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Bacterial Profile and Antimicrobial Resistance Patterns in Wound Infections in Misurata, Libya: A Cross-Sectional Study

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Abstract: Wound infections represent a significant clinical challenge due to their polymicrobial nature and the increasing burden of antimicrobial resistance. This study aimed to investigate the bacterial profile, prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), and antibiotic resistance patterns among wound infections in Misurata, Libya. A total of 120 wound samples were collected, of which 92 (76.7%) yielded bacterial growth. Gram-negative bacteria predominated (58.7%), while Gram-positive bacteria accounted for 41.3% of isolates. *Staphylococcus aureus* was the most frequently isolated organism (41.3%), followed by *Pseudomonas aeruginosa* (16.3%) and *Escherichia coli* (15.2%). Among *S. aureus* isolates, MRSA accounted for 68.4%, indicating a high prevalence of methicillin resistance. MRSA isolates exhibited significantly higher resistance to multiple antibiotics compared to MSSA, particularly against erythromycin (73.1%), ciprofloxacin (65.4%), and clindamycin (53.8%), while no resistance to vancomycin or linezolid was detected. Gram-negative isolates demonstrated high resistance to commonly used antibiotics, especially ampicillin (up to 85.7%), amoxicillin–clavulanate (up to 66.7%), and third-generation cephalosporins, whereas carbapenems showed relatively lower resistance rates. Multidrug resistance was observed in 53.3% of isolates, with 15.2% classified as extensively drug-resistant. The mean multiple antibiotic resistance (MAR) index was highest among MRSA isolates (0.54 ± 0.12), followed by Gram-negative isolates (0.36 ± 0.13). These findings highlight a substantial burden of antimicrobial resistance in wound infections and emphasize the need for continuous surveillance, rational antibiotic use, and effective infection control strategies.

Keywords:Wound infections; MRSA; antimicrobial resistance; Gram-negative bacteria; multidrug resistance; MAR index

Introduction:

The human body possesses multiple defense mechanisms that protect internal tissues against microbial invasion, with the skin serving as the primary physical barrier against pathogens. This barrier not only provides mechanical protection but also exhibits antimicrobial properties through the secretion of fatty acids and other inhibitory substances that limiting microbial colonization [1, 2]. However, any disruption in the integrity of the skin, such as wounds resulting from trauma, surgical procedures, or underlying pathological conditions, compromises this defense system and facilitates microbial invasion and proliferation, ultimately leading to wound infections [3].

Wound infections occur when microorganisms invade damaged tissue and multiply to levels that overwhelming host immune defenses, resulting in delayed healing and tissue destruction. These infections are typically accompanied by classical signs of inflammation, including redness, warmth, swelling, and pain, often progressing to purulent exudate formation due to the accumulation of immune cells, microbial components, and necrotic tissue [4]. Several factors may further complicate wound healing, including chronic diseases such as diabetes mellitus, poor vascular supply, prolonged or inappropriate antibiotics use, and inadequate sterilization of medical equipment. Consequently, wound infections represent a significant clinical challenge and a major burden on healthcare systems worldwide [5].

Wounds are broadly classified into acute and chronic types, each differing in etiology and healing dynamics [6, 7]. According to the Centers for Disease Control and Prevention (CDC), wound infections are categorized into superficial infections affecting the skin and subcutaneous tissue, and deeper infections involving muscles and connective tissues, which may lead to abscess formation and require more invasive interventions [8]. The microbial profile of wound infections is frequently polymicrobial and varies depending on the type of wound, environmental exposure, and patient-related factors.

Among the most frequently isolated pathogens in wound infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* [9]. Of particular concern is *Staphylococcus aureus*, which is associated with a wide range of infections, including skin and soft tissue infections, abscesses, and post-surgical complications [10]. The emergence of antibiotic-resistant strains, especially Methicillin-resistant *Staphylococcus aureus* (MRSA), has significantly complicated the management of wound infections. MRSA strains harbor the mecA gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for β-lactam antibiotics, thereby conferring resistance to methicillin and related drugs [11, 12].

MRSA is recognized as a leading cause of healthcare-associated infections and is associated with increased morbidity, prolonged hospital stays, and higher treatment costs [13]. The rising prevalence of MRSA, coupled with the global increase in antimicrobial resistance, highlights the urgent need for continuous surveillance of bacterial pathogens and their antibiotic susceptibility patterns, particularly in resource-limited settings where local epidemiological data are scarce.

In Libya, particularly in Misurata, limited published data exist regarding the bacterial profile of wound infections and the prevalence of MRSA among clinical isolates. This lack of local evidence hinders the development of effective empirical therapy strategies and infection control policies.

Therefore, the present study aimed to isolate and identify bacterial species associated with wound infections among patients attending the General Surgery Clinic at Misurata Medical Center, to determine the prevalence of MRSA among *Staphylococcus aureus* isolates, and to evaluate the antibiotic susceptibility patterns of these isolates.

Materials and Methods

Study Design and Setting

A cross-sectional study was conducted between May and July 2025 at the General Surgery Clinic of Misurata Medical Center, Libya.

