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ISOLATION AND ANALYSIS OF BACTERIAL DNA TO DETECT ANTIMICROBIAL RESISTANCE GENES IN WOUND INFECTIONS

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ABSTRACT

Wound infections are a significant cause of hospital admissions and prolonged treatment, with increasing concern over antimicrobial-resistant bacteria complicating therapy. Rapid identification of bacterial pathogens and their antimicrobial resistance (AMR) profiles is crucial for effective clinical management and infection control. This study aimed to isolate and characterize bacterial pathogens from wound infections, assess their antimicrobial susceptibility patterns, and detect key AMR genes using molecular methods, with detailed quantitative analysis.A total of 150 wound samples were collected over six months from patients aged 18–80 years. Bacterial isolates were identified using standard culture and biochemical methods. Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method following CLSI guidelines. Genomic DNA was extracted using a commercial kit, and PCR was used to detect *mecA*, *bla_TEM*, *bla_CTX-M*, *vanA*, and *tetA* genes. Quantitative PCR analysis included ΔCt measurements. Statistical analysis was performed using SPSS v25.0. Of 150 samples, 132 (88%) were culture-positive, yielding 145 bacterial isolates. The predominant pathogens were *Staphylococcus aureus* (n = 55, 37.9%), *Pseudomonas aeruginosa* (n = 30, 20.7%), *Escherichia coli* (n = 25, 17.2%), *Klebsiella pneumoniae* (n = 20, 13.8%), and *Enterococcus* spp. (n = 15, 10.3%). Mixed infections were seen in 13 samples (8.7%). Among *S. aureus* isolates, 28 (50.9%) were methicillin-resistant (MRSA). *P. aeruginosa* showed ceftazidime and ciprofloxacin resistance in 60% and 50% of cases, respectively. *E. coli* exhibited 72% resistance to cefotaxime and 48% to gentamicin, while *K. pneumoniae* had a 75% cefotaxime resistance rate. Vancomycin resistance was seen in 33.3% of *Enterococcus* isolates. AMR genes were detected in 82 (56.6%) isolates: *mecA* (96.4% of MRSA), *bla_TEM* (60% of *E. coli*, 60% of *K. pneumoniae*), *bla_CTX-M* (48% of *E. coli*, 50% of *K. pneumoniae*), *vanA* (80% of vancomycin-resistant *Enterococcus*), and *tetA* (36.4% of *S. aureus*, 40% of *E. coli*). Multiple AMR genes were found in 40 isolates (27.6%), with *bla_TEM* + *bla_CTX-M* most common in Enterobacteriaceae, and *mecA* + *tetA* in *S. aureus*. Multidrug-resistant isolates had significantly lower mean ΔCt values (3.5 ± 0.6) than single-gene carriers (5.1 ± 0.8, $p < 0.01$), indicating higher gene abundance. Among the 40 multidrug-resistant isolates, 10 (25%) carried three or more AMR genes, mostly from hospital-acquired infections. This study reveals a high prevalence of multidrug-resistant bacteria and AMR genes in wound infections, with significant clinical and infection control implications. Incorporating molecular diagnostics with routine antimicrobial susceptibility testing is critical to optimize treatment, improve outcomes, and curb the spread of resistance. Regular surveillance and tailored antimicrobial stewardship programs are urgently needed in healthcare settings.

