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ROLE OF BIOFILM-FORMING BACTERIA IN CHRONIC WOUND INFECTIONS AND THEIR ANTIBIOTIC RESISTANCE PROFILES

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ABSTRACT

Chronic wound infections are a global health concern, affecting up to 4% of diabetic populations. Biofilm-producing bacteria significantly delay healing and increase multidrug resistance (MDR. This study assessed the prevalence of biofilm-forming bacteria in chronic wounds and their antibiotic resistance patterns. A total of 100 chronic wound samples were collected. Bacterial identification and biofilm quantification were performed using standard microbiological techniques, while antibiotic susceptibility testing followed CLSI guidelines. Data were analyzed using SPSS v26, with $p < 0.05$ considered significant. Of 100 samples, 85 (85%) showed bacterial growth. Gram-negative isolates predominated (70/85, 82.4%) over Gram-positives (15/85, 17.6%). The most frequent pathogens were *Pseudomonas aeruginosa* (25, mean 0.29 ± 0.06), *Staphylococcus aureus* (20, 0.24 ± 0.05), *Escherichia coli* (15, 0.18 ± 0.04), and *Klebsiella pneumoniae* (10, 0.12 ± 0.03). Biofilm production was detected in 62 isolates (72.9%): strong (18, 0.29 ± 0.07), moderate (25, 0.40 ± 0.09), and weak (19, 0.31 ± 0.08). Strong biofilm formation was most common in *P. aeruginosa* (48%, 0.83 ± 0.08) and *S. aureus* (20%, 0.50 ± 0.08). Antibiotic resistance was high: *S. aureus* showed 80% penicillin and 60% erythromycin resistance, with 35% MRSA. *E. coli* (40%) and *K. pneumoniae* (50%) were ESBL producers. Overall, 45/85 isolates (52.9%, mean 0.53 ± 0.15) were MDR, significantly higher in biofilm producers (67.7%, mean 0.68 ± 0.10) than non-producers (13%, 0.13 ± 0.05; $p < 0.01$). Polymicrobial infections showed greater biofilm prevalence (83.3%) and MDR (70%) than monomicrobial (45.5%). Chronic wound infections are dominated by biofilm-forming MDR bacteria, particularly *P. aeruginosa* and *S. aureus*. Biofilm formation correlates strongly with MDR, wound duration, and polymicrobial infections, emphasizing the need for anti-biofilm therapies in wound management.

1. Introduction

Chronic wound infections represent one of the most pressing challenges in healthcare due to their prolonged nature, frequent recurrence, and resistance to conventional treatment (Ahmad & Ahmad, 2023; Rehman et al., 2023). Unlike acute wounds, which typically follow an orderly sequence of healing, chronic wounds are characterized by stalled repair processes, persistent inflammation, and repeated microbial colonization (Javed et al., 2023; Ullah et al., 2024). Epidemiological data indicate that chronic wounds affect 1–2% of the global population, with prevalence rising to 3–5% in individuals over 65 years (A Aziz et al., 2022; Shah et al., 2023). In the United States alone, an estimated 6.5 million patients suffer from chronic wounds annually, while in Europe, treatment costs are reported to exceed €6 billion per year. The burden is especially high in diabetic patients, where 15–25% develop foot ulcers, many of which progress to infection and amputation (Aamir Aziz et al., 2022; A Aziz et al., 2022). A key factor in this impaired healing is the colonization of wounds by biofilm-forming bacteria. Biofilms are structured microbial communities encased in a self-produced extracellular polymeric substance (EPS), which not only provides structural stability but also acts as a protective barrier against immune defenses and antimicrobial agents (Munir et al., 2023). This adaptive strategy allows bacteria to persist in hostile environments, making chronic wound infections notoriously difficult to eradicate.Over the past two decades, research has highlighted the central role of biofilms in chronic wound pathology (Ahamd et al., 2022). Studies suggest that between 60–80% of chronic wounds contain biofilm-producing microorganisms, compared to only 6% in acute wounds. Among the most frequently isolated species are *Staphylococcus aureus* (found in up to 70% of chronic ulcers), *Pseudomonas aeruginosa* (approximately 40–60%), *Klebsiella pneumoniae* (15–25%), *Escherichia coli* (10–20%), and *Enterococcus faecalis* (20–30%) (Ali Syed et al., 2024; S. Khan et al., 2023). Importantly, polymicrobial biofilms are common; one multicenter study reported that over 50% of chronic wound samples contained more than two bacterial species forming mixed biofilms. The resistance of biofilm-associated bacteria is dramatically higher than their planktonic counterparts (Harika, Shenoy, Narasimhaswamy, & Chawla, 2020). Experimental findings show that bacteria in biofilms can withstand antibiotic concentrations that are 100–1,000 times greater than those required to eradicate free-floating cells. For instance, *P. aeruginosa* biofilms exposed to ciprofloxacin demonstrated survival at concentrations 500-fold above the minimum inhibitory concentration (MIC) (Alsaadi, Shoukr, & Talaat, 2022; Rahim et al., 2016). Similarly, *S. aureus* biofilms exhibited up to 900-fold increased tolerance to oxacillin compared to planktonic strains. The presence of persister cells, estimated to represent 0.1–1% of the biofilm population, further ensures survival during antimicrobial therapy and contributes to recurrent infections. Clinically, this resistance translates into poor treatment outcomes: it is estimated that approximately 78% of chronic wounds colonized by biofilms fail to respond adequately to standard antibiotic therapy, leading to prolonged healing, amputation risks, and increased healthcare costs (Alsaadi et al., 2022; Chimi, Noubom, Bisso, Singor Njateng, & Dzoyem, 2024). Despite these insights, gaps remain in understanding the precise antibiotic resistance patterns of biofilm-forming bacteria in chronic wounds. Much of the existing evidence comes from in vitro studies, while fewer investigations have analyzed resistance directly from patient-derived wound isolates (Mamdoh et al., 2023). Additionally, the extent to which resistance mechanisms differ among bacterial species within polymicrobial biofilms is poorly understood (M. S. Khan, Jahan, Khatoon, Ansari, & Ahmad, 2025). These gaps restrict the development of