Study Population and Sample Size

A total of 120 clinical samples were collected from 120 patients presenting with wound infections of varying severity. The study population included patients with various clinical conditions, including diabetic foot infections, boils, and post-surgical wounds.

Sample Collection and Transportation

Prior to specimen collection, the wound area was gently cleansed using sterile dry cotton swabs to remove superficial contaminants. Sterile cotton swabs moistened with sterile normal saline were then used to collect samples by rotating the swab over the infected tissue. All specimens were immediately transported to the microbiology laboratory under aseptic conditions for prompt processing.

Culture and Isolation of Bacteria

Samples were inoculated onto blood Agar, mannitol-Salt agar and MacConkey Agar plates Bacterial growth was evaluated based on colony morphology, hemolysis patterns, lactose fermentation, pigment production, and other phenotypic characteristics.

Bacterial Identification

Bacterial isolates were identified using standard microbiological methods. Initially, Gram staining was performed to differentiate between Gram-positive and Gram-negative organisms. This was followed by conventional biochemical testing, including catalase, coagulase, oxidase, indole, citrate utilization, and Triple Sugar Iron (TSI) tests, to achieve preliminary identification.

For confirmation, isolates were further identified using the Analytical Profile Index (API) system (bioMérieux, France), including API 20E and API 20NE, according to the manufacturer’s instructions. This approach

ensured accurate and reliable identification of bacterial species.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with the Clinical and Laboratory Standards Institute(CLSI) guidelines [14]. Bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) and uniformly inoculated onto the agar surface using sterile swabs.

For gram-positive isolates (*Staphylococcus aureus*), the antibiotic discs used included cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), linezolid (30 µg), and vancomycin (30µ g). For Gram-negative isolates, the tested antibiotics included ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), meropenem (10 µg), and imipenem (10 µg).

Plates were incubated at 35–37°C for 18–24 hours, after which inhibition zone diameters were measured in millimeters and interpreted according to CLSI breakpoints. Susceptibility to vancomycin was determined using the minimum inhibitory concentration (MIC) method in accordance with CLSI guidelines, which represents the gold standard for detecting vancomycin resistance in *Staphylococcus aureus*.

Detection of MRSA

MRSA was detected using the cefoxitin (30 µg) disk diffusion method, which serves as a surrogate marker for *mecA*-mediated resistance. Following incubation, isolates showing resistance to cefoxitin were classified as MRSA, whereas susceptible isolates were considered Methicillin-sensitive *S. aureus* (MSSA), based on standard interpretive criteria.

Statistical Analysis

Data were analyzed by SPSS v26. Descriptive statistics were used to summarize frequencies, percentages, and distributions of bacterial isolates and antimicrobial resistance patterns.

The association between categorical variables (such as wound type, culture positivity, Gram reaction, and MRSA distribution) was evaluated using the Pearson's chi-square test, while the Fisher's exact test was applied when expected cell counts were less than 5.

Comparisons of antibiotic resistance between MRSA and MSSA isolates were performed using the chi-square test.

The multiple antibiotic resistance (MAR) index was calculated for each isolate as the ratio of the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested. Differences in MAR index values among bacterial groups were assessed using the Kruskal–Wallis test.

A p-value < 0.05 was considered statistically significant.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki (2013). Verbal informed consent was obtained from all participants, and no identifiable patient data were collected.

Results

Culture Positivity According to Wound Type

As shown in Table 1, out of the 120 wound samples included in the study, bacterial growth was detected in 92 samples (76.7%), while 28 samples (23.3%) showed no growth.

The highest culture positivity rate was observed among diabetic foot infections, with 37 out of 42 samples (88.1%) yielding bacterial growth. Post-surgical wounds also demonstrated a high positivity rate, with 39 out of 50 samples (78.0%) being culture-positive. In contrast, boils showed the lowest positivity rate, with only 16 out of 28 samples (57.1%) exhibiting bacterial growth, while 12 samples (42.9%) were culture-negative.

A statistically significant difference in culture positivity was observed among the different wound types ($p = 0.011$).

Table 1. Culture positivity among wound samples according to wound type (n = 120)

Wound type	Total samples n (%)	Positive growth n (%)	No growth n (%)	P-value
Diabetic foot infections	42 (35.0)	37 (88.1)	5 (11.9)	0.011*
Boils	28 (23.3)	16 (57.1)	12 (42.9)	
Post-surgical wounds	50 (41.7)	39 (78.0)	11 (22.0)	
Total	120 (100)	92 (76.7)	28 (23.3)	

*** Significant**

Distribution of Bacterial Isolates

A total of 92 bacterial isolates were recovered from culture-positive wound samples. *Staphylococcus aureus* was the most frequently isolated organism, accounting for 38 isolates (41.3%).

Among Gram-negative bacteria, *Pseudomonas aeruginosa* was the most common species with 15 isolates (16.3%), followed by *Escherichia coli* with 14 isolates (15.2%). Other isolates included *Klebsiella pneumoniae* (9 isolates, 9.8%), *Acinetobacter baumannii* (7 isolates, 7.6%), *Proteus* spp. (5 isolates, 5.4%), and *Enterobacter* spp. (4 isolates, 4.3%).

