID-GN [13] or ID-GP [14] cards for Gram-negative and Gram-positive organisms, respectively.

Antimicrobial susceptibility testing (AST). AST was performed using specific VITEK 2 AST cards based on the organism type.The AST-N280 card [15] was used for Enterobacterales and included: amikacin, ampicillin, amoxicillin-clavulanic acid, cefepime, cefoperazone-sulbactam, ceftriaxone, cefuroxime, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tigecycline, and trimethoprim-sulfamethoxazole.

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- The AST-N281 card [16] was used for non-fermenting bacteria (*e.g., P. aeruginosa*, *A. baumannii*) and included: amikacin, cefepime, cefoperazone-sulbactam, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, levofloxacin, meropenem, minocycline, piperacillin-tazobactam, ticarcillin-tazobactam, tigecycline, and trimethoprim-sulfamethoxazole.
- The AST-P628 card [17] was used for Gram-positive bacteria and included: penicillin, gentamicin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, and cefoxitin.

Lot numbers for all VITEK cards used are listed in Appendix 1. Following inoculation, cards were automatically filled, sealed, and incubated for 6–8 hours, with results subsequently interpreted by the VITEK 2 system software [18].

Data interpretation and quality control. The resulting minimum inhibitory concentrations (MICs) were used to categorize each isolate as susceptible, intermediate, or resistant according to the interpretive criteria in the CLSI M100 guidelines [19]. Quality control (QC) was performed using the reference strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213. QC testing was performed daily per lot until control was established and then reduced to once weekly.

Statistical analysis. Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Chi-square tests were used to compare categorical variables. Statistical significance was defined as a two-sided P-value ≤ 0.05. All reported percentages are isolate-based.

RESULTS

Bacterial culture results. Of the 1,388 wound specimens cultured, 681 (49.1%) yielded bacterial growth. Among these culture-positive specimens, 618 (90.7%) were monomicrobial and 63 (9.3%) were polymicrobial, yielding a total of 744 bacterial isolates for analysis.

Table 1. Distribution of clinical specimens by age group and culture outcome

Age group (years)	Total specimens (n)	Culture-positive (n, %)[1]	Culture-negative (n, %)[2]
0-10	79	31 (4.6)	48 (6.8)
11-20	52	33 (4.8)	19 (2.7)
21-30	156	87 (12.8)	69 (9.8)
31-40	210	99 (14.5)	111 (15.7)
41-50	302	139 (20.4)	163 (23.1)
51-60	231	118 (17.3)	113 (16.0)
61-70	261	120 (17.6)	141 (19.9)
>70	97	54 (7.9)	43 (6.1)
Total	1,388	681 (100.0)	707 (100.0)

[1]Percentages are calculated based on the total number of culture-positive specimens (n=681).

[2]Percentages are calculated based on the total number of culture-negative specimens (n=707).

While the 41–50 age group contributed the highest absolute number of cases, the highest rate of culture positivity was observed in the 21–30 age group (55.8%) and patients >70 years (55.7%) (Table 1). Moreover, no significant association was observed between gender and culture outcome (Table 2). The culture positivity rate was similar between males (49.5%) and females (48.3%); this difference was not statistically significant ($\chi^2(1) = 3.26$, $P = 0.071$).

Table 2. Distribution of culture outcomes by patient gender

Gender	Culture-positive (n, %)	Culture-negative (n, %)	Total (n)
Male	416 (49.5)	424 (50.5)	840
Female	265 (48.3)	283 (51.7)	548
Total	681 (49.1)	707 (50.9)	1,388 (100.0)

Note: Row percentages are shown. There was no significant difference in culture positivity between males and females ($P = 0.071$).

Bacteriological profile of wound infections. Of the 744 bacterial isolates identified, Gram-negative bacteria predominated (81%, n=602), followed by Gram-positive organisms (19%, n=142). Among the Gram-negative isolates, 444 (73.8%) were members of the order *Enterobacterales*, and 158 (26.2%) were non-fermenting Gram-negative bacteria.

E. coli was the most prevalent isolate overall (29.4%, n=219), followed by *S. aureus* and *K. pneumoniae* (each 17.2%, n=128) (Table 3).