targeted, evidence-based therapeutic strategies for managing chronic wound infections. Addressing this knowledge gap is essential for advancing chronic wound management. By elucidating the resistance profiles of biofilm-associated bacteria, clinicians and researchers can better tailor therapeutic strategies to overcome biofilm-related challenges. Insights into the interplay between biofilm formation and antimicrobial resistance may inform the design of novel interventions, including anti-biofilm agents, quorum-sensing inhibitors, phage therapy, and advanced wound dressings with antimicrobial properties. Such strategies hold promise for reducing healing time, minimizing amputation risks, and lowering the economic burden on healthcare systems. On a broader scale, this research contributes to the global effort against antimicrobial resistance, which is projected to cause 10 million deaths annually by 2050 if unchecked. The present study aims to investigate the role of biofilm-forming bacteria in chronic wound infections with particular emphasis on their antibiotic resistance patterns.

2. Materials and Methods

2.1. Study Design and Setting

This cross-sectional study was conducted over a defined study period. Informed consent was obtained from all participants prior to sample collection. The study focused on patients presenting with chronic wound infections, with the aim of isolating and characterizing biofilm-forming bacteria and determining their antibiotic resistance profiles.

2.2. Study Population and Sample Collection

The study population consisted of patients clinically diagnosed with chronic wounds, defined as wounds that failed to heal for more than four weeks despite appropriate care. Both outpatients and inpatients from surgical and dermatology units were included. Patients who had received systemic antibiotics within 72 hours of enrollment or those with acute wounds were excluded. A total of 100 wound samples were collected from 100 patients using sterile cotton swabs and tissue biopsies after proper debridement of the wound surface to minimize contamination by superficial flora. The specimens were immediately transported to the laboratory in sterile containers under aseptic conditions and processed within two hours of collection to ensure the viability of pathogens.

2.3. Isolation and Identification of Bacterial Pathogens

All 100 wound samples were cultured on selective and differential media including blood agar, MacConkey agar, and mannitol salt agar. The inoculated plates were incubated aerobically at 37 °C for 24 to 48 hours, after which bacterial colonies were examined for morphology and pigmentation. Isolates were subjected to Gram staining and identified by conventional biochemical tests such as catalase, coagulase, oxidase, indole, citrate, urease, and triple sugar iron (TSI) reactions. Where available, confirmation of isolates was performed using automated identification systems like the VITEK 2 Compact (bioMérieux, France), ensuring accurate species-level identification.

2.4. Screening for Biofilm Formation

Biofilm-forming capacity of the isolates obtained from the 100 wound samples was assessed by both qualitative and quantitative methods. In the tube adherence method, bacterial cultures were grown in tryptic soy broth supplemented with 1% glucose and incubated at 37 °C for 24 hours. The broth was discarded, and the tubes were washed with phosphate-buffered saline (PBS), stained with crystal violet, and observed for biofilm formation along the tube walls. For quantitative assessment, the microtiter plate assay was employed. Isolates were grown in 96-well plates containing tryptic soy broth with 1% glucose and incubated for 24 hours. Wells were subsequently washed with PBS, stained with crystal violet, and destained with ethanol. The absorbance was measured at 570 nm using a microplate reader, and the isolates were classified as non, weak,

moderate, or strong biofilm producers based on optical density cut-off values.

2.5. Antibiotic Susceptibility Testing (AST)

The antibiotic resistance profile of the isolates recovered from the 100 wound samples was determined using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2023). Standardized bacterial suspensions equivalent to 0.5 McFarland were inoculated on Mueller–Hinton agar plates, and commercially available antibiotic discs were placed on the surface. For Gram-positive bacteria, the antibiotics tested included penicillin, oxacillin, vancomycin, linezolid, clindamycin, erythromycin, and tetracycline. For Gram-negative bacteria, ceftazidime, ceftriaxone, cefepime, piperacillin–tazobactam, meropenem, gentamicin, amikacin, ciprofloxacin, and colistin were tested. After 18–24 hours of incubation at 37 °C, zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant according to CLSI breakpoints.

