

## Bacteriological analysis of wound infections and evaluation of multi-drug resistance. A retrospective study.

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### Abstract

#### Background

Wound infections remain a frequent problem in hospitalized patients and often lead to delayed recovery and prolonged hospital stay. In recent years, the increasing emergence of multidrug-resistant organisms has made treatment more complicated and less predictable.

#### Objective

To evaluate the bacteriological profile of wound infections and determine the prevalence of multidrug resistance in a tertiary care hospital in Odisha.

#### Methods

A retrospective study was carried out at DRIEMS Institute of Health Sciences & Hospital, Tangi, Cuttack, from March to August 2024. Fifty culture-positive wound samples were analyzed. Identification of isolates was performed using standard microbiological procedures. Antimicrobial susceptibility testing was done by the Kirby–Bauer disk diffusion method according to CLSI 2023 guidelines. Multidrug resistance was defined as resistance to at least one drug in three or more antimicrobial classes. Data were analyzed using SPSS version 25. Chi-square test was applied, and  $p < 0.05$  was considered statistically significant.

#### Results

Gram-negative bacteria accounted for 72% of isolates, while 28% were Gram-positive. *Staphylococcus aureus* was the most frequently isolated organism (24%), followed by *Pseudomonas aeruginosa* (20%) and *Escherichia coli* (18%). Overall, 46% of isolates were multidrug resistant. MDR was significantly more common among Gram-negative organisms compared to Gram-positive organisms (58.3% vs 14.3%,  $\chi^2 = 6.42$ ,  $p = 0.01$ ).

#### Conclusion

The study demonstrates a substantial burden of Gram-negative pathogens and multidrug resistance in wound infections. Continuous surveillance and rational antibiotic use are necessary to prevent further escalation of resistance.

**Keywords:** Gram-negative pathogens, rational antibiotic use, wound infections, Surveillance

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### Introduction

Wound infections are among the most common healthcare-associated infections and contribute substantially to prolonged hospitalization, increased treatment costs, and mortality (1). The microbial flora of wound infections varies depending on the type of wound, hospital environment, and geographic location (2).

Acute and chronic wounds provide an ideal environment for microbial colonization and proliferation due to tissue necrosis, poor vascular supply, and impaired host immunity (3). Both Gram-positive and Gram-negative organisms are implicated in wound infections, with increasing reports of polymicrobial involvement (4).

Historically, *Staphylococcus aureus* has been the leading cause of wound infections (5). However, recent trends indicate a rising predominance of Gram-negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* (6,7).

The growing burden of antimicrobial resistance (AMR) has become a global public health crisis (8). The World Health Organization (WHO) has identified AMR as one of the top ten global health threats (9). Multidrug-resistant organisms (MDROs) significantly reduce therapeutic options and worsen clinical outcomes (10).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria are increasingly reported in wound infections (11,12). Carbapenem-resistant Enterobacteriaceae (CRE) and multidrug-resistant *Pseudomonas* further complicate management (13).

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The irrational use of antibiotics, over-the-counter availability, and inadequate infection control practices contribute to rising resistance rates (14,15). Surveillance studies are essential to understand local antimicrobial susceptibility patterns and guide empirical therapy (16).

Several Indian studies have reported high MDR prevalence in wound isolates ranging from 30–60% (17,18). Regional variability necessitates institution-specific antibiograms (19).

This study was undertaken to determine the bacteriological profile of wound infections and assess multidrug resistance patterns in a tertiary care hospital in Odisha.

## Materials and Methods

### Study Design

This investigation was conducted as a hospital-based retrospective observational study. The study involved analysis of microbiological records of patients diagnosed with wound infections during the study period.

### Study Setting

The study was carried out in the Department of Microbiology at DRIEMS Institute of Health Sciences & Hospital, Tangi, Cuttack, Odisha, India. The hospital is a tertiary care teaching institution catering to both rural and urban populations.

### Study Duration

The study covered a period of six months, from March 2024 to August 2024.

### Study Population and Sample Size

A total of 50 culture-positive wound samples were included in the study. These samples were obtained from patients admitted to various clinical departments including general surgery, orthopedics, and medicine.

### Inclusion Criteria

Patients with clinical evidence of wound infection (e.g., purulent discharge, redness, swelling, tenderness).

Wound specimens that yielded significant bacterial growth on culture.

### Exclusion Criteria

- Wound samples showing fungal growth only.
- Duplicate isolates from the same patient.
- Samples with incomplete laboratory documentation.
- Contaminated specimens.

### Sample Collection and Transport

Wound specimens were collected under aseptic precautions by trained healthcare personnel. Depending on the wound type, either sterile cotton swabs or aspirated pus samples were obtained. Samples were transported immediately to the microbiology laboratory for processing. In cases of delay, specimens were preserved in appropriate transport media to prevent desiccation and contamination.