Overall, Gram-negative bacteria predominated, representing 54 isolates (58.7%), whereas Gram-positive bacteria accounted for 38 isolates (41.3%), as presented in Figure 1.

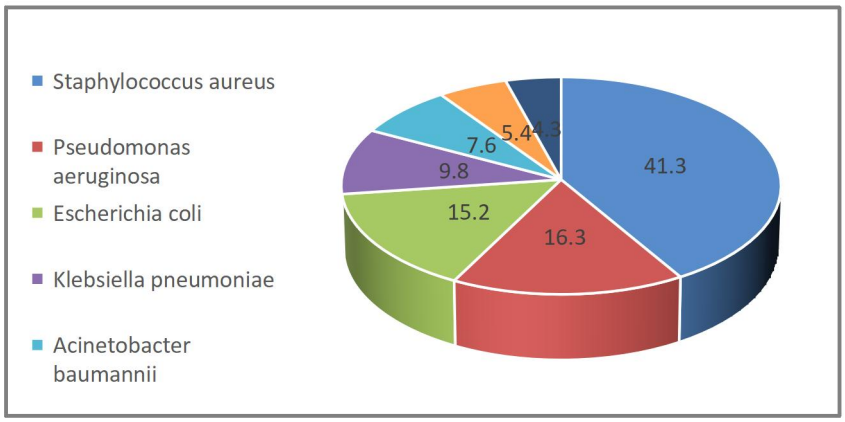

Fig 1. Distribution of bacterial isolates by species and Gram reaction (n = 92)

Distribution of Gram Reaction Across Wound Types

As shown in Table 2, the distribution of Gram-positive and Gram-negative isolates varied across different wound types.

In diabetic foot infections, Gram-negative bacteria predominated, accounting for 23 out of 37 isolates (62.2%), while Gram-positive bacteria represented 14 isolates (37.8%). Similarly, in post-surgical wounds, Gram-negative isolates were more frequent, with 27 out of 39 isolates (69.2%), compared to 12 isolates (30.8%) of Gram-positive bacteria. In contrast, boils showed a higher proportion of Gram-positive bacteria, with 12 out of 16 isolates (75.0%), whereas Gram-negative bacteria accounted for only 4 isolates (25.0%).

Overall, Gram-negative bacteria constituted 54 isolates (58.7%), while Gram-positive bacteria accounted for 38 isolates (41.3%). A statistically significant difference in the distribution of Gram reaction across wound types was observed ($p = 0.009$).

Table 2. Distribution of Gram reaction across wound types (n = 92)

Wound type	Gram-positive n (%)	Gram-negative n (%)	Total	P-value
Diabetic foot infections	14 (37.8)	23 (62.2)	37	0.009*
Boils	12 (75.0)	4 (25.0)	16	
Post-surgical wounds	12 (30.8)	27 (69.2)	39	
Total	38 (41.3)	54 (58.7)	92	

*** Significant**

Distribution of Bacterial Isolates According to Wound Type

Figure 2 reveals the distribution of bacterial isolates varied across different wound types. In diabetic foot infections, *Staphylococcus aureus* was the most frequently isolated organism, accounting for 14 out of 37 isolates (37.8%), followed by *Pseudomonas aeruginosa* (8 isolates, 21.6%) and *Escherichia coli* (5 isolates, 13.5%). Other isolates included *Klebsiella pneumoniae* (4 isolates, 10.8%), *Acinetobacter baumannii* (3 isolates, 8.1%), *Proteus* spp. (2 isolates, 5.4%), and *Enterobacter* spp. (1 isolate, 2.7%).

Among boils, *Staphylococcus aureus* was the predominant organism, representing 12 out of 16 isolates (75.0%), while the remaining isolates were distributed among *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* spp., and *Enterobacter* spp., each accounting for 1 isolate (6.3%). No isolates of *Klebsiella pneumoniae* or *Acinetobacter baumannii* were detected in this group.

In post-surgical wounds, *Staphylococcus aureus* was also the most common isolate (12 out of 39 isolates, 30.8%), followed by *Escherichia coli* (8 isolates, 20.5%) and *Pseudomonas aeruginosa* (6 isolates, 15.4%). Other isolates included *Klebsiella pneumoniae* (5 isolates, 12.8%), *Acinetobacter baumannii* (4 isolates, 10.3%), *Proteus* spp. (2 isolates, 5.1%), and *Enterobacter* spp. (2 isolates, 5.1%).