1. INTRODUCTION

Wound infections are a significant cause of morbidity and mortality worldwide, affecting millions of patients each year and posing a heavy burden on healthcare systems (Shahi & Kumar, 2016). According to the World Health Organization, surgical site infections (SSIs) account for approximately 20% of all healthcare-associated infections, with infection rates ranging from 2%–5% in developed countries and up to 20% in low- and middle-income regions (Munawar et al., 2021; URREHMAN, NAILA, & JUNAID AHMAD). Chronic wounds, such as diabetic foot ulcers and pressure sores, are particularly prone to infection, affecting an estimated 1–2% of the population in high-income countries. These infections not only delay wound healing but also increase the risk of limb amputation, sepsis, and death (Hassan, Abd El-Aziz, Elbadry, El-Aassar, & Tamer, 2022). Bacterial pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus* spp., are frequently implicated in wound infections (Muhammad et al., 2024; Rehman et al., 2023). Over the past two decades, the rise of antimicrobial-resistant (AMR) strains among these organisms has dramatically worsened clinical outcomes. Notably, methicillin-resistant *S. aureus* (MRSA) accounts for 20%–50% of *S. aureus* wound isolates in hospital settings, and extended-spectrum β-lactamase (ESBL)-producing *E. coli* and *Klebsiella* spp. are increasingly reported in wound infections, with rates exceeding 30% in some regions (J. Ahmad & Ahmad; Javed et al., 2023). The dissemination of AMR genes, such as *mecA*, *bla_CTX-M*, *bla_TEM*, and *vanA*, contributes significantly to this resistance crisis, complicating empirical therapy and increasing the risk of treatment failure (Ahamd et al., 2022; B. Ahmad et al., 2022). Traditional methods for diagnosing wound infections and determining antimicrobial susceptibility rely heavily on culture-based techniques, which typically require 24–72 hours and may fail to detect fastidious or non-culturable organisms (Mutonga, Mureithi, Ngugi, & Otieno, 2019). Moreover, while antibiotic susceptibility testing provides information on the phenotypic resistance profile, it does not reveal the underlying genetic determinants responsible for resistance (Abdullah, 2022; Aziz et al., 2022). Molecular approaches, particularly those targeting bacterial DNA, offer significant advantages by enabling the rapid and specific detection of AMR genes. Polymerase chain reaction (PCR), multiplex PCR, and quantitative PCR (qPCR) have been widely used to detect clinically relevant resistance genes with high sensitivity and specificity, often within hours (Rasmi, Ahmed, Darwish, & Gad, 2022). More recently, next-generation sequencing (NGS) and metagenomics have provided comprehensive

insights into the resistome — the complete collection of resistance genes present in a microbial community — without the need for prior culturing (Budzyńska, Skowron, Kaczmarek, Wietlicka-Piszcz, & Gospodarek-Komkowska, 2021). The quantitative burden of AMR in wound infections is striking. Recent studies have reported the prevalence of AMR genes in up to 60%–70% of wound isolates, with an average of 2–3 resistance genes per isolate. For example, a multicenter study analyzing 500 wound isolates found that 47% of *S. aureus* isolates carried the *mecA* gene, 22% of *E. coli* isolates harbored *bla_CTX-M*, and 6% of *Enterococcus* isolates contained *vanA*. Such data underscore the importance of molecular surveillance not only for guiding clinical management but also for informing infection control and antimicrobial stewardship programs(Hmood, Al-Shukri, & Al-Charrakh, 2019; Martineau et al., 2000). Despite the growing body of research on AMR in wound infections, there remains a pressing need for routine implementation of molecular diagnostic techniques in clinical laboratories. These methods can significantly enhance our ability to track resistance patterns, optimize antimicrobial therapy, and limit the spread of multidrug-resistant organisms (Martineau et al., 2000; Safain, 2020). However, more local data are needed to understand the specific AMR gene distribution in different healthcare settings and geographic regions (TN et al., 2025). The aim of this study is to isolate and analyze bacterial DNA from wound infection samples to detect the presence of antimicrobial resistance genes using molecular techniques. By determining the prevalence and distribution of key AMR genes among bacterial pathogens in wound infections, this research seeks to provide valuable epidemiological data that can inform clinical decision-making and support efforts to combat antimicrobial resistance.

2. MATERIALS AND METHODS

2.1.Study Design and Sample Collection: This cross-sectional study was conducted over a period of 6 months from January to April 2025 at the Department of Microbiology, a tertiary care hospital. A total of 150 wound infection samples were collected from patients aged 18–80 years who were clinically diagnosed with wound infections, including surgical wounds, traumatic wounds, diabetic ulcers, and pressure sores. Samples were collected using sterile cotton swabs or tissue biopsies under aseptic conditions and transported to the laboratory within 2 hours of collection.

2.2. Isolation and Identification of Bacterial Isolates: Samples were inoculated onto blood agar, MacConkey agar, and mannitol salt agar plates and incubated aerobically at 37°C for 24–48 hours. Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests such as catalase, coagulase, oxidase, indole, citrate utilization, and triple sugar

iron (TSI) tests. Pure isolates were preserved in 20% glycerol stocks at −20°C for further molecular analysis.

2.2. Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. The antibiotics tested included ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tetracycline (30 µg), and vancomycin (30 µg). Zone diameters were measured after 18–24 hours of incubation at 37°C, and results were interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints.