### Microbiological Processing

Upon receipt in the laboratory, samples were inoculated onto:

#### Blood agar & MacConkey agar

The inoculated plates were incubated aerobically at 37°C for 18–24 hours. After incubation, bacterial growth was assessed for colony morphology, hemolytic characteristics, pigmentation, and lactose fermentation. Identification of isolates was performed using:

#### Gram staining

**Standard biochemical tests** (catalase, coagulase, oxidase test)

IMViC tests for Enterobacteriaceae

Triple Sugar Iron (TSI) agar reactions

Urease and citrate utilization tests

Motility testing where applicable

Only clinically significant bacterial growth was considered for further analysis.

### Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.

The antibiotic panel included representatives from major antimicrobial classes:

- Beta-lactams (penicillins, cephalosporins)
- Carbapenems
- Aminoglycosides
- Fluoroquinolones
- Tetracyclines
- Glycopeptides (for Gram-positive organisms)

After incubation at 37°C for 16–18 hours, zones of inhibition were measured in millimeters and interpreted as Sensitive, Intermediate, or Resistant according to CLSI criteria.

Quality control strains were used periodically to ensure accuracy of testing procedures.

## Definition of Multidrug Resistance (MDR)

Multidrug resistance was defined as resistance to at least one agent in three or more antimicrobial categories, in accordance with internationally accepted definitions.

## Data Collection

Data were retrieved from laboratory registers and electronic medical records. The following information was collected:

- Patient age and gender
- Type of bacterial isolate
- Gram classification
- Antibiotic susceptibility results
- MDR status

All data were anonymized before analysis to maintain patient confidentiality.

## Statistical Analysis

The demographic distribution is presented in **Table 1**.

Table 1: Demographic Distribution of Study Population (n=50)

Variable	Number	Percentage (%)
<b>Gender</b>		
Male	32	64
Female	18	36
<b>Age Group (Years)</b>		
18–40	20	40
41–60	18	36
>60	12	24

## Distribution of Bacterial Isolates

Out of the 50 isolates, Gram-negative organisms accounted for 36 (72%), while Gram-positive organisms constituted 14 (28%).

All data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 25. Categorical variables such as gender distribution, type of bacterial isolate, Gram classification, and multidrug resistance status were expressed as frequencies and percentages. The association between Gram staining characteristics (Gram-positive vs Gram-negative) and multidrug resistance was evaluated using the Chi-square test. A p-value of less than 0.05 was considered statistically significant. In the present study, multidrug resistance was observed in 21 of 36 (58.3%) Gram-negative isolates compared to 2 of 14 (14.3%) Gram-positive isolates. The difference was statistically significant ( $\chi^2 = 6.42$ , degrees of freedom = 1,  $p = 0.01$ ), indicating a strong association between Gram-negative organisms and higher multidrug resistance rates.

## Ethical Considerations

As the study was retrospective and based on laboratory records, direct patient interaction was not involved. Patient identifiers were removed during data extraction. Institutional ethical standards were followed in accordance with hospital research guidelines.

## Results

### Demographic Characteristics

A total of 50 culture-positive wound samples were analyzed. Among these, 32 patients (64%) were males and 18 (36%) were females, yielding a male-to-female ratio of 1.7:1. The majority of patients belonged to the 18–40 years age group (40%), followed by 41–60 years (36%) and >60 years (24%).

The most frequently isolated organism was *Staphylococcus aureus* (24%), followed by *Pseudomonas aeruginosa* (20%) and *Escherichia coli* (18%). Among Gram-negative bacteria, *Klebsiella pneumoniae* (14%), *Proteus* spp. (12%), and *Acinetobacter* spp. (8%) were also identified. *Enterococcus* spp. accounted for 4% of isolates. The detailed distribution is shown in **Table 2**, and the graphical representation is provided in **Figure 1**.

Table 2: Distribution of Bacterial Isolates (n=50)

Organism	Number	Percentage (%)
Staphylococcus aureus	12	24
Pseudomonas aeruginosa	10	20
Escherichia coli	9	18
Klebsiella pneumoniae	7	14
Proteus spp.	6	12
Acinetobacter spp.	4	8
Enterococcus spp.	2	4