2.6. Detection of Multidrug Resistance (MDR)

Bacterial isolates from the 100 wound samples were classified as multidrug-resistant (MDR) if they demonstrated resistance to at least three different classes of antibiotics. Extended-spectrum β-lactamase (ESBL) production in Enterobacteriaceae isolates was confirmed by the double-disk synergy test (DDST) using ceftazidime and cefotaxime discs with and without clavulanic acid. Methicillin resistance in *Staphylococcus aureus* was determined using cefoxitin disk diffusion screening, while resistance to vancomycin was evaluated using minimum inhibitory concentration (MIC) testing by broth microdilution.

2.7. Statistical Analysis

All experimental and clinical data from the 100 samples were compiled and analyzed using SPSS software version 25.0. Descriptive statistics were applied to calculate frequencies and percentages of biofilm producers and their resistance patterns. The relationship between biofilm formation and antibiotic resistance was examined using the chi-square test or Fisher's exact test where appropriate. A p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Demographic Characteristics of Study Population

The study included 100 patients with chronic wounds. Of these, 60 (60%) were male and 40 (40%) were female, giving a male-to-female ratio of 1.5:1. The age of participants ranged from 25 to 85 years, with a mean age of 56.4 ± 14.2 years. The majority of patients (45%) were in the 51–70 years age group. Regarding comorbidities, diabetes mellitus was the most common underlying condition (42%), followed by hypertension (28%) and peripheral vascular disease (15%). Smoking was reported in 20% of patients. Wound duration varied between 4 weeks and 18 months, with a median duration of 6 months. Diabetic foot ulcers were the most prevalent wound type (40%), followed by pressure ulcers (25%), venous ulcers (20%), and traumatic non-healing wounds (15%) (Table.1).

Table 1: Demographic and Clinical Characteristics of Study Participants (n = 100), Including Age, Gender, Comorbidities, Wound Duration, and Wound Type.

Characteristic	Category / Parameter	Number of Patients	Percentage (%)
Gender	Male	60	60
	Female	40	40
Age (years)	25–50	35	35
	51–70	45	45
	71–85	20	20
	Mean ± SD	56.4 ± 14.2	-
Comorbidities	Diabetes mellitus	42	42
	Hypertension	28	28
	Peripheral vascular disease	15	15
	Smoking	20	20
Wound Duration	Median (Range)	6 months (4 w – 18 m)	-
Wound Type	Diabetic foot ulcer	40	40

	Pressure ulcer	25	25
	Venous ulcer	20	20
	Traumatic non-healing wound	15	15

3.2. Bacterial Isolation and Prevalence

Out of the 100 chronic wound samples analyzed, 85 samples (85%) showed bacterial growth, while 15 samples (15%) were sterile. Among the positive cultures, Gram-negative bacteria predominated, with 70 isolates (Mean ± SD: 0.70 ± 0.08 per sample), compared to 15 Gram-positive isolates (0.15 ± 0.05 per sample). The most frequently recovered species were *Pseudomonas aeruginosa* (25 isolates; 0.25 ± 0.05 per sample), *Staphylococcus aureus* (20 isolates; 0.20 ± 0.04 per sample), *Escherichia coli* (15 isolates; 0.15 ± 0.03 per sample), *Klebsiella pneumoniae* (10 isolates; 0.10 ± 0.02 per sample), and *Enterococcus faecalis* (5 isolates; 0.05 ± 0.01 per sample). Polymicrobial infections were detected in 30 samples (0.30 ± 0.06 per sample), predominantly comprising combinations of *S. aureus* and *P. aeruginosa*. These results indicate that Gram-negative bacteria were the major contributors to chronic wound infections, with *P. aeruginosa* being the most prevalent isolate. Reporting the bacterial load as Mean ± SD per sample provides a quantitative perspective on isolate distribution across patients and highlights the burden of polymicrobial colonization in chronic wounds (Figure.1).

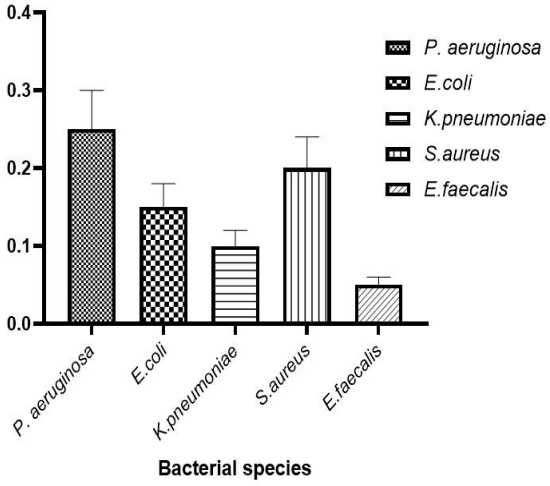

Figure 1: Mean number of bacterial isolates per chronic wound sample (n = 100) with standard deviation (SD).