Antibiotic susceptibility patterns of pathogens

A) Susceptibility of *Enterobacterales* isolates

The antimicrobial susceptibility patterns of 444 *Enterobacterales* isolates to twelve selected antibiotics are presented in Figure 1. Overall, susceptibility rates varied widely, ranging from only 3% for ampicillin to a high of 67% for amikacin. High rates of susceptibility were also observed for tigecycline (61%) and meropenem (57%), whereas resistance was most common for

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ampicillin (3% susceptible) and amoxicillin-clavulanic acid (11% susceptible).

Table 3. Distribution of bacterial isolates from wound specimens (n = 744)

Bacterial isolate	Frequency (n, %)
E. coli	219 (29.4)
S. aureus	128 (17.2)
K. pneumoniae	128 (17.2)
P. aeruginosa	93 (12.5)
A. baumannii	59 (7.9)
Enterobacter cloacae complex	39 (5.2)
Proteus mirabilis	25 (3.4)
Citrobacter freundii complex	15 (2.0)
Enterococcus spp.	14 (1.9)
Morganella morganii	13 (1.7)
Serratia marcescens	4 (0.5)
Others	7 (0.9)
Total	744 (100.0)

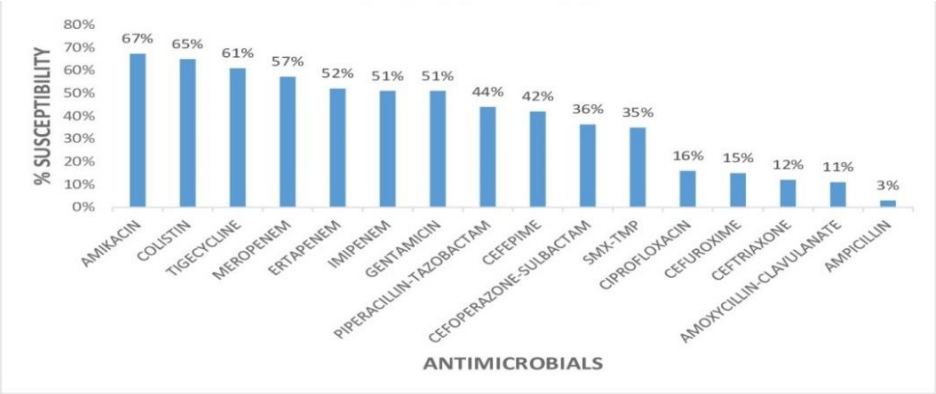

Fig. 1. Antibiotic susceptibility patterns of *Enterobacterales* isolates

B) Susceptibility of non-fermenting Gram-negative bacteria

The antimicrobial susceptibility patterns of *P. aeruginosa* (n=93) and *A. baumannii* (n=59) are detailed in Figure 2. *P. aeruginosa* exhibited its highest susceptibility rates to meropenem (57%) and imipenem (54%). Excluding intrinsic resistance patterns, *P. aeruginosa* exhibited its lowest susceptibility rate to ceftazidime (14%). *A. baumannii* showed its highest susceptibility to tigecycline (25%) but demonstrated low susceptibility rates to most other agents tested. Direct comparison showed that *P. aeruginosa* was significantly more susceptible to most antimicrobials ($P < 0.05$), except ceftazidime ($P = 0.064$) (Table 4).

Susceptibility to most of the tested antimicrobials differed significantly between the two organisms, with the exceptions of ceftazidime, trimethoprim-sulfamethoxazole, and colistin, for which the differences were not statistically significant (Table 4).

C) Susceptibility of Gram-positive cocci

Antimicrobial susceptibility patterns for the two most common Gram-positive isolates are presented in Figure 3. *S. aureus* (n=128) isolates demonstrated high susceptibility to linezolid (91%), tigecycline (91%), teicoplanin (88%), and vancomycin (85%). Moderate susceptibility was observed for daptomycin (80%), tetracycline (80%), gentamicin (55%), and clindamycin (54%). Susceptibility was poor for erythromycin (23%), ciprofloxacin (23%), and penicillin (2%). Based on cefoxitin resistance, the overall rate of methicillin-resistant *S. aureus* (MRSA) was 62%.

Enterococcus sp. (n=14), comprising *E. faecium* and *E. faecalis*, showed very high susceptibility to linezolid, teicoplanin, vancomycin, and tigecycline (92% each). Susceptibility was lower for gentamicin (62%), penicillin (62%), daptomycin (46%), tetracycline (31%), and levofloxacin (31%), with poor activity observed for ciprofloxacin (23%), erythromycin (23%), and clindamycin (8%).