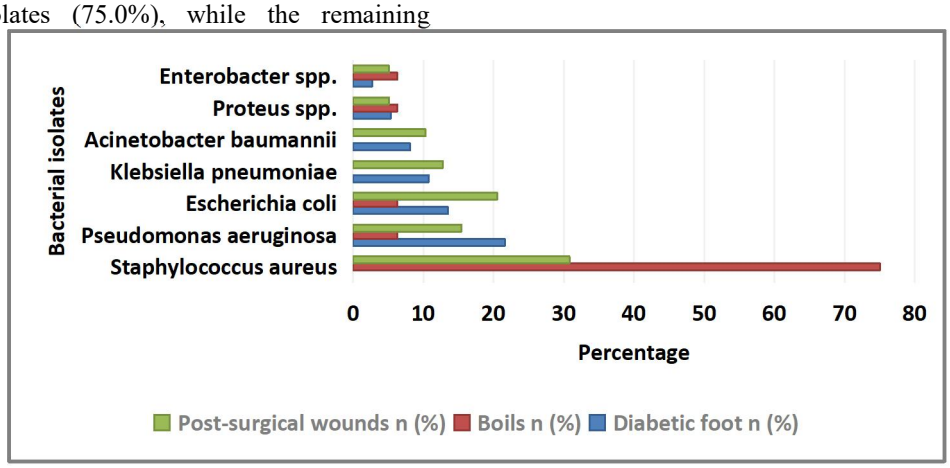

Fig2. Distribution of bacterial isolates according to wound type (n = 92)

Distribution of MRSA and MSSA According to Wound Type

Table 3 shows a total of 38 *Staphylococcus aureus* isolates were identified, of which 26 isolates (68.4%) were classified as MRSA and 12 isolates (31.6%) as MSSA.

Among diabetic foot infections, MRSA accounted for 11 out of 14 isolates (78.6%), while MSSA represented 3 isolates (21.4%). In boils, MRSA was detected in 8 out of 12 isolates (66.7%), compared to 4 isolates (33.3%) of MSSA. Similarly, in post-surgical wounds, MRSA was identified in 7 out of 12 isolates (58.3%), whereas MSSA accounted for 5 isolates (41.7%).

No statistically significant difference in the distribution of MRSA and MSSA across the different wound types was observed (p = 0.535).

Table 3. Distribution of MRSA and MSSA according to wound type (n = 38)

Wound type	*S. aureus* n	MRSA n (%)	MSSA n (%)	P-value
Diabetic foot infections	14	11 (78.6)	3 (21.4)	0.535 (NS)
Boils	12	8 (66.7)	4 (33.3)	
Post-surgical wounds	12	7 (58.3)	5 (41.7)	
Total	38	26 (68.4)	12 (31.6)	

NS, not significant

Antibiotic Resistance Pattern of *Staphylococcus aureus* Isolates

As shown in Table 4, all MRSA isolates (100%) were resistant to cefoxitin, whereas none of the MSSA isolates showed resistance to this antibiotic. High resistance rates among MRSA isolates were observed for erythromycin (19/26, 73.1%) and ciprofloxacin (17/26, 65.4%). Moderate resistance was detected against clindamycin (14/26, 53.8%), tetracycline (12/26, 46.2%), and gentamicin (11/26, 42.3%). In comparison, MSSA isolates demonstrated lower resistance rates across most antibiotics, including erythromycin (4/12, 33.3%), ciprofloxacin (2/12, 16.7%), clindamycin (2/12, 16.7%), tetracycline (3/12, 25.0%), and gentamicin (1/12, 8.3%). No resistance to vancomycin or linezolid was detected among either MRSA or MSSA isolates. Statistically significant differences between MRSA and MSSA were observed for cefoxitin ($p < 0.001$), ciprofloxacin ($p= 0.004$), gentamicin ($p = 0.028$), erythromycin ($p= 0.021$), and clindamycin ($p= 0.033$), while no significant difference was found for tetracycline ($p = 0.189$).

Table 4. Antibiotic resistance pattern of *Staphylococcus aureus* isolates (MRSA vs MSSA)

Antibiotic	MRSA Resistant n (%)	MSSA Resistant n (%)	P-value
Cefoxitin	26 (100)	0 (0.0)	<0.001*
Ciprofloxacin	17 (65.4)	2 (16.7)	0.004*
Gentamicin	11 (42.3)	1 (8.3)	0.028*
Erythromycin	19 (73.1)	4 (33.3)	0.021*
Clindamycin	14 (53.8)	2 (16.7)	0.033*
Tetracycline	12 (46.2)	3 (25.0)	0.189
Vancomycin	0 (0.0)	0 (0.0)	—
Linezolid	0 (0.0)	0 (0.0)	—

*** Significant**

Antibiotic Resistance Among Gram-Negative Isolates

Table 5 presents antibiotic resistance patterns varied among Gram-negative bacterial species.

Among *Pseudomonas aeruginosa* isolates (n = 15), resistance was most frequently observed to ceftazidime (8/15, 53.3%) and ciprofloxacin (7/15, 46.7%), followed by gentamicin (5/15, 33.3%). Lower resistance rates were noted for meropenem (2/15, 13.3%) and imipenem (1/15, 6.7%).

For *Escherichia coli* isolates (n = 14), high resistance was observed to ampicillin (12/14, 85.7%) and amoxicillin–clavulanate (8/14, 57.1%), followed by cefotaxime (7/14, 50.0%) and ceftazidime (6/14, 42.9%). Resistance to ciprofloxacin and gentamicin was detected in 5 (35.7%) and 4 (28.6%) isolates, respectively, while lower resistance rates were observed for meropenem (1/14, 7.1%) and no resistance to imipenem.