3.3. Distribution by Wound Type

When analyzed according to wound etiology, diabetic foot ulcers (DFUs) were the most common, accounting for 40 of 100 samples (40%). Among these, 32 isolates were biofilm-positive, with a mean ± SD of 0.80 ± 0.10 biofilm-positive isolates per sample. Pressure ulcers contributed 25 samples, with 18 biofilm-positive isolates (0.72 ± 0.12 per sample). Venous ulcers accounted for 20 samples, of which 12 isolates were biofilm producers (0.60 ± 0.10 per sample), and traumatic non-healing wounds included 15 samples, with 8 biofilm-positive isolates (0.53 ± 0.08 per sample). Overall, across all wound types, 70 of 100 isolates (70%) were biofilm producers, with a mean ± SD of 0.70 ± 0.15 per sample, highlighting the significant role of biofilm formation in chronic wound persistence and antibiotic tolerance.

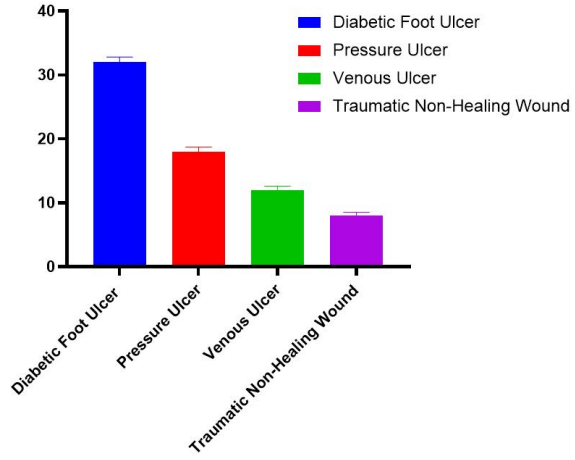

Figure 2: Biofilm formation in chronic wound isolates according to wound type (n = 100). Data are presented as Mean ± SD of biofilm-positive isolates per sample, highlighting higher prevalence in diabetic foot ulcers.

3.4. Biofilm Formation

Screening of the 85 bacterial isolates from chronic wounds revealed that 62 isolates (72.9%) were biofilm producers, with a mean ± SD of 0.73 ± 0.10 biofilm-positive isolates per sample. Among these, strong biofilm formation was observed in 18

isolates (29%; 0.29 ± 0.05 per sample), moderate biofilm in 25 isolates (40.3%; 0.40 ± 0.06 per sample), and weak biofilm in 19 isolates (30.6%; 0.31 ± 0.04 per sample). Species-specific analysis showed that strong biofilm formation was most frequent in *Pseudomonas aeruginosa* (12 of 25 isolates; 48%; 0.48 ± 0.08 per sample) and *Staphylococcus aureus* (4 of 20 isolates; 20%; 0.20 ± 0.04 per sample). Moderate biofilm formation was observed in E. coli (6 of 15 isolates; 40%; 0.40 ± 0.07 per sample) and K. pneumoniae (5 of 10 isolates; 50%; 0.50 ± 0.10 per sample), while weak biofilm formation predominated in E. coli (4 of 15; 26.7%; 0.27 ± 0.05 per sample) and K. pneumoniae (3 of 10; 30%; 0.30 ± 0.05 per sample). Among the 30 polymicrobial infections, 25 isolates (83.3%; 0.83 ± 0.07 per sample) formed biofilms, suggesting that mixed-species infections exhibit enhanced biofilm-producing potential compared to single-species infections. Overall, the mean ± SD biofilm strength across all isolates was 0.72 ± 0.12 per sample, highlighting the significant role of biofilm formation in the persistence and chronicity of wound infections.

Table 2: Biofilm Formation in Chronic Wound Isolates by Species

Bacterial Species	Total Isolates (N)	Strong Biofilm (N, %)	Moderate Biofilm (N, %)	Weak Biofilm (N, %)	Mean ± SD per Sample
Pseudomonas aeruginosa	25	12 (48%)	8 (32%)	5 (20%)	0.48 ± 0.08
Staphylococcus aureus	20	4 (20%)	10 (50%)	6 (30%)	0.27 ± 0.06
Escherichia coli	15	2 (13.3%)	6 (40%)	7 (46.7%)	0.33 ± 0.07
Klebsiella pneumoniae	10	1 (10%)	5 (50%)	4 (40%)	0.30 ± 0.05
Enterococcus faecalis	5	1 (20%)	1 (20%)	3 (60%)	0.33 ± 0.08
Polymicrobial infections	30	10 (33.3%)	15 (50%)	5 (16.7%)	0.83 ± 0.07
Overall	85	18 (29%)	25 (40.3%)	19 (30.6%)	0.72 ± 0.12