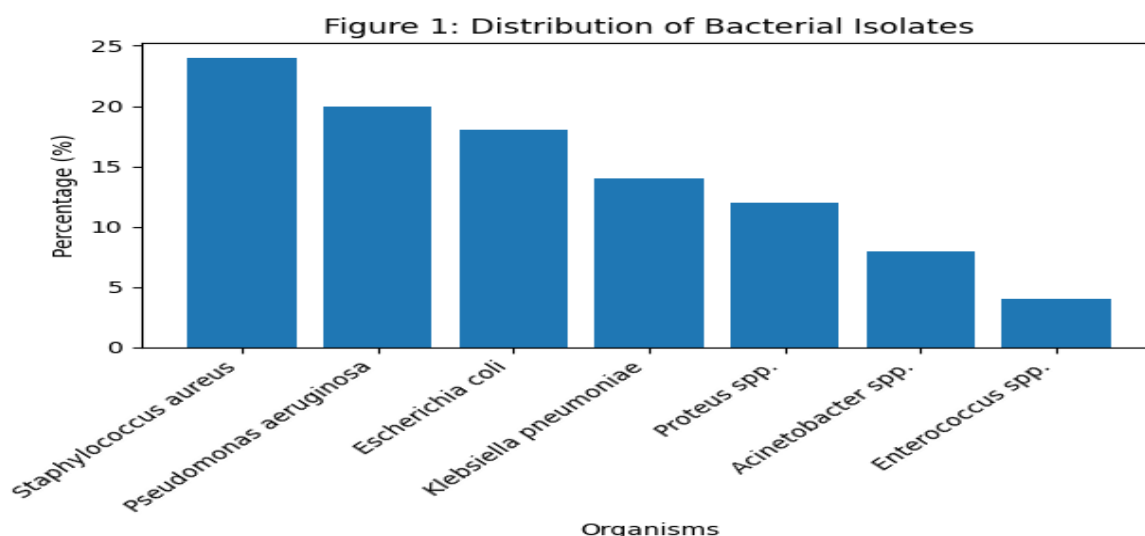


Figure 1: Distribution of Bacterial Isolates

### Multidrug Resistance (MDR) Pattern

Out of 50 isolates, 23 were multidrug resistant, giving an overall MDR prevalence of 46%.

Among individual organisms:

*Acinetobacter* spp. showed the highest MDR rate (75%)

*Pseudomonas aeruginosa* – 60%

*Klebsiella pneumoniae* – 57.1%

*Escherichia coli* – 55.6%

*Proteus* spp. – 50%

*Staphylococcus aureus* – 16.7%

No MDR was observed in *Enterococcus* spp. The detailed organism-wise MDR distribution is shown in **Table 3**.

Table 3: Organism-wise Multidrug Resistance Pattern

Organism	Total Isolates	MDR (n)	MDR (%)
Staphylococcus aureus	12	2	16.7
Pseudomonas aeruginosa	10	6	60
Escherichia coli	9	5	55.6
Klebsiella pneumoniae	7	4	57.1
Proteus spp.	6	3	50
Acinetobacter spp.	4	3	75
Enterococcus spp.	2	0	0

Overall MDR prevalence = 23/50 (46%)

### Comparison of MDR in Gram-positive and Gram-negative Isolates

MDR among Gram-negative isolates was 21 out of 36 (58.3%), whereas only 2 out of 14 Gram-positive isolates (14.3%) were multidrug resistant.

Chi-square ( $\chi^2$ ) = 6.42

Degrees of freedom = 1

p-value = 0.01

Since  $p < 0.05$ , the difference was statistically significant, indicating a significantly higher MDR rate among Gram-negative isolates.

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Chi-square test was applied to determine statistical significance.

This comparison is illustrated in Figure 2.