Klebsiella pneumoniae isolates (n = 9) showed resistance to amoxicillin–clavulanate in 6 isolates (66.7%), cefotaxime in 5 isolates (55.6%), and ceftazidime in 4 isolates (44.4%). Resistance to ciprofloxacin and gentamicin was detected in 3 (33.3%) and 2 (22.2%) isolates, respectively, with lower resistance to meropenem (1/9, 11.1%) and no resistance to imipenem.

Among *Acinetobacter baumannii* isolates (n = 7), resistance was highest to cefotaxime (5/7, 71.4%), followed by ceftazidime and ciprofloxacin (4/7, 57.1% each), and gentamicin (3/7, 42.9%). Resistance to carbapenems was lower, with 2 isolates (28.6%) resistant to meropenem and 1 isolate (14.3%) resistant to imipenem.

Proteus spp. isolates (n = 5) demonstrated high resistance to ampicillin (4/5, 80.0%) and amoxicillin–clavulanate (3/5, 60.0%), while lower resistance was observed for cefotaxime and ceftazidime (2/5, 40.0% each), ciprofloxacin (1/5, 20.0%), and gentamicin (1/5, 20.0%), with no resistance detected to carbapenems.

Finally, *Enterobacter* spp. isolates (n = 4) showed resistance to cefotaxime in 2 isolates (50.0%), while resistance to ceftazidime, ciprofloxacin, and gentamicin was observed in 1 isolate each (25.0%), with no resistance to carbapenems.

Table 5. Antibiotic resistance among Gram-negative isolates by species

Species	AMP	AMC	CTX	CAZ	CIP	GEN	MEM	IPM
Pseudomonas aeruginosa (n=15)	NT	NT	NT	8 (53.3)	7 (46.7)	5 (33.3)	2 (13.3)	1 (6.7)
E. coli (n=14)	12 (85.7)	8 (57.1)	7 (50.0)	6 (42.9)	5 (35.7)	4 (28.6)	1 (7.1)	0 (0.0)
Klebsiella (n=9)	NT	6 (66.7)	5 (55.6)	4 (44.4)	3 (33.3)	2 (22.2)	1 (11.1)	0 (0.0)
Acinetobacter (n=7)	NT	NT	5 (71.4)	4 (57.1)	4 (57.1)	3 (42.9)	2 (28.6)	1 (14.3)
Proteus (n=5)	4 (80.0)	3 (60.0)	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)
Enterobacter (n=4)	NT	NT	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)

Distribution of Multidrug Resistance Among Isolates

Multidrug resistance was observed in a substantial proportion of the isolates. A total of 49 out of 92 isolates (53.3%) were classified as multidrug-resistant (MDR), while 14 isolates (15.2%) were identified as extensively drug-resistant (XDR) as showed in Table 6. In contrast, 29 isolates (31.5%) were categorized as non-MDR.

Table 6. Multidrug resistance (MDR) distribution among isolates (n = 92)

Category	Number of isolates	Percentage (%)
Non-MDR	29	31.5
MDR	49	53.3
XDR	14	15.2
Total	92	100

Mean MAR Index Across Bacterial Groups

The mean multiple antibiotic resistance (MAR) index varied among the different bacterial groups. MRSA isolates demonstrated the highest mean MAR index (0.54 ± 0.12), followed by Gram-negative isolates (0.36 ± 0.13). In contrast, MSSA isolates exhibited the lowest mean MAR index (0.18 ± 0.09) as revealed in Table 7. A statistically significant difference in MAR index values was observed among the studied groups (p = 0.003).

Table 7. Mean MAR index across bacterial groups

Bacterial group	Mean MAR ± SD	P-value
MRSA	0.54 ± 0.12	0.003*
MSSA	0.18 ± 0.09	
Gram-negative isolates	0.36 ± 0.13	
Overall comparison		

* Significant

Discussion

Wound infections represent a complex clinical entity influenced by host factors, microbial diversity, and antimicrobial resistance dynamics. The present study provides a detailed characterization of the bacterial profile and resistance patterns in wound infections within a Libyan healthcare setting, highlighting significant variations in microbial distribution, a predominance of Gram-negative pathogens, and a substantial burden of multidrug resistance, particularly among MRSA and Gram-negative isolates. These findings collectively reflect an evolving epidemiological landscape shaped by both local healthcare practices and global antimicrobial resistance trends.

Culture Positivity and Clinical Implications

The high culture positivity observed in this study is consistent with previous reports indicating that chronic and healthcare-associated wounds provide a favorable environment for bacterial colonization and persistence [15, 16]. This phenomenon is particularly evident in diabetic and post-surgical wounds, where impaired host defenses and prolonged exposure to external environments facilitate microbial establishment [9, 17].