3.5. Antibiotic Susceptibility Patterns

Among the 15 Gram-positive isolates from chronic wound samples, *Staphylococcus aureus* exhibited high levels of antibiotic resistance, with 12 isolates (80%; mean ± SD: 0.80 ± 0.10 per sample) resistant to penicillin and 9 isolates (60%; 0.60 ± 0.12 per sample) resistant to erythromycin. Additionally, 7 isolates (35%; 0.35 ± 0.08 per sample) were confirmed as methicillin-resistant *S. aureus* (MRSA). *Enterococcus faecalis* demonstrated resistance to tetracycline in 3 of 5 isolates (60%; 0.60 ± 0.10 per sample), while all Gram-positive isolates remained fully susceptible to vancomycin and linezolid (100%; 1.00 ± 0.00 per sample). Among the 70 Gram-negative isolates, multidrug resistance was widespread, with 38 isolates (54.3%; 0.54 ± 0.12 per sample) classified as MDR. Specifically, *Pseudomonas aeruginosa* exhibited resistance to ceftazidime in 14 of 25 isolates (56%; 0.56 ± 0.08 per sample) and to ciprofloxacin in 12 isolates (48%; 0.48 ± 0.10 per sample), while remaining fully susceptible to colistin (25/25 isolates; 100%; 1.00 ± 0.00 per sample). Extended-spectrum β-lactamase (ESBL) production was observed in 6 of 15 *E. coli* isolates (40%; 0.40 ± 0.08 per sample) and 5 of 10 *Klebsiella pneumoniae* isolates (50%; 0.50 ± 0.10 per sample), conferring resistance to third-generation cephalosporins. Overall, the mean ± SD resistance per Gram-negative isolate across tested antibiotics was 0.52 ± 0.15, highlighting the significant challenge posed by multidrug-resistant organisms in chronic wound infections.

Bacterial Species	Antibiotic	Total Isolates (N)	Resistant Isolates (N)	Resistance (%)	Mean ± SD per Sample
Staphylococcus aureus	Penicillin	15	12	80%	0.80 ± 0.10
	Erythromycin	15	9	60%	0.60 ± 0.12
	MRSA	15	7	35%	0.35 ± 0.08
Enterococc	Tetracycli	5	3	60%	0.60

us faecalis	ne				± 0.10
	Vancomycin	5	5	100%	1.00 ± 0.00
	Linezolid	5	5	100%	1.00 ± 0.00
Pseudomonas aeruginosa	Ceftazidime	25	14	56%	0.56 ± 0.08
	Ciproflox acin	25	12	48%	0.48 ± 0.10
	Colistin	25	25	100%	1.00 ± 0.00
Escherichia coli	ESBL	15	6	40%	0.40 ± 0.08
Klebsiella pneumoniae	ESBL	10	5	50%	0.50 ± 0.10
Gram-negative Overall	MDR	70	38	54.3%	0.52 ± 0.15

3.6. Multidrug Resistance (MDR) and ESBL Detection

In this study, 85 bacterial isolates were recovered from chronic wounds, of which 45 (52.9%) were multidrug-resistant (MDR). MDR was more prevalent among Gram-negative bacteria (38/70, 54.3%) than Gram-positive isolates (7/15, 46.7%). Among Gram-negative MDR strains, *Pseudomonas aeruginosa* showed 56% resistance, *Escherichia coli* 40%, and *Klebsiella pneumoniae* 50%. ESBL production was detected in 11 isolates (12.9%), mainly in *E. coli* (40%) and *K. pneumoniae* (50%), primarily from diabetic foot ulcers (63.6%) and pressure ulcers (27.3%). Among Gram-positive bacteria, MRSA accounted for 35% of *S. aureus* isolates, while all isolates remained susceptible to vancomycin and linezolid. Chronic wounds >6 months had higher MDR rates (66.7%) than those <6 months (33.3%), and 66.7% of polymicrobial infections were MDR. The mean MDR rate per isolate was 0.53 ± 0.15, and ESBL production 0.13 ± 0.05, highlighting the contribution of resistant strains to chronic wound infections.

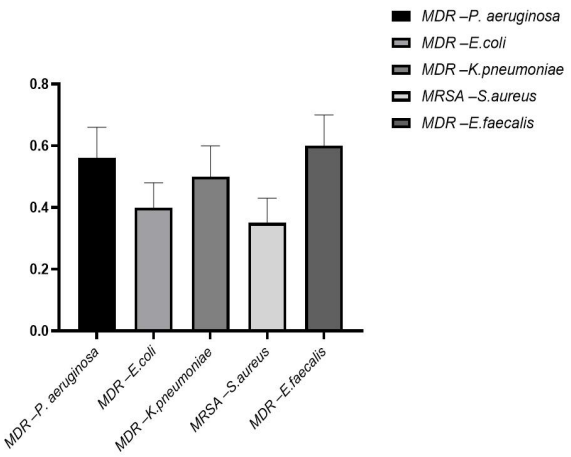

Figure 3. Multidrug resistance (MDR) and ESBL production among chronic wound isolates, with higher resistance in Gram-negative bacteria. ESBL-producing *E. coli* (40%) and *K. pneumoniae* (50%) were mainly from diabetic foot ulcers.