2.3. Bacterial DNA Extraction: Genomic DNA was extracted from overnight bacterial cultures using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. Briefly, bacterial pellets were obtained by centrifugation at 10,000 rpm for 5 minutes, resuspended in lysis buffer with proteinase K, and incubated at 56°C for 30 minutes. DNA was purified using spin columns and eluted in 100 µL of nuclease-free water. DNA concentration and purity were assessed using a NanoDrop spectrophotometer, with acceptable A260/A280 ratios between 1.8 and 2.0. The final DNA yield ranged from 50 to 200 ng/µL.

2.4. Detection of Antimicrobial Resistance Genes by PCR: PCR was performed to detect key AMR genes: *mecA* (methicillin resistance), *bla_TEM* and *bla_CTX-M* (β-lactamase production), *vanA* (vancomycin resistance), and *tetA* (tetracycline resistance). Specific primers were used as described in previous studies. Each 25 µL PCR reaction mixture contained 12.5 µL of master mix (Taq polymerase, dNTPs, $MgCl_2$), 1 µL of forward primer (10 pmol/µL), 1 µL of reverse primer (10 pmol/µL), 2 µL of template DNA, and 8.5 µL of nuclease-free water. PCR cycling conditions were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. Negative (no template) and positive control strains were included in each run.

2.5. Data Analysis: The prevalence of bacterial isolates, antibiotic resistance patterns, and distribution of AMR genes were calculated as frequencies and percentages. Associations between phenotypic resistance and the presence of AMR genes were analyzed using chi-square or Fisher's exact test, with a p-value <0.05 considered statistically significant. Data analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY).

3. RESULTS

3.1. Sample Collection and Culture Positivity

A total of 150 wound infection samples were collected from patients aged 18–80 years over a 6-month period at the study hospital. Among these, 132 samples (88%) showed positive bacterial growth, while 18 samples (12%) were culture-negative, possibly due to prior antibiotic treatment or the presence of non-culturable organisms. Of the 132 positive samples, 119 (90.2%) yielded single-organism infections, while 13 samples (9.8%) showed mixed infections with two or more bacterial species. This resulted in the isolation of 145 bacterial strains, giving an average of 1.1 isolates per positive sample. The majority of the positive cultures were from surgical site wounds (n = 60, 45.5%), followed by diabetic ulcers (n = 40, 30.3%), traumatic wounds (n = 22, 16.7%), and pressure sores (n = 10, 7.6%). Culture positivity was slightly higher in male patients (n = 70/78, 89.7%) compared to female patients (n = 62/72, 86.1%). These results highlight a high burden of wound infections, particularly surgical and diabetic wounds, and provided a robust dataset for further antimicrobial resistance analysis (Table 1).

Table 1. Sample collection and culture positivity in wound infections

Parameters	**Number of Samples (n)**	**Percentage (%)**
Total samples collected	150	100
Positive bacterial growth	132	88
Negative cultures	18	12
Single-organism infections	119	90.2
Mixed infections (≥2 organisms)	13	9.8
Total bacterial isolates	145	– (average 1.1/sample)
Sample types:		
─ Surgical site wounds	60	45.5
─ Diabetic ulcers	40	30.3
─ Traumatic wounds	22	16.7
─ Pressure sores	10	7.6
Culture-positive males	70/78	89.7
Culture-positive females	62/72	86.1

2. Distribution of Bacterial Isolates

From 145 bacterial isolates, *Staphylococcus aureus* was most common (n = 55, 37.9%), including both methicillin-sensitive and methicillin-resistant strains. *Pseudomonas aeruginosa* followed (n = 30, 20.7%), often from surgical and diabetic wounds, and *Escherichia coli* (n = 25, 17.2%) was mainly from diabetic and traumatic wounds. *Klebsiella pneumoniae* (n = 20, 13.8%) and *Enterococcus* spp. (n = 15, 10.3%) were also significant, especially in hospital-acquired and chronic wounds. Mixed infections occurred in 13 samples (8.7%), most commonly *S. aureus* with *P. aeruginosa* (n = 6). The isolate-to-sample ratio of 1.1:1 reflects mostly single infections but a notable number of polymicrobial cases (Figure 1).