From a pathophysiological perspective, chronic wounds are characterized by tissue hypoxia, reduced vascular supply, and impaired immune responses, all of which contribute to increased susceptibility to infection [16, 18]. In diabetic patients, hyperglycemia further compromises neutrophil function, reduces chemotaxis, and impairs phagocytosis, thereby enhancing bacterial survival and colonization [15, 16].

In addition, post-surgical wounds are particularly vulnerable to infection due to disruption of the skin barrier and exposure to hospital-associated microorganisms, which increases the likelihood of contamination during or after surgical procedures [9, 19]. Similar findings have been reported in studies of surgical site infections, where high bacterial recovery rates were associated with both environmental contamination and host-related factors [18, 20].

The observed variation in culture positivity across wound types further supports the concept that microbial burden is influenced by underlying clinical and environmental conditions rather than being uniformly distributed across all wound categories [15].

Predominance of Gram-Negative Bacteria

The predominance of Gram-negative bacteria observed in this study reflects an important epidemiological shift in wound infections, particularly in hospital-associated and chronic wound settings [18, 20]. Similar patterns have been reported in regional studies, including Libyan data demonstrating increased isolation of Gram-negative organisms in clinical wound infections [21].

This shift can be explained by several microbiological and ecological factors. Gram-negative bacteria possess an outer membrane containing lipopolysaccharide, which acts as a permeability barrier and reduces antibiotic penetration, thereby enhancing intrinsic resistance [1]. In addition, these organisms frequently produce β-lactamases, including extended-spectrum β-lactamases (ESBLs), which contribute to resistance against commonly used antibiotics [9, 19].

Another critical factor is the ability of Gram-negative bacteria to form biofilms, which play a central role in chronic wound infections. Biofilm formation enables bacterial communities to adhere to tissue surfaces and produce an extracellular matrix that protects them from both host immune responses and antimicrobial agents [15, 17]. This mechanism is particularly relevant in chronic wounds, where persistent infection is often driven by biofilm-associated bacteria [18].

Furthermore, the hospital environment represents a major reservoir for Gram-negative pathogens, especially *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which are well adapted to survive on moist surfaces, medical devices, and disinfectant-resistant niches [9, 19]. This ecological advantage explains their higher prevalence in post-surgical wounds, where exposure to healthcare settings is more pronounced.

Consistent with these observations, recent Libyan data on *Pseudomonas aeruginosa* demonstrated high levels of antimicrobial resistance in conjunction with virulence traits such as biofilm formation, further emphasizing the clinical significance of Gram-negative pathogens in persistent infections [22].

Collectively, these findings support the concept that Gram-negative bacteria are increasingly dominant in wound infections due to their adaptive resistance mechanisms, environmental persistence, and ability to establish chronic, biofilm-mediated infections [15].

Bacterial Distribution Across Wound Types

The variation in bacterial distribution across wound types observed in this study reflects the influence of distinct ecological niches and host-related factors on microbial colonization. The predominance of *Staphylococcus aureus* in superficial infections such as boils is consistent with its role as a common skin commensal and opportunistic pathogen capable of causing localized infections following disruption of the skin barrier [11, 15].

In contrast, chronic wounds, particularly diabetic and post-surgical wounds, demonstrated a more diverse microbial profile, indicating a polymicrobial nature of infection. This pattern has been widely reported in previous studies, where chronic wounds were characterized by the coexistence of multiple bacterial species, including both Gram-positive and Gram-negative organisms [17]. Such polymicrobial communities are known to enhance pathogenicity through synergistic interactions, leading to increased virulence and persistence within the wound environment [9, 15].

From a mechanistic perspective, polymicrobial infections contribute to biofilm formation, where different bacterial species interact to produce a structured extracellular matrix that enhances resistance to antimicrobial agents and host immune responses [18]. This phenomenon is particularly relevant in diabetic wounds, where impaired immune function and reduced tissue perfusion facilitate the establishment of complex microbial communities [9, 16].

Additionally, the presence of Gram-negative bacteria in post-surgical wounds reflects the role of environmental contamination and healthcare-associated exposure in shaping microbial profiles. Similar observations have been reported in studies of surgical site infections, where Gram-negative organisms were frequently associated with hospital environments and invasive procedures [19, 20].

In the Libyan context, similar findings have been reported, demonstrating a diverse bacterial distribution in diabetic foot infections with a notable predominance of Gram-negative pathogens [21]. In addition, clinical isolates of *Pseudomonas aeruginosa* from healthcare-associated infections have been shown to exhibit both high antimicrobial resistance and virulence potential, further emphasizing the critical role of Gram-negative organisms in complex wound infections [22].

Taken together, these findings highlight that bacterial distribution in wound infections is not random but rather determined by a combination of host factors, wound characteristics, and environmental exposure, all of which contribute to the development of polymicrobial and persistent infections [15].

Prevalence and Distribution of MRSA

The high prevalence of methicillin-resistant *Staphylococcus aureus* observed in this study reflects the increasing burden of MRSA in both hospital-associated and community-acquired infections. This finding is consistent with global reports identifying MRSA as a major contributor to wound infections and antimicrobial resistance [11, 23].