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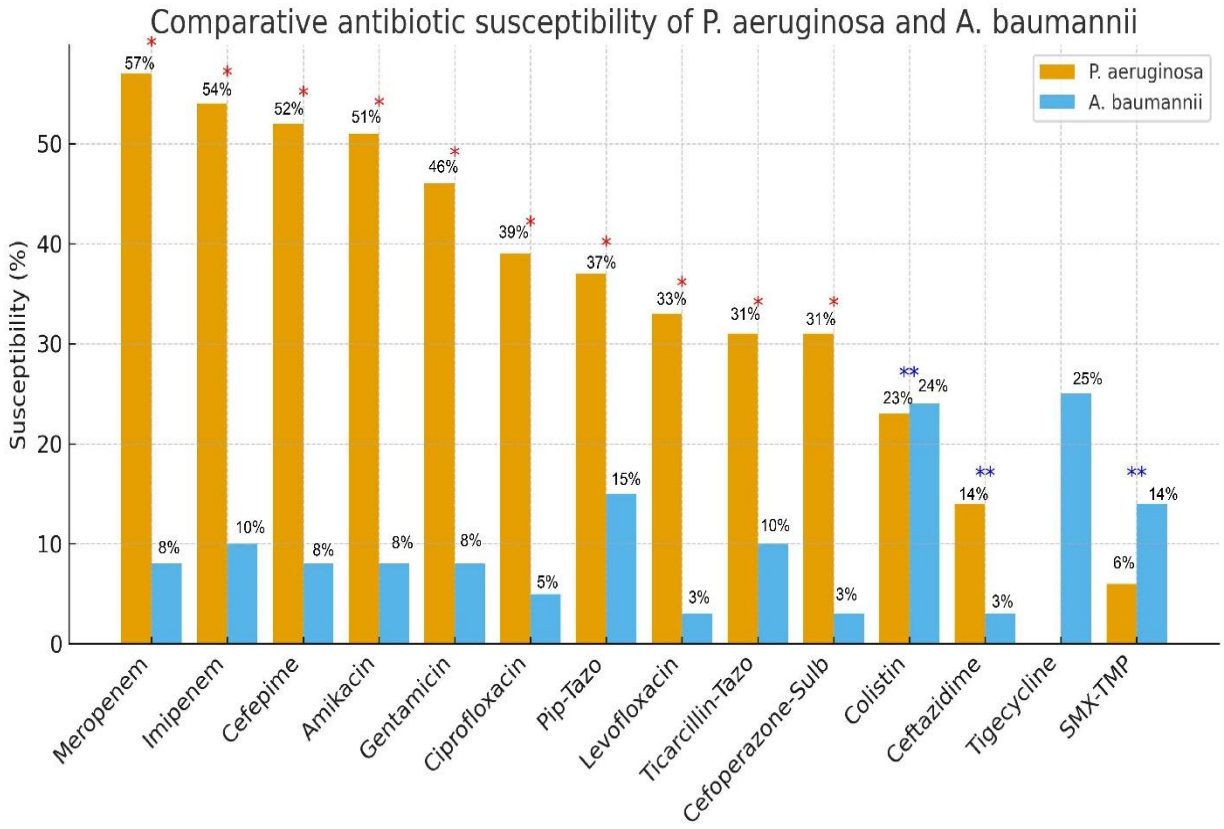

Fig.2. Comparative antibiotic susceptibility of *P. aeruginosa* and *A. baumannii*. *Antibiotic abbreviations:* Pip-Tazo, piperacillin-tazobactam; Cefoperazone-Sulb, cefoperazone-sulbactam; Ticarcillin-Tazo, ticarcillin-tazobactam; SMX-TMP, sulfamethoxazole-trimethoprim. Note: Tigecycline susceptibility was not determined for *P. aeruginosa*.

Table 4. Comparison of antimicrobial susceptibility rates between *P. aeruginosa* and *A. baumannii* isolates

Antimicrobial agent	*P. aeruginosa* (n=93) susceptible (n, %)	*A. baumannii* (n=59) susceptible (n, %)	*P*- value
Gentamicin	43 (46)	5 (8)	<0.001*
Amikacin	47 (51)	5 (8)	<0.001*
Cefepime	48 (52)	5 (8)	<0.001*
Ceftazidime	13 (14)	2 (3)	0.064
Piperacillin-tazobactam	34 (37)	9 (15)	0.008*
Cefoperazone-sulbactam	29 (31)	2 (3)	<0.001*
Meropenem	53 (57)	5 (8)	<0.001*
Imipenem	50 (54)	6 (10)	<0.001*
Tigecycline[a]	NA	15 (25)	NA
Levofloxacin	31 (33)	2 (3)	<0.001*
Ciprofloxacin	36 (39)	3 (5)	<0.001*
Ticarcillin-tazobactam	29 (31)	6 (10)	0.016*
Trimethoprim-sulphamethoxazole	5 (6)	8 (14)	0.120
Colistin	22 (23)	14 (23)	0.990

[a]Tigecycline susceptibility was not determined for *P. aeruginosa* isolates; NA, not applicable.
*Statistically significant difference.