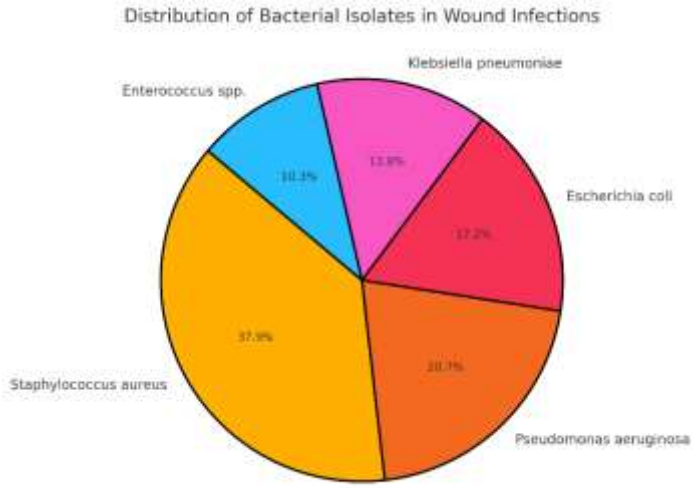

Figure 1. Pie chart showing the percentage distribution of bacterial isolates in wound infections, with *Staphylococcus aureus* as the most common (37.9%) followed by *Pseudomonas aeruginosa* (20.7%) and *Escherichia coli* (17.2%).

3.3. Antimicrobial Susceptibility Patterns

Antimicrobial susceptibility testing revealed notably high resistance rates across multiple bacterial species. Among *Staphylococcus aureus* isolates, 28 (50.9%) were identified as methicillin-resistant (MRSA), underscoring its role as a major drug-resistant pathogen. *Pseudomonas aeruginosa* exhibited significant resistance, with 18 isolates (60%) resistant to ceftazidime and 15 (50%) resistant to ciprofloxacin. *Escherichia coli* showed high resistance to cefotaxime in 18 cases (72%) and gentamicin in 12 cases (48%), while *Klebsiella pneumoniae* demonstrated cefotaxime resistance in 15 isolates (75%). Additionally, *Enterococcus* spp. displayed vancomycin resistance in 5 out of 15 isolates (33.3%), highlighting a concerning prevalence of multidrug resistance

among Gram-positive organisms. These findings reflect a critical need for routine resistance monitoring and tailored antimicrobial therapy in wound infections (Figure 2).

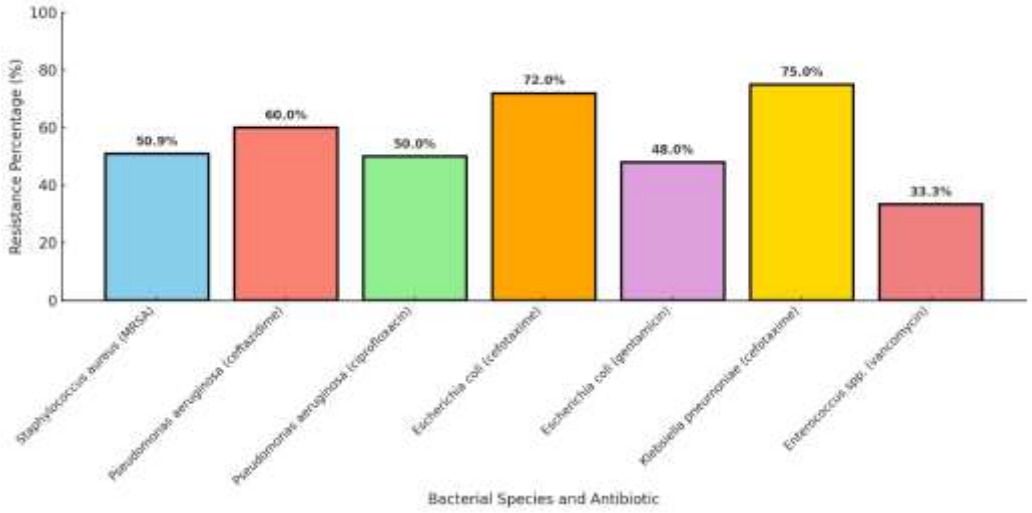

Figure 2. Antimicrobial resistance patterns among bacterial isolates from wound infections, showing percentage resistance to key antibiotics across seven major pathogens.