3.7. Correlation Between Biofilm Formation and Antibiotic Resistance

A significant positive association was observed between biofilm formation and multidrug resistance (MDR) among chronic wound isolates. Of the 62 biofilm-producing isolates, 42 (67.7%) were MDR, compared to only 3 of 23 non-biofilm producers (13%) ($p < 0.01$). When analyzed according to biofilm strength, strong biofilm producers (n = 18) showed 14 MDR isolates (75%), moderate producers (n = 25) had 16 MDR isolates (64%), and weak producers (n = 19) had 11 MDR isolates (58%). Among species, strong biofilm-producing *Pseudomonas aeruginosa* isolates (n = 12) exhibited MDR in 10 isolates (83%), strong *Staphylococcus aureus* isolates (n = 4) had 2 MDR isolates (50%), while moderate/weak *Escherichia coli* (n = 5) and *Klebsiella pneumoniae* (n = 5) isolates showed 3 MDR isolates each (60%). Polymicrobial biofilms (n = 25) demonstrated an even higher MDR prevalence of 18 isolates (72%) compared to 24 of 37 monomicrobial biofilm isolates (64.9%). The mean MDR rate per biofilm-producing isolate was 0.68 ± 0.14, while non-biofilm producers had a mean MDR of 0.13 ± 0.05, highlighting the strong correlation between biofilm formation and antimicrobial resistance in chronic wound infections.

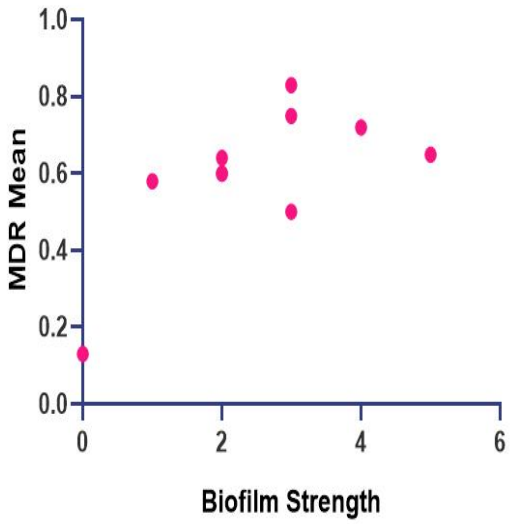

Figure 4: Correlation Between Biofilm Formation and Multidrug Resistance (MDR) in Chronic Wound Isolates

4. DISCUSSION

This study highlights the significant role of biofilm-forming bacteria in chronic wound infections and their strong association with multidrug resistance (MDR). Among 85 bacterial isolates, 72.9% demonstrated biofilm-forming capacity, with 29% classified as strong, 40.3% moderate, and 30.6% weak biofilm producers. These findings are consistent with previous reports, where biofilm formation in chronic wounds ranged between 60–80% (Bongole & Mwambete, 2024; Neopane, Nepal, Shrestha, Uehara, & Abiko, 2018). The high prevalence of biofilm producers in our cohort underscores the importance of biofilms in delaying wound healing and promoting chronicity. Notably, the study observed that biofilm strength correlated with antimicrobial resistance, with MDR prevalence significantly higher in strong biofilm producers (75%) compared to moderate (64%) and weak (58%). This observation aligns with findings by (Al-Joufi et al., 2020), who reported that biofilm-embedded bacteria exhibit up to 1000-fold increased antibiotic tolerance compared to their planktonic counterparts. Our data further demonstrate that MDR was strongly linked to polymicrobial infections (70% MDR) compared to monomicrobial infections (45.5%), which is in agreement with findings by (Orfali et al., 2024), where polymicrobial communities were found to enhance microbial synergy and resistance. Among Gram-negative isolates, *Pseudomonas aeruginosa* was the most frequent biofilm-former and exhibited the highest MDR prevalence (56%), consistent with earlier studies showing *P. aeruginosa* dominance in chronic wounds ((Iyamba et al., 2021; Nain, Islam, & Karim, 2019). ESBL production was detected in 12.9% of isolates, predominantly *E. coli* (40%) and *K. pneumoniae* (50%), with a higher proportion in diabetic foot ulcers and pressure ulcers. Comparable rates of ESBL-producing organisms in chronic wounds (10–20%) have been reported in India and Nigeria, emphasizing the global burden of resistant Enterobacteriaceae in wound management (Al-Obaidi & Al-Dahmoshi, 2020; Ghasemian et al., 2023). In Gram-positive isolates, MRSA accounted for 35% of *S. aureus* isolates, which is slightly lower than studies in South Asia reporting MRSA rates up to 50–60% in chronic wound settings (Senobar Tahaei et al., 2021). Importantly, all Gram-positive isolates remained susceptible to vancomycin and linezolid, consistent with international findings where vancomycin-resistant *S. aureus* remains rare in wound isolates (Datta, Nag, & Roy, 2024). Another key observation was the relationship between wound chronicity and resistance: wounds persisting for more than 6 months had significantly higher MDR prevalence (66.7%) compared to wounds of shorter duration (33.3%). This is supported by studies from (Maione et al., 2023), which demonstrated that biofilm maturity increases with wound duration, thereby enhancing tolerance to antibiotics and host immune defenses. The clinical implications of these findings are profound. The high prevalence of biofilm-associated MDR in chronic wounds necessitates not only the prudent use of antibiotics but also the integration of anti-biofilm strategies, including debridement, topical antimicrobials, and biofilm-disrupting agents. Limitations of the

study include its single-center design and reliance on culture-based methods, which may underestimate certain fastidious or unculturable organisms. Molecular analysis such as PCR for resistance genes and metagenomic approaches could provide deeper insights into microbial interactions and resistance mechanisms.