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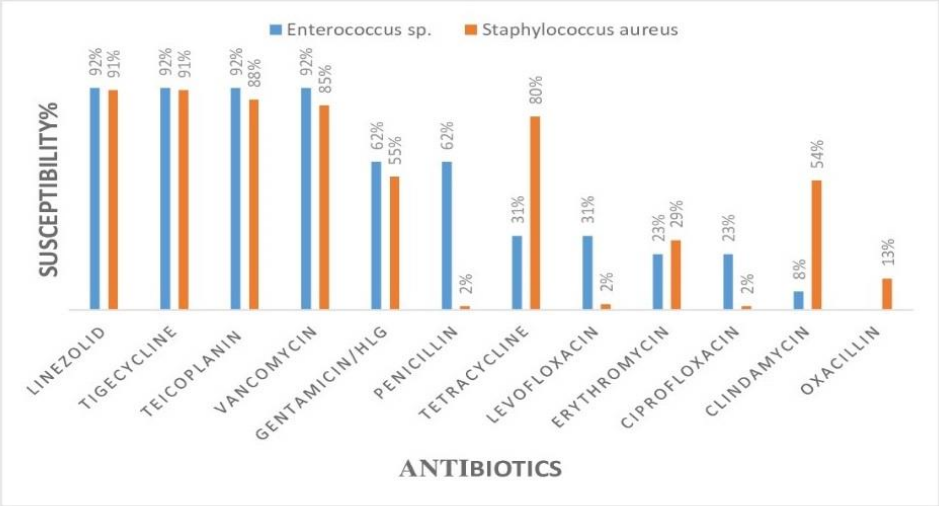

Fig. 3. Antibiotic susceptibility of Gram-positive cocci.

DISCUSSION

Effective wound management relies on identifying causative organisms and selecting appropriate antimicrobials. This study generated a comprehensive local antibiogram of aerobic wound pathogens to guide empirical therapy. Antibiograms are essential tools recommended in antimicrobial stewardship guidelines to monitor resistance trends and promote the appropriate use of antimicrobials.

In this study, the overall culture positivity rate was 49%. This finding is comparable to rates reported by Mahat *et al*. (49%) [6] and Prabhakar *et al*. (43.7%) [20], though Tameez-ud-Din *et al*. reported a higher rate of 59% [21] and other studies have reported even higher positivity rates of 66–85% [11, 22, 23]. The nearly 51% of culture-negative specimens suggest the potential involvement of other etiological agents not detected by our methods, such as anaerobic bacteria (*e.g*., *Bacteroides*, *Clostridium* spp.) or fungi, which are known to cause wound infections, particularly in chronic or deep wounds.

The analysis of demographic factors did not reveal a statistically significant association between patient age and culture positivity (P = 0.075). The highest absolute number of culture-positive cases was observed in the 41–50-year group (20.4%), while the highest positivity rates were seen in the 21–30 (55.8%) and >70 (55.7%) age groups. This finding aligns with previous research and may reflect a higher incidence of comorbidities, trauma, or surgical procedures, which predispose individuals to infection [2, 24, 25]. In addition, gender was not a major determinant of bacterial growth in this cohort.

Our study found that 9% of positive cultures were polymicrobial. Polymicrobial infections can delay wound healing and promote the development of antibiotic-resistant strains. Bacterial synergy, in which multiple species enhance each other's pathogenicity, is a well-documented mechanism that complicates the eradication of infection and promotes antimicrobial resistance [26]. The complexity of these polymicrobial communities highlights the challenge of treatment, where topical agents may serve as adjuncts to systemic therapy and debridement in select cases, such as chronic wounds [27].