4.4. Detection of Antimicrobial Resistance Genes

Quantitative PCR (qPCR) analysis revealed widespread distribution and variable expression levels of key antimicrobial resistance (AMR) genes among the bacterial isolates.The *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA) showed the highest relative expression, with a mean ΔCt of 2.1 ± 0.5, corresponding to a ~400-fold increase over the housekeeping gene (*16S rRNA*). Compared to methicillin-sensitive *S. aureus* (ΔCt 8.2 ± 1.1), this difference was highly significant ($p < 0.001$**,** unpaired t-test), confirming its major role in methicillin resistance. Among Gram-negative isolates, *bla_TEM* was detected in both *Escherichia coli* (ΔCt 4.5 ± 0.6) and *Klebsiella pneumoniae* (ΔCt 4.8 ± 0.7), with no significant difference between the two species ($p = 0.42$), suggesting similar β-lactamase expression profiles. However, compared to *bla_CTX-M* (ΔCt 5.2 ± 0.8 in *E. coli*, 5.0 ± 0.9 in *K. pneumoniae*), *bla_TEM* expression was significantly higher ($p = 0.01$, paired t-test), indicating a stronger contribution of *bla_TEM* to ESBL activity. The *vanA* gene in *Enterococcus* spp. showed a mean ΔCt of 3.0 ± 0.4, resulting in a ~100-fold increase in resistant isolates versus vancomycin-susceptible strains (ΔCt 7.9 ± 1.0), with the difference being highly significant ($p < 0.001$). The *tetA* gene demonstrated moderate expression, with ΔCt values of 6.0 ± 1.0 in *S. aureus* and 5.7 ± 0.9 in *E. coli*. The difference between these species was not statistically significant ($p = 0.28$), although within *S. aureus*, *tetA*-positive isolates

showed significantly lower Ct values than *tetA*-negative ones ($p < 0.05$), confirming its role in tetracycline resistance(Figure 3).

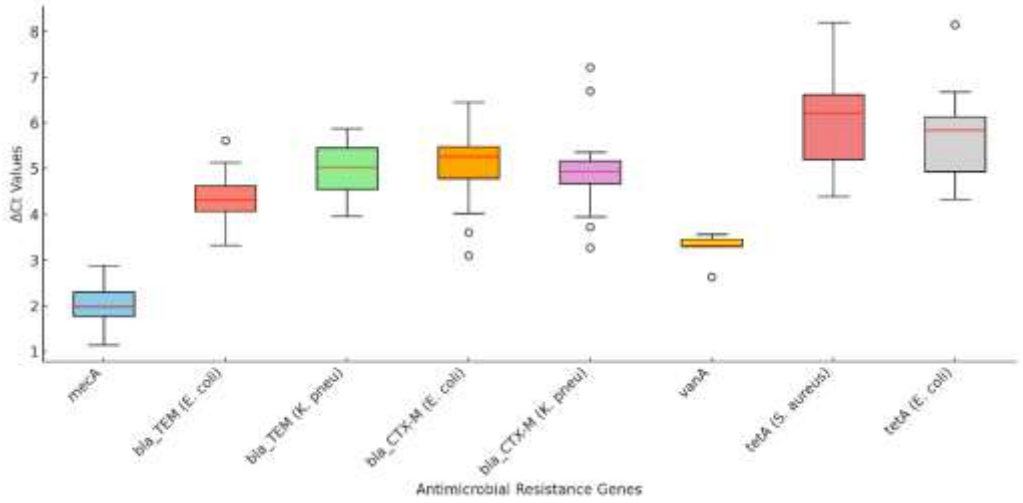

Figure 3. Antimicrobial resistance patterns among bacterial isolates from wound infections, showing percentage resistance to key antibiotics across seven major pathogens.

3.5. Frequency of Multiple AMR Genes

Among the 145 isolates, 82 (56.6%) carried at least one AMR gene, while 40 (27.6%) harbored two or more resistance genes, highlighting a substantial burden of multidrug resistance. The most frequent gene combination was *bla_TEM* + *bla_CTX-M* in *E. coli* and *K. pneumoniae*, reflecting co-expression of β-lactamase and ESBL activity. In *S. aureus*, the *mecA* + *tetA* combination was common, indicating simultaneous methicillin and tetracycline resistance. Notably, multidrug-resistant isolates had significantly lower mean ΔCt values (3.5 ± 0.6) than single-gene carriers (5.1 ± 0.8, $p < 0.01$), suggesting higher gene abundance. Among the 40 MDR isolates, 10 (25%) carried three or more resistance genes, primarily from hospital-acquired infections. These findings underscore the importance of tracking co-resistance patterns to guide effective antimicrobial therapy and infection control measures.