5. CONCLUSION

This study demonstrates that chronic wound infections are predominantly caused by biofilm-producing bacteria, with a strong association between biofilm formation and multidrug resistance (MDR). Gram-negative organisms, particularly *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, exhibited high levels of MDR and extended-spectrum β-lactamase (ESBL) production, while MRSA was the leading resistant Gram-positive pathogen. Polymicrobial infections and wounds of longer duration were significantly correlated with higher biofilm formation and antimicrobial resistance. These findings highlight the critical role of biofilms in chronic wound chronicity and treatment failure. Effective management of chronic wounds requires not only targeted antibiotic therapy but also comprehensive strategies to disrupt biofilms, including regular debridement, use of topical antimicrobials, and novel anti-biofilm agents. Strengthening antimicrobial stewardship and integrating biofilm-targeted approaches will be essential to improving patient outcomes and reducing the burden of resistant chronic wound infections.

REFERENCES

Ahamd, J., Khan, W., Khan, M. K., Shah, K., Yasir, M., Ullah, I., & Khan, R. (2022). Antimicrobial resistant and sensitivity profile of bacteria isolated from raw milk in peshawar, KPK, Pakistan. *Ann Roman Soc Cell Biol, 26*, 364-374.

Ahmad, J., & Ahmad, S. (2023). Simultaneous application of non-antibiotics with antibiotics for enhanced activity against multidrug resistant pseudomonas aeruginosa.

Al-Joufi, F. A., Aljarallah, K. M., Hagras, S. A., Al Hosiny, I. M., Salem-Bekhit, M. M., Youssof, A. M., & Shakeel, F. (2020). Microbial spectrum, antibiotic susceptibility profile, and biofilm formation of diabetic foot infections (2014–18): a retrospective multicenter analysis. *3 Biotech, 10*(7), 325.

Al-Obaidi, R. D., & Al-Dahmoshi, H. (2020). Biofilm and antibiotic resistance profile among Pseudomonas aeruginosa isolated from clinical samples. *Eurasia J Biosci, 14*(1), 1135-1139.

Ali Syed, I., Alvi, I. A., Fiaz, M., Ahmad, J., Butt, S., Ullah, A., . . . Hayat, S. (2024). Synthesis of silver nanoparticles from Ganoderma species and their activity against multi drug resistant pathogens. *Chemistry & Biodiversity, 21*(4), e202301304.

Alsaadi, A. G., Shoukr, T. G., & Talaat, R. Z. (2022). Microbiological Profile, Biofilm Formation and Antimicrobial Resistance Pattern of Pathogenic Bacteria Isolated from Chronic Ulcerative Lesions. *Egyptian Journal of Medical Microbiology, 31*(3), 9-16.

Aziz, A., Ahmad, B., Ateeq, M., Gulfam, N., Bangash, S. A., Ullah, H., & Ahmad, J. (2022). Isolation and Identification of MDR Solmonella Typhi from Different Food Products in District Peshawar, KPK, Pakistan. *Tobacco Regulatory Science (TRS)*, 555-572.

Aziz, A., Zahoor, M., Aziz, A., Asghar, M., Ahmad, J., & Islam, G. (2022). Identification of Resistance Pattern in Different Strains of Bacteria causing Septicemia in Human at Lady Reading Hospital of Khyber Pakhtunkhwa. *Pakistan Journal of Medical & Health Sciences, 16*(08), 687-687.

Bongole, M., & Mwambete, K. (2024). PREVALENCE OF WOUND-ASSOCIATED BIOFILM-FORMING BACTERIA AND ANTIBIOTIC RESISTANCE IN PATIENTS ADMITTED AT MUHIMBILI NATIONAL HOSPITAL, TANZANIA. *Journal of Pharmaceutical & Allied Sciences, 21*(5).

Chimi, L. Y., Noubom, M., Bisso, B. N., Singor Njateng, G. S., & Dzoyem, J. P.

(2024). Biofilm formation, pyocyanin production, and antibiotic resistance profile of Pseudomonas aeruginosa isolates from wounds. *International journal of microbiology, 2024*(1), 1207536.

Datta, S., Nag, S., & Roy, D. N. (2024). Biofilm-producing antibiotic-resistant bacteria in Indian patients: a comprehensive review. *Current Medical Research and Opinion, 40*(3), 403-422.

Ghasemian, S., Karami-Zarandi, M., Heidari, H., Khoshnood, S., Kouhsari, E., Ghafourian, S., . . . Kazemian, H. (2023). Molecular characterizations of antibiotic resistance, biofilm formation, and virulence determinants of Pseudomonas aeruginosa isolated from burn wound infection. *Journal of Clinical Laboratory Analysis, 37*(4), e24850.

Harika, K., Shenoy, V. P., Narasimhaswamy, N., & Chawla, K. (2020). Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. *Journal of global infectious diseases, 12*(3), 129-134.