The absence of a statistically significant association between MRSA distribution and wound type suggests that MRSA is widely disseminated across different clinical conditions, supporting the concept of endemic circulation within healthcare settings [9, 24].

Importantly, these findings are supported by recent data from Libya indicating that MRSA carriage among healthcare workers remains substantial, even in the presence of infection control interventions, highlighting its persistence within hospital environments [25]. In addition, a high prevalence of MRSA colonization has been reported among radiology technicians in Misurata, emphasizing the role of healthcare personnel as potential reservoirs for MRSA transmission within clinical settings [26].

The convergence of these findings suggests that MRSA transmission is likely maintained through continuous interaction between patients, healthcare workers, and the hospital environment, reinforcing its endemic nature in the region [21, 25].

From a mechanistic perspective, the persistence of MRSA is primarily attributed to the presence of the mecA gene, which encodes an altered penicillin-binding protein (PBP2a), reducing the efficacy of β-lactam antibiotics [11]. In addition, MRSA strains possess virulence factors that enhance adhesion, colonization, and biofilm formation, enabling prolonged survival on tissues and abiotic surfaces [1, 15].

Moreover, antibiotic selection pressure plays a central role in maintaining MRSA within clinical environments. The widespread use of antibiotics, particularly in empirical therapy, facilitates the survival and expansion of resistant strains, thereby increasing their prevalence over time [9].

Taken together, these findings indicate that MRSA is not only prevalent but also epidemiologically entrenched in the study setting,

supported by both local evidence and global trends [25].

Antibiotic Resistance in MRSA

The resistance pattern observed among MRSA isolates reflects their well-established multidrug-resistant phenotype, which extends beyond β-lactam antibiotics to multiple antimicrobial classes [23, 24]. This broad resistance profile is a defining characteristic of MRSA and represents a major challenge in clinical management of wound infections.

The significantly higher resistance observed in MRSA compared to MSSA indicates that methicillin resistance is frequently associated with co-resistance to other antibiotics, a phenomenon widely reported in previous studies [23]. This association is primarily driven by the accumulation of resistance determinants on mobile genetic elements, such as plasmids and transposons, which facilitate the horizontal transfer of multiple resistance genes [1].

In addition to genetic factors, selective antibiotic pressure plays a critical role in shaping resistance patterns. The widespread use of antibiotics in clinical settings promotes the survival of resistant strains while eliminating susceptible populations, thereby contributing to the persistence and spread of MRSA [5, 9].

Importantly, the absence of resistance to vancomycin and linezolid observed in this study suggests that these agents remain effective therapeutic options for MRSA infections. Similar findings have been reported in a recent Libyan study, where no vancomycin-resistant MRSA isolates were detected despite a high prevalence of methicillin resistance [25]. This indicates that glycopeptides and oxazolidinones continue to play a critical role in the management of MRSA infections in the region.

Furthermore, the persistence of susceptibility to vancomycin may reflect its controlled use and its status as a last-line antibiotic, which reduces selective pressure compared to more commonly used agents [11]. However, global reports of emerging vancomycin-intermediate and resistant *S. aureus* strains emphasize the need for cautious use and continuous monitoring [11].

These findings collectively highlight that MRSA resistance is driven by a combination of genetic adaptability and antibiotic pressure, while the preserved activity of last-line agents underscores their continued importance in clinical practice [23, 25].

Antibiotic Resistance Among Gram-Negative Bacteria

The elevated resistance observed among Gram-negative isolates, particularly to β-lactam antibiotics and cephalosporins, reflects a well-established global trend of increasing antimicrobial resistance among these organisms [18, 19]. This pattern is especially pronounced in hospital-associated infections, where Gram-negative bacteria are frequently exposed to multiple antibiotic classes.

This resistance profile is largely driven by the production of β-lactamases, including extended-spectrum β-lactamases (ESBLs), which hydrolyze a wide range of β-lactam antibiotics and significantly reduce their clinical effectiveness [1]. In addition, Gram-negative bacteria possess intrinsic resistance mechanisms such as reduced outer membrane permeability and active efflux pumps, which further limit antibiotic penetration and efficacy.

The relatively lower resistance observed to carbapenems indicates that these agents remain among the most effective treatment options for Gram-negative infections. This finding is consistent with recent regional data, where carbapenems demonstrated higher activity compared to other antibiotic classes [19]. However, the emergence of resistance even at lower levels is clinically significant, as it may indicate the early development of carbapenem-resistant strains.

In the Libyan context, high resistance rates have been reported among *Pseudomonas aeruginosa* isolates, particularly to ceftazidime, ciprofloxacin, and gentamicin, supporting the resistance patterns observed in the present study. This consistency suggests a regional trend driven by similar selective pressures and healthcare practices [22].

Moreover, a significant association between virulence factors, particularly biofilm formation, and increased antimicrobial resistance has been documented, indicating that resistance is not solely a genetic phenomenon but is also influenced by phenotypic adaptations. Biofilm-producing isolates demonstrated higher resistance levels due to the protective extracellular matrix, which limits antibiotic penetration and enhances bacterial survival [22].