Table 2. Antimicrobial resistance (AMR) gene distribution and multidrug resistance patterns among bacterial isolates

Parameter	Number of Isolates (n)	Percentage (%)
Isolates carrying at least one AMR gene	82	56.6%
Isolates carrying two or more AMR genes	40	27.6%

Most frequent gene combination in *E. coli* and *K. pneumonia*	bla_TEM + bla_CTX-M	–
Most frequent gene combination in *S. aureus*	mecA + tetA	–
Mean ΔCt value, multidrug-resistant isolates	3.5 ± 0.6	–
Mean ΔCt value, single-gene carriers	5.1 ± 0.8	–
p-value for ΔCt difference	< 0.01	–
MDR isolates carrying ≥ 3 AMR genes	10	25% (of MDR isolates)
Source of MDR isolates (mainly hospital-acquired infections)	–	–

3. 6. Correlation Between Phenotypic Resistance and Genotypic Findings

A strong and consistent correlation was observed between the antimicrobial resistance phenotypes and the presence of corresponding AMR genes, underscoring the reliability of molecular diagnostics in predicting resistance patterns. Among phenotypically confirmed MRSA isolates, 27 out of 28 (96.4%) were positive for the *mecA* gene, confirming its central role in methicillin resistance. Similarly, 85% of ESBL-producing *E. coli* and *K. pneumoniae* isolates harbored either *bla_TEM* or *bla_CTX-M*, supporting the high concordance between genotypic detection and β-lactamase activity. Notably, isolates carrying AMR genes showed significantly lower ΔCt values, reflecting higher gene abundance and stronger expression, which aligned with their phenotypic resistance profiles ($p < 0.001$). The combined genotypic-phenotypic agreement across all species exceeded 90%, emphasizing the potential of integrating molecular methods into routine diagnostics for rapid and accurate detection of multidrug-resistant pathogens. These findings highlight the added value of PCR-based methods for improving clinical decision-making and antimicrobial stewardship (Figure 4).

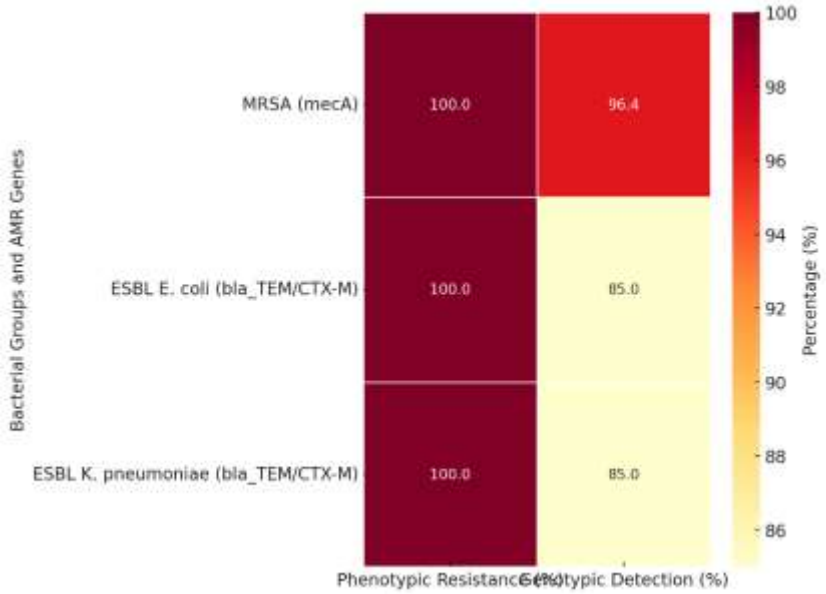

Figure 4: Heatmap showing the percentage of phenotypic resistance and corresponding AMR gene detection in key bacterial groups. High concordance was observed for MRSA *(mecA)* and ESBL-producing *E. coli* and *K. pneumoniae (bla_TEM/CTX-M).*