Figure 2: Comparison of MDR Between Gram-negative and Gram-positive Iso

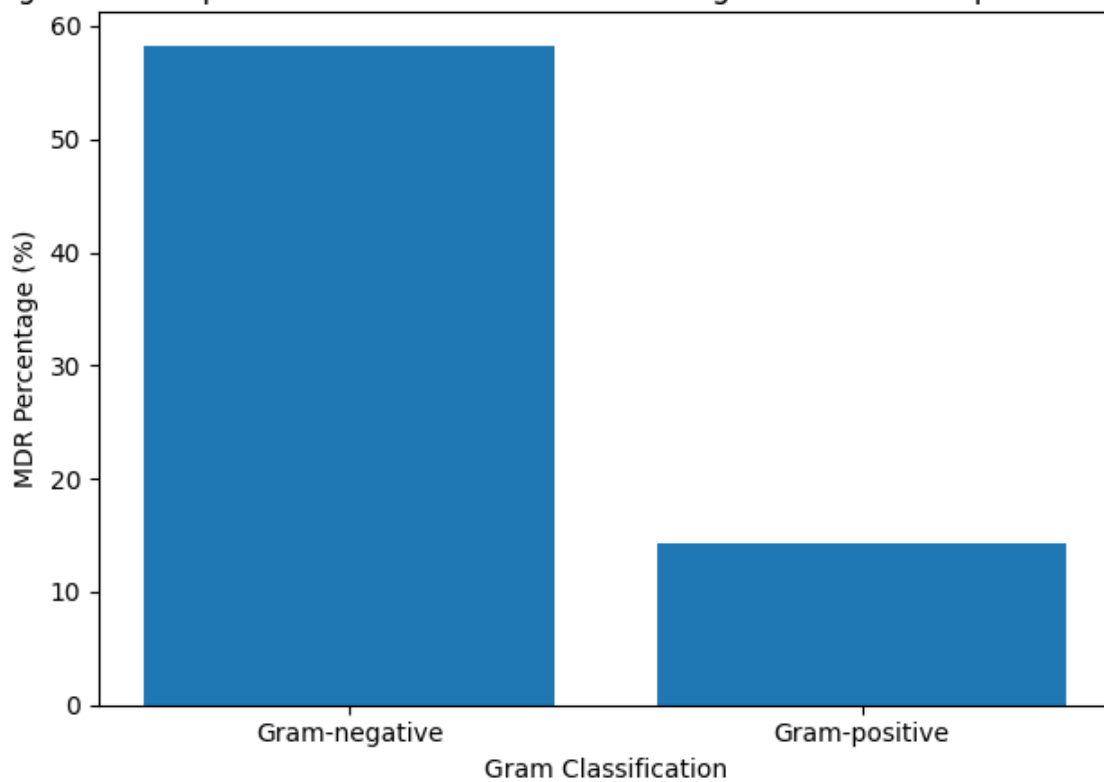


Figure 2: Comparison of MDR Between Gram-positive and Gram-negative Isolates

### Summary of Key Findings

The present study demonstrated a predominance of Gram-negative bacteria (72%) in wound infections, with Gram-positive organisms accounting for 28% of isolates. *Staphylococcus aureus* (24%) was the most frequently isolated individual pathogen, followed by *Pseudomonas aeruginosa* (20%) and *Escherichia coli* (18%). The overall prevalence of multidrug resistance was 46% (23/50 isolates). Among individual organisms, the highest MDR rates were observed in *Acinetobacter spp.* (75%) and *Pseudomonas aeruginosa* (60%). Importantly, multidrug resistance was significantly higher among

Gram-negative isolates compared to Gram-positive isolates (58.3% vs 14.3%,  $p = 0.01$ ). These findings highlight the increasing burden of Gram-negative pathogens and the substantial prevalence of multidrug resistance in wound infections within the study setting.

### Discussion

The present study demonstrated a predominance of Gram-negative organisms (72%) over Gram-positive isolates in wound infections. Similar trends have been documented in recent regional studies, where Gram-negative bacilli have increasingly replaced Gram-positive cocci as the dominant pathogens in hospital-based wound infections.

This epidemiological shift may be attributed to selective antibiotic pressure and hospital environmental factors.

Although *Staphylococcus aureus* remained the most common individual isolate (24%) in our study, the collective burden of Gram-negative organisms was significantly higher. Saha et al. (20) reported comparable findings, emphasizing the growing importance of non-fermenting Gram-negative bacilli in wound infections. Likewise, Golia et al. (21) observed a predominance of *Pseudomonas aeruginosa* and Enterobacteriaceae in tertiary care settings.

The overall multidrug resistance (MDR) rate of 46% observed in the present study is consistent with previous Indian reports. Tiwari et al. (22) documented MDR prevalence rates exceeding 40% among wound isolates, highlighting the emerging therapeutic challenge. High resistance levels among *Klebsiella pneumoniae* and *Escherichia coli* in our study parallel findings by Basak et al. (23), who reported significant ESBL-mediated resistance in Gram-negative isolates.

The markedly higher MDR rate among Gram-negative isolates compared to Gram-positive isolates (58.3% vs 14.3%,  $p=0.01$ ) reflects global concerns regarding resistance mechanisms such as ESBL production and carbapenemase activity. According to Magiorakos et al. (24), standardized definitions of multidrug resistance have enabled better comparison across surveillance studies, revealing increasing global MDR trends.

The high resistance observed among *Acinetobacter* spp. in our study is particularly concerning. Nordmann et al. (25) described the rapid dissemination of carbapenem-resistant organisms worldwide, significantly limiting treatment options. These pathogens are known for their capacity to persist in hospital environments and acquire multiple resistance determinants.

The findings of the present study reinforce the urgent need for continuous microbiological surveillance and institution-specific antibiogram development. Strict adherence to antimicrobial stewardship principles is essential to curb the rising burden of multidrug-resistant organisms.

## Conclusion

This study revealed a predominance of Gram-negative organisms and a high prevalence of multidrug resistance in wound infections. Targeted antibiotic therapy guided by culture sensitivity testing is essential. Regular surveillance and antimicrobial stewardship programs are strongly recommended.

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