The predominance of Gram-negative bacteria (81%) in our study is consistent with other regional reports [4, 28]. This finding is consistent with their role as both common colonizers of skin wounds and significant agents of hospital-acquired infections (HAIs), including organisms such as *Klebsiella* spp., *A. baumannii*, *P. aeruginosa*, and *E. coli* [6, 29]. In our cohort, *E. coli* (29.4%) was the most frequently isolated pathogen, followed by *S. aureus* (17.2%) and *K. pneumoniae* (17%). While some studies corroborate *E. coli* as the leading pathogen [30, 31], others report *S. aureus* or *P. aeruginosa* as more common, highlighting the regional and institutional variability in wound infection epidemiology [6, 10, 26].

Among *Enterobacterales* isolates, high susceptibility rates to amikacin, tigecycline, and meropenem were observed, while resistance to ampicillin and amoxicillin-clavulanate was widespread. This pattern, consistent with reports from other Indian centers [21, 30], likely reflects the high prevalence of extended-spectrum β-lactamases (ESBLs) driven by the overuse of cephalosporins and fluoroquinolones [32].

Notably, *Acinetobacter baumannii* isolates demonstrated widespread resistance, with susceptibility rates to key antimicrobials like tigecycline (25%) and imipenem (10%) being markedly lower than those reported elsewhere [2, 24]. This high resistance level may be driven by factors prevalent in the region, such as poor adherence to treatment regimens and the indiscriminate use of antibiotics [33]. Because tigecycline is a reserve antibiotic, its use is limited, which may explain its relatively preserved activity compared to more commonly

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used agents [34]. This finding, contrasted with the relatively higher susceptibility of *P. aeruginosa*, underscores the challenge posed by multidrug-resistant *A. baumannii* in our setting.

The 62% prevalence of methicillin-resistant *S. aureus* (MRSA) in our study is a critical finding. This rate is higher than the national average of 44.5% reported by the Indian Council of Medical Research (ICMR) Antimicrobial Resistance Surveillance Network (AMRSN) but is comparable to other regional reports [23, 32]. The high prevalence of MRSA is a significant concern for both community and hospital-acquired infections and may be attributed to factors such as patient carriage, poor hand hygiene practices among healthcare workers, and the widespread misuse of antimicrobials [33]. While our MRSA isolates remained highly susceptible to linezolid (91%) and vancomycin (85%), similar to findings in some studies [35, 36], the high prevalence necessitates stringent infection control and stewardship. The susceptibility of *S. aureus* to tetracycline in our study (80%) was lower than the 96.3% susceptibility to doxycycline reported by Bhalchandra *et al*. [10].

Based on the observed susceptibility patterns, local empirical therapy guidelines for severe wound infections should prioritize amikacin or carbapenem for suspected Gram-negative infections and linezolid or vancomycin for Gram-positive coverage. Given the high rates of resistance, agents such as cephalosporins and fluoroquinolones should be used with caution, and all empirical regimens should be de-escalated based on culture and susceptibility results to support antimicrobial stewardship. The high prevalence of MRSA (62%) underscores the urgent need for robust infection control measures, including hand hygiene and patient screening, to curb its spread within the hospital.

In conclusion, his study has several limitations. First, its scope was restricted to aerobic and facultative anaerobic bacteria, as anaerobic culture facilities were not available at our center. Second, the single-center, retrospective design may limit the generalizability of our findings. Therefore, multi-center, prospective studies are essential to provide a more comprehensive picture of wound infection epidemiology in the region.

This study highlights the predominance of Gram-negative bacteria, particularly *E. coli*, in wound infections at our institution and reveals a high prevalence of multidrug-resistant organisms, including a 62% MRSA rate. The local antimicrobial susceptibility data presented here are crucial for guiding empirical therapeutic choices, refining antimicrobial stewardship policies, and improving patient outcomes in the face of growing resistance.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AI DISCLOSURE

No AI tools were used in this study.

DATA AVAILABILITY

Raw data of this study is available from the corresponding author upon reasonable request.

AUTHOR'S CONTRIBUTION

CK: Conceptualization; Investigation; Project administration; Supervision; Writing – original draft; Writing – review & editing. PS: Investigation; Formal analysis; Writing – review & editing. SS: Supervision; Investigation; Writing – review & editing. All authors read and approved the final manuscript.

ETHICS STATEMENT

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by the Institutional Ethics Committee (approval number: SGRD/IEC/2023-200). As a retrospective study utilizing anonymized data from the hospital microbiology laboratory database, the need for individual patient informed consent was waived by the Institutional Ethics Committee. All patient identifiers were removed to ensure privacy.

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