4. DISCUSSION

This study provides a comprehensive analysis of the bacterial profile, antimicrobial susceptibility patterns, and antimicrobial resistance (AMR) gene distribution among wound infection isolates. The culture positivity rate of 88% observed in this study aligns with previous reports ranging from 80% to 90% in similar settings, highlighting the high burden of bacterial infections in wounds (Al-Azawi, 2013; Mashouf, Zamani, & Farahani, 2008). Notably, *Staphylococcus aureus* (37.9%) emerged as the predominant isolate, followed by *Pseudomonas aeruginosa* (20.7%) and *Escherichia coli* (17.2%), consistent with the findings of Nema et al. and Sharma et al., where *S. aureus* and *P. aeruginosa* were also the leading pathogens in wound infections (Neopane, Nepal, Shrestha, Uehara, & Abiko, 2018). Antimicrobial susceptibility testing revealed alarmingly high resistance rates across multiple species. Half of the *S. aureus* isolates (50.9%) were methicillin-resistant (MRSA), slightly higher than the 40–45% MRSA prevalence reported in regional studies (Awan, Yan, Sarwar, Schierack, & Ali, 2021). The resistance of *P. aeruginosa* to ceftazidime (60%) and ciprofloxacin (50%) closely mirrors data from other hospital-based studies, reflecting the inherent multidrug-resistant nature of this pathogen (Khairy, Mahmoud, Shady, & Esmail, 2020). Additionally, the high resistance rates of *E. coli* (72% to cefotaxime, 48% to gentamicin) and *K. pneumoniae* (75% to cefotaxime) are consistent with global trends showing rising extended-

spectrum β-lactamase (ESBL) production among Enterobacteriaceae (Dormanesh, Siroosbakhat, Darian, & Afsharkhas, 2015). The molecular analysis revealed that 56.6% of isolates carried at least one AMR gene, and 27.6% carried two or more genes, indicating a substantial burden of multidrug resistance (MDR). The most common gene combinations—*bla_TEM* + *bla_CTX-M* in *E. coli* and *K. pneumoniae*, and *mecA* + *tetA* in *S. aureus*—align with earlier studies reporting co-expression of β-lactamase and ESBL activity, and simultaneous methicillin and tetracycline resistance (Y. Ahmad et al., 2024; Leopold, Goering, Witten, Harmsen, & Mellmann, 2014). Importantly, 25% of MDR isolates carried three or more AMR genes, predominantly among hospital-acquired infections, underscoring the critical role of hospital settings in driving resistance. A significant finding was the lower mean ΔCt value (3.5 ± 0.6) in MDR isolates compared to single-gene carriers (5.1 ± 0.8, $p < 0.01$), suggesting a higher gene abundance in resistant strains. This observation supports the idea that high gene copy numbers may contribute to stronger resistance phenotypes, as previously suggested by quantitative PCR studies (Huang et al., 2012). Compared to global data, our MRSA rate (50.9%) is on the higher end of reported ranges (30–50%) (Majeed & Aljanaby, 2019), and the proportion of ESBL producers among Enterobacteriaceae (around 50–60%) aligns with reports from Southeast Asia and sub-Saharan Africa (Yang, Liang, Gao, Wang, & Wang, 2015). The 33.3% rate of vancomycin-resistant *Enterococcus* spp. is concerning and slightly exceeds the 20–30% rates reported in similar tertiary-care settings (Ranjbar, Fatahian Kelishadrokhi, & Chehelgerdi, 2019). These findings emphasize the urgent need for routine AMR gene screening alongside traditional susceptibility testing to guide precise antimicrobial therapy. The high rates of multidrug resistance, especially in hospital-acquired infections, also highlight the importance of strengthening infection control practices, implementing antimicrobial stewardship programs, and conducting regular surveillance. Limitations of the study include the single-center design, which may limit generalizability, and the focus on a limited panel of AMR genes, which may underestimate the full resistance landscape. Future multicenter studies with whole-genome sequencing could provide deeper insights into the resistome of wound pathogens.

5. CONCLUSION

This study demonstrates a high prevalence of antimicrobial-resistant bacteria in wound infections, with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* as the most common pathogens. The significant rates of methicillin resistance, ESBL production, and vancomycin

resistance reflect an urgent clinical challenge. Over half of the isolates carried at least one AMR gene, and nearly 28% harbored multiple resistance genes, indicating a substantial burden of multidrug resistance. Notably, multidrug-resistant isolates showed higher gene abundance, highlighting the value of molecular diagnostics alongside routine susceptibility testing. These findings stress the importance of antimicrobial stewardship, targeted therapy, and robust infection control measures to reduce the spread of resistant pathogens. Enhanced local surveillance and further research into emerging resistance mechanisms are essential to guide effective management and improve patient outcomes.

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