The ability of Gram-negative bacteria to form biofilms is particularly relevant in wound infections, where chronic exposure to antimicrobial agents and host defenses promotes the selection of highly resistant bacterial populations [15, 17]. This mechanism contributes to persistent infections and complicates treatment strategies.

Additionally, the hospital environment plays a crucial role in the dissemination of resistant Gram-negative organisms. These bacteria can survive on medical devices, moist surfaces, and hospital equipment, facilitating their transmission between patients and contributing to healthcare-associated infections [9, 18].

Taken together, these findings indicate that antimicrobial resistance among Gram-negative bacteria is driven by a combination of enzymatic degradation of antibiotics, reduced drug permeability, biofilm formation, and environmental persistence, all of which contribute to their dominance and clinical significance in wound infections [1, 22].

Multidrug Resistance and MAR Index

The high prevalence of multidrug resistance observed in this study reflects the cumulative impact of sustained antibiotic exposure within clinical settings, particularly in hospital-associated infections [9, 16]. Multidrug-resistant organisms emerge as a result of continuous selective pressure, where repeated and often empirical antibiotic use favors the survival of resistant strains over susceptible populations.

The elevated MAR index observed among bacterial isolates further supports this interpretation, as it serves as a quantitative indicator of prior antibiotic exposure and environmental risk. High MAR values are typically associated with high-risk sources, such as hospital environments where antibiotics are frequently used, leading to the accumulation of resistance traits within microbial populations [9].

The significantly higher MAR index observed among MRSA and Gram-negative isolates suggests that these organisms have been exposed to multiple antimicrobial agents, enabling them to acquire and maintain diverse resistance mechanisms. This observation is consistent with previous studies reporting higher MAR indices in hospital-acquired infections compared to community-acquired cases [16, 18].

In the Libyan context, similar findings have demonstrated a measurable reduction in the MAR index following the implementation of infection control interventions, highlighting the direct relationship between antibiotic pressure and resistance burden [25]. This evidence further supports the role of healthcare practices in shaping resistance patterns.

Moreover, the coexistence of multidrug resistance with virulence traits, such as biofilm formation, has been demonstrated in Gram-negative pathogens, particularly *Pseudomonas aeruginosa*, where resistant isolates exhibited enhanced virulence potential [22]. This relationship suggests that resistance and pathogenicity are often interconnected, leading to more persistent and clinically challenging infections.

From a clinical perspective, the presence of multidrug-resistant organisms is associated with limited therapeutic options, increased treatment failure, prolonged hospitalization, and higher healthcare costs [9, 19]. These outcomes underscore the importance of early detection and targeted antimicrobial therapy.

Furthermore, the findings emphasize the critical need for antimicrobial stewardship programs aimed at optimizing antibiotic use and reducing unnecessary exposure. Effective stewardship strategies, combined with infection control measures such as hand hygiene and environmental decontamination, have been shown to reduce both antimicrobial resistance and MAR index values in clinical settings [25].

Overall, the high prevalence of multidrug resistance and elevated MAR index observed in this study highlight the significant role of antibiotic pressure in shaping resistance patterns and underscore the urgent need for coordinated strategies to mitigate the spread of resistant pathogens [9].

Conclusion

In conclusion, this study provides important insights into the epidemiology of wound infections in a Libyan healthcare setting, demonstrating a complex microbial profile characterized by a predominance of Gram-negative bacteria and a high prevalence of methicillin-resistant *Staphylococcus aureus*. The findings highlight a substantial burden of antimicrobial resistance, with a considerable

proportion of isolates exhibiting multidrug-resistant phenotypes and elevated MAR index values, reflecting significant antibiotic pressure within the clinical environment.

The observed resistance patterns, particularly among MRSA and Gram-negative pathogens, underscore the increasing challenge in the management of wound infections and the potential risk of treatment failure when empirical therapy is not guided by local susceptibility data. At the same time, the preserved susceptibility to last-line agents such as vancomycin and linezolid emphasizes their continued clinical importance, while also indicating the need for their cautious and regulated use.

Overall, these findings reinforce the urgent need for continuous microbiological surveillance, evidence-based antibiotic policies, and strengthened infection control strategies to limit the spread of resistant pathogens and improve clinical outcomes in wound management.

Implications, Recommendations, and Limitations

The findings of this study emphasize the need for strengthened antimicrobial stewardship programs and strict infection control measures, particularly in hospital settings where antibiotic pressure and transmission risk are high. Routine microbiological testing and susceptibility profiling should be encouraged to guide targeted therapy and reduce reliance on empirical treatment. Continuous monitoring of MRSA and multidrug-resistant organisms among both patients and healthcare workers is essential to limit transmission.

Future research should focus on molecular characterization of resistance mechanisms, including key resistance genes and biofilm-associated traits, as well as longitudinal surveillance to track resistance trends over time. In addition, evaluation of infection control interventions and alternative therapeutic strategies may provide further insights into effective management approaches.

However, the findings of this study should be interpreted in light of certain limitations, including its single-center design, cross-sectional nature, and the absence of molecular and biofilm-related analyses, which may limit the generalizability and depth of resistance characterization.

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