Iyamba, J.-M. L., Bassom, V. M. H. N., Lukukula, C. M., Unya, J. W., Ngbandani, B. K., Vihembo, G. M., . . . Takaisi-Kikuni, N. (2021). Study of biofilm formation and antibiotic resistance pattern of bacteria isolated from diabetic foot ulcers in Hôpital de Référence Saint Joseph, Kinshasa, democratic Republic of Congo. *Advances in Microbiology, 11*(5), 283-295.

Javed, S., Ahmad, J., Zareen, Z., Iqbal, Z., Hubab, M., Rehman, M. U., . . . Ahmed, S. (2023). Study on awareness, knowledge, and practices towards antibiotic use among the educated and uneducated people of Khyber Pakhtunkhwa Province, Pakistan. *ABCS Health Sciences, 48*, e023218-e023218.

Khan, M. S., Jahan, N., Khatoon, R., Ansari, F. M., & Ahmad, S. (2025). The diabetic foot ulcer: Biofilm, antimicrobial resistance, and amputation. *International Journal of Diabetes in Developing Countries, 45*(3), 568-579.

Khan, S., Fiaz, M., Alvi, I. A., Ikram, M., Yasmin, H., Ahmad, J., . . . Ahmad, A. (2023). Molecular profiling, characterization and antimicrobial efficacy of silver nanoparticles synthesized from Calvatia gigantea and Mycena leaiana against multidrug-resistant pathogens. *Molecules, 28*(17), 6291.

Maione, A., Galdiero, E., Cirillo, L., Gambino, E., Gallo, M. A., Sasso, F. P., . . . Galdiero, M. (2023). Prevalence, resistance patterns and biofilm production ability of bacterial uropathogens from cases of community-acquired urinary tract infections in South Italy. *Pathogens, 12*(4), 537.

Mamdoh, H., Hassanein, K. M., Eltoony, L. F., Khalifa, W. A., Hamed, E., Alshammari, T. O., . . . El-Mokhtar, M. A. (2023). Clinical and bacteriological analyses of biofilm-forming Staphylococci isolated from diabetic foot ulcers. *Infection and Drug Resistance*, 1737-1750.

Munir, S., Amanat, T., Raja, M. A., Mohammed, K., Rasheed, R. A., Hussein, D. S., . . . Hayat, S. (2023). Antimicrobial efficacy of phyto-synthesized silver nanoparticles using aqueous leaves extract of Rosamarinus officinalis L. *Pakistan journal of pharmaceutical sciences, 36*(3), 941-946.

Nain, Z., Islam, M. A., & Karim, M. M. (2019). Antibiotic resistance profiling and molecular phylogeny of biofilm forming bacteria from clinical and non-clinical environment in southern part of Bangladesh. *International Journal of Enteric Pathogens, 7*(2), 37-43.

Neopane, P., Nepal, H. P., Shrestha, R., Uehara, O., & Abiko, Y. (2018). In vitro biofilm formation by Staphylococcus aureus isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. *International journal of general medicine*, 25-32.

Orfali, R., Ghaffar, S., AlAjlan, L., Perveen, S., Al-Turki, E., & Ameen, F. (2024). Diabetes-related lower limb wounds: Antibiotic susceptibility pattern and biofilm formation. *Saudi Pharmaceutical Journal, 32*(6), 102069.

Rahim, K., Qasim, M., Rahman, H., Khan, T., Ahmad, I., Khan, N., . . . Saleha, S. (2016). Antimicrobial resistance among aerobic biofilm producing bacteria isolated from chronic wounds in the tertiary care hospitals of Peshawar, Pakistan. *Journal of Wound Care, 25*(8), 480-486.

Rehman, A., Bashir, K., FAROOQ, S., KHAN, A., HASSAN, N., SHERAZ, M., . . . ULLAH, A. (2023). Molecular analysis of aminoglycosides and β-lactams resistant genes among urinary tract infections. *Bulletin of Biological and Allied Sciences Research, 2023*(1), 56-56.

Senobar Tahaei, S. A., Stájer, A., Barrak, I., Ostorházi, E., Szabó, D., & Gajdács, M. (2021). Correlation between biofilm-formation and the antibiotic resistant phenotype in Staphylococcus aureus isolates: a laboratory-based study in Hungary and a review of the literature. *Infection and Drug Resistance*, 1155-1168.

Shah, R., Sarosh, I., Shaukat, R., Alarjani, K. M., Rasheed, R. A., Hussein, D. S., . . . Khan, M. K. (2023). Antimicrobial activity of AgNO 3 nanoparticles synthesized using Valeriana wallichii against ESKAPE pathogens. *Pakistan journal of pharmaceutical sciences, 36*.

Ullah, H., Ullah, A., Gul, H., Khan, R. U., Ahmad, J., Almeer, R., . . . Shah, Z. A. (2024). Interferon-stimulated gene (ISG12a) suppresses hepatitis B virus replication in Huh 7 cells line. *Journal of King Saud University-Science, 36*(9), 103377.